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Editorial

"Dental and Medical Problems": Where we are and where we are going

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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The important thing is not to stop questioning. Curiosity has its own reason for existence. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvelous structure of reality. It is enough if one tries merely to comprehend a little of this mystery each day.

Albert Einstein

Obtaining the Journal Impact Factor (JIF) by "Dental and Medical Problems" (*Dent Med Probl*) has resulted in an influx of scientific manuscripts of high quality and relevance from the world's leading dental schools, e.g., Harvard School of Dental Medicine or ACTA (Academisch Centrum Tandheelkunde Amsterdam) – a collaboration between the Faculty of Dentistry of the University of Amsterdam and the Vrije Universiteit Amsterdam.[1–4] This phenomenon has great scientific and image significance, and indeed raises the position and prestige of our journal, which in turn is of great importance for the Faculty of Dentistry of Wroclaw Medical University, as well as for Polish dentistry in general.

Considering the most frequently cited articles in 2023 and 2024, the key topics seem to be sleep disorders (especially sleep-related breathing disorders), bruxism, temporomandibular disorders, orofacial pain, and headache.[5–10] What is also noticeable is the appearance of articles from the frontiers of dentistry and medicine.[11,12] Indeed, *Dent Med Probl* is registered in the Dentistry, Oral Surgery & Medicine (Q1) and Medicine, General & Internal (Q1) (Web of Science), and General Dentistry (Q2) and General Medicine (Q2) (Scopus) categories. Thus, it strongly represents a multidisciplinary approach, and a comprehensive view of different general disorders and conditions which can involve the oral and facial region. This type of attitude appears to be optimal from the perspective of human well-being.

In 2024, we introduced guidelines for authors on data sharing and the use of artificial intelligence (AI) while preparing manuscripts for submission, as well as guidelines for reviewers on the use of AI in reviewing manuscripts. In the coming years, special emphasis should be put on raising the reporting quality of studies, and further internationalizing the journal. We think that large international cohort studies and translational studies, of which there have been a few in *Dent Med Probl* to date, will also play a major role, as well as

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research on digital dentistry and the use of AI in dentistry. We also hope that the number of manuscripts presenting Mendelian randomization (MR) and genome-wide association studies (GWAS) will increase in the future.

We would like to acknowledge the editorial staff, section editors, Editorial Board members, reviewers, authors, and readers for their contribution to the development of our journal. We do believe that *Dent Med Probl* will continue to advance with the help of the scientific community from around the world.

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References

1. Sedky RAF, Ben Dor B, Mustafa DS, et al. Self-assessment skills of undergraduate students in operative dentistry: Preclinical performance and gender [published online as ahead of print on September 27, 2024]. *Dent Med Probl*. doi:10.17219/dmp/175276
2. Thymi M, Farzan A, Ahlberg J, Manfredini D, Lobbezoo F. Qualitative suggestions for the further development of the Standardized Tool for the Assessment of Bruxism (STAB). *Dent Med Probl*. 2024;61(3):323–333. doi:10.17219/dmp/183692
3. Machado-Santos L, Baroudi K, Silikas N, et al. Physical analysis of an acrylic resin modified by metal and ceramic nanoparticles. *Dent Med Probl*. 2023;60(4):657–664. doi:10.17219/dmp/171844
4. Yamakami SA, Faraoni JJ, Lia NSND, Regula FB, Ohyama H, Palma-Dibb RG. Effect of an experimental chitosan/casein gel on demineralized enamel under a cariogenic challenge. *Dent Med Probl*. 2022;59(4):531–538. doi:10.17219/dmp/146038
5. 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
6. 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
7. 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
8. Görürgöz C, İçen M, Kurt MH, et al. Degenerative changes of the mandibular condyle in relation to the temporomandibular joint space, gender and age: A multicenter CBCT study. *Dent Med Probl*. 2023;60(1):127–135. doi:10.17219/dmp/147514
9. 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
10. 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
11. Dewan M, Pandit AK, Goyal L. Association of periodontitis and gingivitis with stroke: A systematic review and meta-analysis. *Dent Med Probl*. 2024;61(3):407–415. doi:10.17219/dmp/158793
12. Nasroen SL, Maskoen AM, Soedjana H, Hilmanto D, Gani BA. *IRF6* rs2235371 as a risk factor for non-syndromic cleft palate only among the Deutero-Malay race in Indonesia and its effect on the *IRF6* mRNA expression level. *Dent Med Probl*. 2022;59(1):59–65. doi:10.17219/dmp/142760

Original paper

Assessment of the effectiveness of a rotatable shank toothbrush compared to a conventional handle toothbrush: A multicenter, single-blind, randomized controlled trial

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Abstract

Background. The persistence of dental plaque is attributable to the inaccessibility to all surfaces of the oral cavity. Thus, an integrated team designed an innovative toothbrush comprising a brush head assembly with an upper end and a lower end, and a handle rotatably configured with the lower end of the brush head assembly. The brush head is connected to the handle through a socket–ball joint, which allows the shank and the handle to rotate at any angle between 0° and 360° with respect to one another around an axis. Additionally, the brush head bends toward the handle, maintaining a bending angle of 15°.

Objectives. The aim of the present randomized controlled trial (RCT) was to analyze and assess the effectiveness of a toothbrush with a rotatable shank in comparison to toothbrushes with flexible and straight handles with respect to supragingival plaque and gingival health outcomes. The secondary objective of the study was to evaluate the feedback of individuals who used the rotatable shank toothbrush.

Material and methods. Three toothbrushes – one with a rotatable shank, one with a flexible handle and one with a straight handle – were compared in terms of efficacy in plaque and gingivitis control at 3 clinical centers (a multicenter trial). A total of 270 patients, aged 18–65 years, were included in the study. The collected data was analyzed and compared using the analysis of variance (ANOVA) with Tukey's post hoc test.

Results. All groups demonstrated improvement in gingival health and a reduction in the plaque index (PI) scores. Nonetheless, the improvement was more pronounced in the group that used the toothbrush with a rotatable shank.

Conclusions. The enhanced plaque removal and improved gingival health at all surfaces achieved with the rotatable shank toothbrush are ascribable to the incorporation of 2 features: the ability to rotate the toothbrush neck along its axis; and an inclination that facilitates access to all surfaces.

Keywords: ergonomics, toothbrush, plaque removal, rotatable shank

Introduction

Periodontitis is a chronic multifactorial inflammatory disease that is associated with dysbiotic dental biofilm. It is characterized by the progressive destruction of the tooth-supporting apparatus and, if left untreated, leads to tooth loss. Periodontitis is a vital public health issue due to its high prevalence, which accounts for a substantial proportion of edentulism and masticatory dysfunction, and has a negative impact on general health.[1]

Mechanical plaque control is one of the most important health promotion strategies in dentistry, and is of utmost importance in the prevention of gingivitis and periodontitis.[2] Studies on the subject emphasize the importance of oral hygiene in reducing the incidence of tooth loss, the number of new decayed surfaces, and periodontal attachment loss, thereby reinforcing its importance in preventive dentistry.[3–6]

Manual toothbrushes are the most frequently used devices for the regular removal of plaque. A wide variety of toothbrushes are currently available on the market.[7]

In conjunction with the appropriately employed brushing technique, a brush should enable complete plaque removal. The advancement of the toothbrush design has been a continual process through the centuries, with the innovations aimed at enhancing efficiency and promoting dental health. The literature on the design of toothbrushes is unequivocal in its assertion that no single design is superior to another in achieving the most effective removal of dental plaque. The analysis of the investigations assessing the cleaning efficiency of different toothbrushes used with the same brushing method does not provide conclusive evidence to determine which toothbrush is superior to others.[8,9]

The trial conducted by Sripriya and Shaik Hyder Ali, which compared the efficacy of 4 different types of commercially available manual toothbrushes, concluded that although some minor differences in the plaque removal efficacy of the brushes were observed, they were not statistically significant, implying that none of the toothbrush designs was effective in terms of complete plaque removal.[10] In a study evaluating the plaque removal efficacy of 4 different designs of manual toothbrushes, Sial et al. hypothesized that, despite the introduction of various toothbrush designs to the market, no single toothbrush was found to be more effective than the others in removing plaque.[11] In a study conducted by Claydon et al., no superior design of a manual toothbrush was identified in the removal of plaque when 8 manual toothbrushes were compared by a professional tooth brusher.[12]

When adults are asked to manually perform oral hygiene procedures with conventional toothbrushes, to the best of their abilities, clinical studies have documented the persistence of a considerable amount of plaque. This is because the majority of the population is either not trained or suffers from a lack of skill to follow the recommendations, which limits the clinical effectiveness of self-performed oral hygiene.[13] Most individuals tend to brush surfaces that are easily accessible and neglect areas that are more challenging to reach. The buccal surfaces and the anterior teeth are most thoroughly cleaned and exhibit the lowest plaque accumulation, whereas the lingual/palatal aspects of the teeth demonstrate the greatest plaque accumulation.[14,15]

In light of these observations, it was pivotal to design a toothbrush that would provide better access to all areas, thereby improving an overall plaque reduction. Hence, an attempt was made to develop and evaluate a novel, ergonomic, rotatable toothbrush. The process of concept generation entailed an insightful understanding of user needs and the conversion of these needs into product requirements. The concept of keeping the bristles in a similar configuration to that of a standard toothbrush and incorporating a ball–socket mechanism at the shank, enabling it to rotate through 360° and bend up to 15°, was developed through a rapid ideation and prototyping process in conjunction with users.

Therefore, the purpose of the present study was to assess the safety and effectiveness of the rotatable shank toothbrush in the elimination of supragingival plaque at all sites of the oral cavity, with a particular focus on gingival health, in comparison to a flexible handle toothbrush and a straight handle toothbrush. The secondary objective of the study was to evaluate the feedback and experience of individuals who used the rotatable shank toothbrush. This could prove beneficial for the general public in selecting the more efficacious toothbrush among all commercially available options. The null hypothesis of the study stated that the rotatable shank toothbrush is equally effective in removing dental plaque by laypeople after 30 days of twice-daily use compared to conventional straight and flexible handle toothbrushes.

Material and methods

Study design

This experimental, multicenter, single-blind, randomized controlled trial (RCT) was conducted at Rural Dental College (Loni, India), Rajasthan Dental College and Hospital (Jaipur, India), and Chhattisgarh Dental College and Research Institute (Rajnandgaon, India). The study was designed in accordance with the CONSORT (Consolidated Standards of Reporting Trials) 2010 criteria (Fig. 1), approved by the Ethics Committee at the Pravara Institute of Medical Sciences (Deemed to be University) (PIMS-DU), Ahmednagar, India (approval No. PIMS/DR/RDC/2022/138), and registered with Clinical Trials Registry – India (CTRI) under the identification No. CTRI/2022/02/049838.

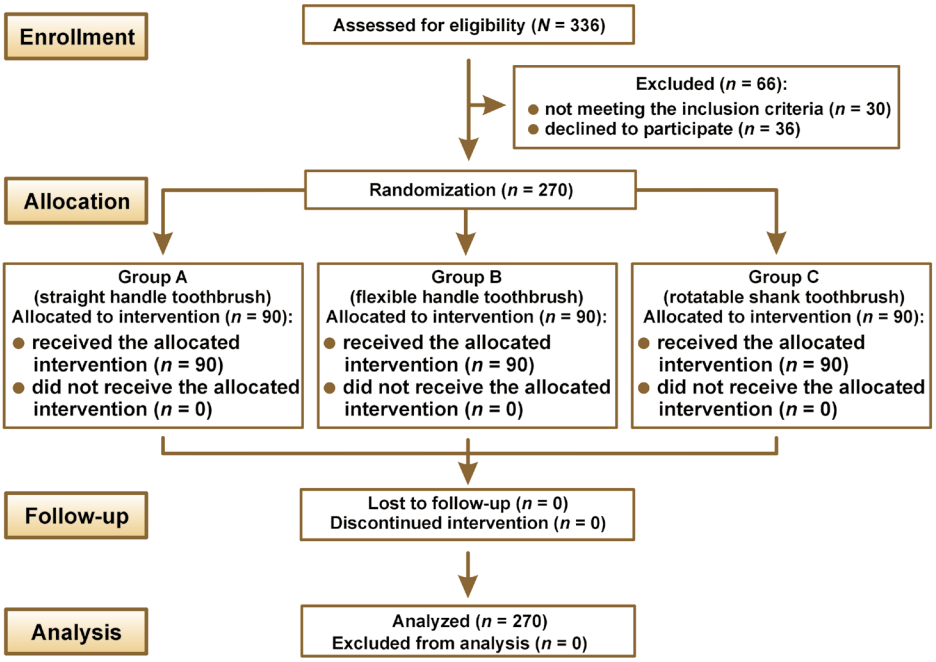

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) 2010 flowchart

Patient recruitment and eligibility criteria

The participants were recruited from the outpatient clinics of the periodontology departments at Rural Dental College, Rajasthan Dental College and Hospital, and Chhattisgarh Dental College and Research Institute, after signing a fully descriptive informed consent form. The patients were enrolled in the study in accordance with the established eligibility criteria. The participants were dental laypeople (i.e., individuals without any dental background), aged between 18 and 65 years, of either gender, having at least 20 natural, scorable teeth, with visible plaque accumulation represented by a continuous band of plaque (up to 1 mm) at the cervical margin on at least 30% of all facial tooth surfaces. The measurements were made using the Turesky modification of the Quigley–Hein plaque index (TMQHPI) (a score ≥2) and the Löe and Silness gingival index (GI) (a score ≤2 – redness, edema and glazing with bleeding on probing (BoP)). The participants exhibited signs of moderate inflammation and sought treatment for teeth scaling (cleaning) during the study period. The patients were excluded if they had undergone any surgical, chemical or antibiotic/dental prophylaxis procedures in the experimental area within 3 months prior to the study, had any major tissue lesions, had orthodontic banding or an intraoral prosthesis, or were pregnant. Additionally, bidi or cigarette smokers, oral tobacco or gutkha users, subjects with irregular brushing frequency (≤2 times/day), incomplete dentition, carious teeth, and those with periodontitis (probing depth (PD) ≥4 mm, clinical attachment loss (CAL) ≥1–2 mm and horizontal bone loss)[16] were excluded from the study.

Sample size calculation

The requisite sample size was estimated using the G*Power software, v. 3.1.9.7 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). With an effect size of 0.8, the minimum calculated sample size in each group was 20. With a 10% loss to follow-up, the sample size was 22 in each group. These values were rounded up, and a minimum of 25 participants were included in each group at each center, resulting in a total of 75 participants per center.

The total sample size was calculated to be 225. However, following the screening process, 336 participants were assessed for eligibility to participate in the study. A total of 30 individuals were excluded from the study due to failure to meet the inclusion criteria, while 36 participants declined to participate. Hence, a final excess of 45 participants were included (Fig. 1).

Sample grouping

Eventually, 90 individuals were selected at each center for the current study. The participants were further divided into 3 groups of 30 individuals each, according to the designated toothbrush, as follows: group A – the straight handle toothbrush; group B –the flexible handle toothbrush; and group C – the rotatable shank toothbrush. The assessments were conducted at baseline, and at 15 days and 30 days following the usage of the investigated toothbrushes.

All of the toothbrushes had identical bristles (tapered, round-ended, with a medium thickness diameter of 0.23–0.29 mm),[17,18] and all the individuals were advised to use the same toothpaste with the Bass toothbrushing technique.

Randomization, allocation concealment and blinding

The participants were randomly assigned to the treatment groups following a simple randomization procedure, specifically the lottery method. A total of 270 chits were created as per the final sample size. Each participant was asked to select a chit following the baseline examination from the investigator. The chits were divided into 3 groups, designated as group A, group B and group C, with 90 participants in each group.

For allocation concealment, the sequentially numbered, opaque, sealed envelope (SNOSE) method was used. The group name was written on a chit and kept in an opaque, sealed envelope. The envelope was labeled with a serial number. Once the patient had consented to participate, the investigator opened the sealed envelope and assigned the treatment group accordingly.

In this single-blind trial, all procedures were conducted by 2 dental examiners at each center. The first examiner (the primary investigator) was responsible for all clinical procedures, whereas the other one was responsible for the assessment of plaque and gingivitis. The primary investigator and the participants were not blinded in this process. However, to ensure the reliability of the results, 3 examiners (one at each center) were blinded to the type of toothbrush the participants were using.

Intervention

Once the initial assessments and randomization were completed, the participants were led to a separate room, equipped with a washbasin and a mirror. Toothbrushes were given to the subjects by the study coordinator. The same toothpaste was provided to all participants. The use of a toothbrush was demonstrated in accordance with the standard operating procedure (SOP) for toothbrushing. Subsequently, the participants were requested to use the provided toothbrush for the first time at the investigation center under the supervision of a study coordinator to confirm that it was being used correctly. A video demonstrating the proper toothbrushing technique was prepared and shared on the WhatsApp group for the participants to reinforce the technique. The participants were instructed to use the assigned toothbrush with toothpaste in accordance with the demonstrated technique at home on a twice-daily basis for 30 days (D1–D30). At the end of each week, the participants were contacted via telephone for a follow-up interview.

The objectives and procedures of the study were elucidated to the participants in their vernacular language. The study participants were free to withdraw at any point in time, and doing so would not affect the treatment they received at hospital. Throughout the entire duration of the study, the participants were instructed to refrain from any oral hygiene procedures other than brushing. They were also informed that they would be excluded from the study in the occurrence of any circumstances that might affect plaque accumulation, including oral prophylaxis, the placement of a restoration, any course of antibiotics, the use of mouthwash, the use of dental floss, and any systemic illness.

A follow-up visit for a clinical assessment was scheduled at 15 days (D15) and the participants were asked to return to the study center on D30. It was recommended that the participants refrain from any oral hygiene procedures, as well as from eating, drinking and gum chewing, for a period of 4 h before each visit. The clinical evaluations performed on D1 (the assessment of the plaque index (PI)) were repeated on D15 and D30. On D30, the subjects from group C, who were acquainted with the rotatable shank toothbrush, were invited to respond to a subjective evaluation questionnaire regarding their acceptability of the toothbrush and its efficacy.

The following instructions for use were given to the participants of the study:

– wet the toothbrush bristles and apply a small amount of the assigned toothpaste;
– place the toothbrush bristles in contact with the tooth at an angle of 45° to the gingiva;
– brush each tooth (or 2–3 at a time) using a gentle vibratory motion;
– brush each tooth well and, when finished, flick the toothbrush down the tooth, away from the gum line (rotate the brush shank in case of a rotatable shank toothbrush);
– maintain an inclination of 45° and constant contact with the teeth during brushing;
– be sure to clean all surfaces of your teeth, including the inner and outer surfaces, the chewing surfaces, and the ones behind the back teeth; do not forget about your tongue;
– guide the toothbrush slowly from tooth to tooth, following the curve of the teeth and gums;
– brush your teeth for approx. 2 min.

Assessment

Three dental examiners, one at each center, recorded the participants' dental status, and carried out the assessments of plaque and gingivitis independently. The intra- and inter-examiner reliability coefficients were 0.83 and 0.88, respectively.

The assessments of plaque and gingivitis were conducted using TMQHPI and the Löe and Silness GI, respectively.

According to the TMQHPI assessment procedure,[19] the subject's mouth was rinsed with the plaque disclosing solution (MIRA-2-TON®; Hager & Werken, Duisburg, Germany) for 1 min to disclose any accumulated plaque. All natural teeth were assessed. The labial/buccal and lingual/palatal aspects of all teeth were scored on a scale

from 0 to 5, as follows: score 0 – no plaque; score 1 – separate flecks of plaque at the gingival margin of the crown; score 2 – a thin continuous band of plaque at the gingival margin of the crown; score 3 – a band of plaque wider than 1 mm, but covering less than ⅓ of the crown; score 4 – plaque covering less than ⅔ of the crown; and score 5 – plaque covering more than ⅔ of the crown. The PI for the subject was calculated by summing the indices for all surfaces (labial/buccal and lingual/palatal) and dividing the result by the number of surfaces examined. The aforementioned scale was used to score both anterior teeth and posterior teeth (overall PI, PI in the region of first and second molars, and PI for the labial/palatal surfaces of anterior teeth).

The gingival status was evaluated according to the criteria established by Löe and Silness[20]: score 0 – absence of inflammation; score 1 – a slight change in color and a little change in texture; score 2 – moderate redness, edema and hypertrophy, and BoP; and score 3 – marked redness and hypertrophy, and a tendency to spontaneous bleeding. The GI for the subject was calculated by summing the values for each tooth and dividing the result by the number of teeth examined.

Toothbrushes tested

The toothbrushes tested in the study included a toothbrush with a straight handle, a toothbrush with a flexible handle and a toothbrush with a rotatable shank.

The toothbrush with a rotatable shank comprises a brush head assembly with an upper end and a lower end, a plurality of brush bristles configured on the surface of the upper end, and a handle rotatably configured with the lower end of the brush head assembly. The brush head is connected to the handle through a socket–ball joint, enabling the shank and the handle to rotate with respect to one another (Fig. 2).

The brush head assembly comprises 2 distinct components – a replaceable brush head and a component rotatably configured with the handle. The replacement of the brush head is a simple, plug-and-play process. The brush head assembly is designed to rotate at any angle between 0° and 360° with respect to the handle around an axis. Additionally, the brush head bends toward the handle, maintaining a bending angle of 15° (Fig. 3).

In other words, one of the components – the brush head assembly or the handle – may be in a fixed position, while the other one may rotate with respect to the stationary element. When the brush head assembly rotates with respect to the handle, the rotating axis is parallel to the length of the handle. Holding the handle between the thumb and the index finger while rotating it facilitates three-dimensional (3D) rotation, thereby making the toothbrush more user-friendly.

The present invention provides an improved toothbrush design, as the rotation and bending of the brush

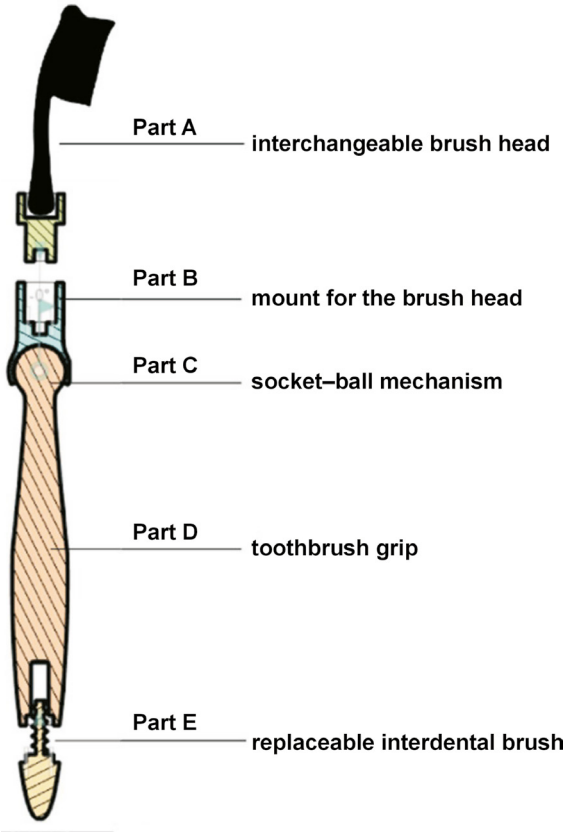

Fig. 2. Components of the rotatable shank toothbrush

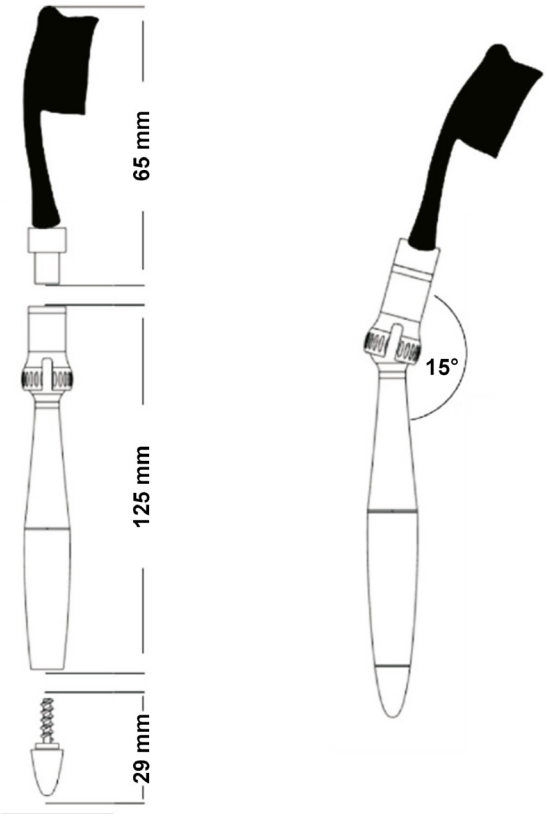

Fig. 3. Size of the components of the rotatable shank toothbrush and the bending angle

head enables adaptation and facilitates the brushing of the lingual/palatal surfaces of teeth, which are difficult to reach with a wrist and arm motion. The improved toothbrush with an ergonomic grip enables users to maneuver the toothbrush with their thumb and index finger while the other 3 fingers are used to press the handle against the palm to acquire a firm grip of the toothbrush. As a result, sweeping the brush downward and upward through a wrist and arm movement is avoided.

Statistical analysis

The obtained data was entered into Microsoft Excel, v. 13 (Microsoft Corporation, Redmond, USA). The data was subjected to statistical analysis using the IBM SPSS Statistics for Windows software, v. 21.0 (IBM Corp., Armonk, USA). For categorical data, the frequency and percentage values were obtained, and for continuous data, the mean and standard deviation ($M \pm SD$) values were obtained.

The primary endpoint was the comparison of groups A, B and C for changes in PI (overall, in the molar region and on the lingual/palatal surfaces of the anterior teeth) and GI at baseline, day 15 and day 30. The mean reduction in the plaque and gingival scores from baseline to 15 days and from baseline to 30 days was assessed. To analyze the data, we used the analysis of variance (ANOVA) with Tukey's post hoc test for pairwise comparisons. The analysis was conducted based on the pre–post values obtained for each group.

All statistical tests were performed with a 95% confidence interval (*CI*). A p-value <0.05 was considered statistically significant.

Results

In the present study, there were 160 (59.3%) male participants and 110 (40.7%) female participants. The difference in the proportion of male and female participants was statistically significant ($p < 0.05$) (Table 1). The majority of the participants were in the age group of 18–34 years, and only 10 patients were above 65 years of age (Table 2).

The participants from group C (the rotatable shank toothbrush group) completed a questionnaire, which revealed that most of them had previously used a straight handle toothbrush, followed by a flexible handle toothbrush.

Table 1. Distribution of the study participants according to gender ($N = 270$)

Gender	*n* (%)	*p*-value
M	160 (59.3)	
F	110 (40.7)	0.000*
Total	270 (100.0)	

M – male; F – female;* statistically significant (χ^2 test).

Table 2. Distribution of the study participants according to age ($N = 270$)

Age [years]	*n* (%)	*p*-value
<18	35 (13.0)	
18–24	70 (26.0)	
25–34	70 (26.0)	
35–44	33 (12.2)	0.000*
45–54	33 (12.2)	
55–64	19 (7.0)	
≥65	10 (3.7)	

* statistically significant (χ^2 test).

Following the use of our toothbrush, the individuals indicated a preference for the rotatable shank toothbrush, as it offered them an overall better experience. They also reported that the rotatable shank toothbrush provided greater accessibility to posterior teeth. The majority of the study participants ascertained that the rotatable shank toothbrush was more effective in removing the lodged food. On the other hand, the participants reported bleeding when using the straight handle toothbrush (Table 3).

In the present study, we compared PI (overall, in the molar region and on the lingual/palatal surfaces of anterior teeth) and GI at baseline, day 15 and day 30 in all 3 groups (Table 4).

In group A, the mean values for PI (overall, in the molar region and on the lingual/palatal surfaces of anterior teeth) and GI at baseline were 2.42 ±0.30, 2.42 ±0.59, 2.42 ±0.59, and 1.65 ±0.17, respectively. The results of ANOVA showed that after using the straight handle toothbrush for a period of 30 days, the PI and GI values decreased to 1.43 ±0.58, 1.36 ±0.46, 1.38 ±0.50, and 1.43 ±0.16, respectively. The reduction in the PI and GI values was statistically significant ($p < 0.05$).

In group B, the mean values for PI (overall, in the molar region and on the lingual/palatal surfaces of anterior teeth) and GI at baseline were 2.38 ±0.35, 2.57 ±0.55, 2.57 ±0.55, and 1.57 ±0.33, respectively. A reduction in the PI and GI values was observed between baseline, day 15 and day 30. The results of ANOVA stated that at day 30, the PI and GI values decreased to 1.18 ±0.41, 1.23 ±0.56, 1.30 ±0.67, and 1.23 ±0.33, respectively. The reduction in the PI and GI values was statistically significant ($p < 0.05$).

Similarly, in group C, the results of ANOVA showed that the mean PI (overall, in the molar region and on the lingual/palatal surfaces of anterior teeth) and GI values had decreased from 2.40 ±0.29, 2.17 ±0.55, 2.16 ±0.55, and 1.59 ±0.30 at baseline to 0.53 ±0.33, 0.80 ±0.45, 0.80 ±0.45, and 0.69 ±0.14 at day 30, respectively. The reduction in the PI and GI values was statistically significant ($p < 0.05$).

The overall comparison of groups A, B and C revealed statistically significant differences in the values measured ($p < 0.05$).

Table 3. Feedback provided by the study participants who used the toothbrush with a rotatable shank ($n = 90$)

Questions inquired	Straight handle toothbrush	Flexible handle toothbrush	Rotatable shank toothbrush	*p*-value
Type of toothbrush used before	54 (60.0)	36 (40.0)	–	–
Toothbrush with better handle and grip	10 (11.1)	22 (24.4)	58 (64.4)	0.000*
Preferable neck design	11 (12.2)	26 (28.9)	53 (58.9)	0.000*
Toothbrush with better accessibility to posterior teeth	6 (6.7)	22 (24.4)	62 (68.9)	0.000*
Toothbrush better in removing the lodged food	3 (3.3)	31 (34.4)	56 (62.2)	0.000*
Toothbrush causing bleeding while brushing	47 (52.2)	29 (32.2)	14 (15.6)	0.000*
Toothbrush with an overall better experience	13 (14.4)	26 (28.9)	51 (56.7)	0.000*

Data presented as frequency (percentage) (*n* (%)).
* statistically significant (ANOVA).

Table 4. Comparisons within and between the study groups with regard to the plaque index (PI) and gingival index (GI) scores

Variable		Group A			Group B			Group C			*p*-value
		baseline	day 15	day 30	baseline	day 15	day 30	baseline	day 15	day 30	
Overall PI	*M* ±*SD*	2.42 ±0.30	1.92 ±0.47	1.43 ±0.58	2.38 ±0.35	1.67 ±0.38	1.18 ±0.41	2.40 ±0.29	1.19 ±0.39	0.53 ±0.33	0.000*
	min	2.00	0.90	0.20	1.90	0.50	0.30	2.00	0.30	0.06	
	max	2.97	2.80	2.50	3.50	2.80	2.40	2.96	2.40	1.70	
	p-value		0.000*			0.000*			0.000*		
PI in the molar region	*M* ±*SD*	2.42 ±0.59	1.84 ±0.52	1.36 ±0.46	2.57 ±0.55	1.64 ±0.60	1.23 ±0.56	2.17 ±0.55	1.21 ±0.48	0.80 ±0.45	0.000*
	min	1.00	0.60	0.50	1.30	0.50	0.40	1.00	0.30	0.10	
	max	3.70	3.00	2.60	3.80	3.10	2.80	3.50	2.10	2.00	
	p-value		0.000*			0.000*			0.000*		
PI on the lingual/palatal surfaces of anterior teeth	*M* ±*SD*	2.42 ±0.59	1.84 ±0.52	1.38 ±0.50	2.57 ±0.55	1.70 ±0.62	1.30 ±0.67	2.16 ±0.55	1.22 ±0.50	0.80 ±0.45	0.000*
	min	1.00	0.60	0.50	1.30	0.50	0.40	1.00	0.30	0.10	
	max	3.70	3.10	3.00	3.80	3.10	3.00	3.50	2.40	2.00	
	p-value		0.000*			0.000*			0.000*		
GI	*M* ±*SD*	1.65 ±0.17	1.54 ±0.16	1.43 ±0.16	1.57 ±0.33	1.41 ±0.32	1.23 ±0.33	1.59 ±0.30	0.87 ±0.19	0.69 ±0.14	0.000*
	min	1.20	1.00	0.80	1.00	0.90	0.80	1.10	0.50	0.40	
	max	2.00	1.90	1.80	3.20	3.10	2.90	2.90	1.70	1.10	
	p-value		0.000*			0.000*			0.000*		

Groups: A – straight handle toothbrush; B – flexible handle toothbrush; C – rotatable shank toothbrush; *M* – mean; *SD* – standard deviation; min – minimum; max – maximum; * statistically significant (ANOVA).

The pairwise comparisons of the mean PI (overall, in the molar region and on the lingual/palatal surfaces of anterior teeth) and GI values for straight, flexible and rotatable shank toothbrushes with regard to baseline, day 15 and day 30 were performed using Tukey's post hoc test (Table 5).

In group A, there was a statistically significant reduction in the PI (overall, in the molar region and on the lingual/palatal surfaces of anterior teeth) and GI values from baseline to 15 days (0.50, 0.58, 0.58, and 0.11, respectively) ($p < 0.05$). Similarly, Tukey's post hoc test displayed a reduction in the PI and GI values from baseline to 30 days (0.99, 1.06, 1.04, and 0.22, respectively), which was statistically significant ($p < 0.05$). The comparison of the PI and GI values between 15 and 30 days also depicted a decrease (0.49, 0.48, 0.46, and 0.11, respectively), with the difference being statistically significant ($p < 0.05$).

The post hoc analysis of group B revealed a statistically significant reduction in the PI (overall, in the molar region and on the lingual/palatal surfaces of the anterior teeth) and GI values from baseline to 15 days (0.71, 0.93, 0.87, and 0.16, respectively) ($p < 0.05$). The reduction in the PI and GI values from baseline to 30 days was also statistically significant (1.20, 1.34, 1.27, and 0.34, respectively) ($p < 0.05$). Additionally, the post hoc analysis revealed that the plaque and gingival scores decreased significantly between 15 and 30 days, and the mean difference was 0.49, 0.41, 0.40, and 0.18, respectively ($p < 0.05$).

In group C, the post hoc analysis revealed a mean reduction in the PI (overall, in the molar region and on the lingual/palatal surfaces of anterior teeth) and GI values from baseline to 15 days (1.21, 0.96, 0.94, and 0.72, respectively), from baseline to 30 days (1.87, 1.37, 1.36, and 0.90, respectively), and between 15 and 30 days (0.66,

Table 5. Pairwise comparisons of the plaque index (PI) and gingival index (GI) scores within the study groups

Dependent variable			Overall PI		PI in the molar region		PI on the lingual/palatal surfaces of anterior teeth		GI	
			mean difference	*p*-value	mean difference	*p*-value	mean difference	*p*-value	mean difference	*p*-value
Group A	baseline	15 days	0.50	0.000*	0.58	0.000*	0.58	0.000*	0.11	0.000*
		30 days	0.99	0.000*	1.06	0.000*	1.04	0.000*	0.22	0.000*
	15 days	30 days	0.49	0.000*	0.48*	0.000*	0.46	0.000*	0.11	0.000*
Group B	baseline	15 days	0.71	0.000*	0.93	0.000*	0.87	0.000*	0.16	0.000*
		30 days	1.20	0.000*	1.34	0.000*	1.27	0.000*	0.34	0.000*
	15 days	30 days	0.49	0.000*	0.41	0.000*	0.40	0.000*	0.18	0.000*
Group C	baseline	15 days	1.21	0.000*	0.96	0.000*	0.94	0.000*	0.72	0.000*
		30 days	1.87	0.000*	1.37	0.000*	1.36	0.000*	0.90	0.000*
	15 days	30 days	0.66	0.000*	0.41	0.000*	0.42	0.000*	0.18	0.000*

Groups: A – straight handle toothbrush; B – flexible handle toothbrush; C – rotatable shank toothbrush; * statistically significant (Tukey's post hoc test).

0.41, 0.42, and 0.18, respectively). The reduction in the PI and GI values at different time intervals was statistically significant ($p < 0.05$).

The ANOVA comparisons of the pre–post scores between baseline and day 15 depicted that group C (rotatable shank toothbrush) exhibited the greatest reduction in the PI and GI values. Similarly, the comparisons of the pre–post scores between baseline and day 30 demonstrated the greatest reduction in the PI and GI scores in the rotatable shank toothbrush group, followed by the flexible handle toothbrush group and the straight handle toothbrush group ($p < 0.05$) (Table 6).

The pairwise comparisons of the pre–post changes in the PI and GI values from baseline to 15 days and from baseline to 30 days were conducted using Tukey's post hoc test (Table 7).

The results indicated that in the pre–post comparison at day 15 between groups A and B, the overall PI was reduced significantly in the flexible handle toothbrush group (mean difference: –0.21). With regard to groups A and C, the latter exhibited a greater reduction of the overall PI (mean difference: –0.71). The difference in mean between groups B and C demonstrated that the reduction was more pronounced for the rotatable shank toothbrush group (mean difference: –0.50). All the differences were statistically significant ($p < 0.05$).

The mean differences in PI in the molar region depicted a similar observation, whereby the pre–post comparisons

Table 6. Mean pre–post change in the plaque index (PI) and gingival index (GI) scores for the study groups

Variable		Reduction between baseline and 15 days			Reduction between baseline and 30 days			*p*-value
		group A	group B	group C	group A	group B	group C	
Overall PI	*M* ±*SD*	0.50 ±0.39	0.71 ±0.38	1.21 ±0.42	0.99 ±0.50	1.20 ±0.44	1.87 ±0.42	0.000*
	min	0.05	0.04	0.28	0.10	0.20	1.00	
	max	1.90	2.46	2.42	2.24	2.56	2.70	
	p-value		0.000*			0.000*		
PI in the molar region	*M* ±*SD*	0.58 ±0.28	0.93 ±0.56	0.96 ±0.30	1.06 ±0.36	1.34 ±0.62	1.37 ±0.40	0.000*
	min	–0.50	0.10	0.40	0.20	0.20	0.50	
	max	1.40	2.40	1.70	1.90	1.60	2.20	
	p-value		0.000*			0.000*		
PI on the lingual/palatal surfaces of anterior teeth	*M* ±*SD*	0.58 ±0.28	0.87 ±0.60	0.94 ±0.29	1.04 ±0.36	1.27 ±0.65	1.36 ±0.40	0.000*
	min	–0.50	0.10	0.10	0.20	0.20	0.50	
	max	1.40	1.10	1.70	1.90	1.60	2.20	
	p-value		0.000*			0.000*		
GI	*M* ±*SD*	0.11 ±0.06	0.16 ±0.13	0.72 ±0.30	0.22 ±0.09	0.34 ±0.24	0.90 ±0.31	0.000*
	min	–0.20	0.03	0.10	–0.12	0.00	0.15	
	max	0.30	0.20	1.80	0.80	0.50	2.32	
	p-value		0.000*			0.000*		

Groups: A – straight handle toothbrush; B – flexible handle toothbrush; C – rotatable shank toothbrush; * statistically significant (ANOVA).

Table 7. Pairwise comparisons of pre–post changes in the plaque index (PI) and gingival index (GI) scores between the study groups

Dependent variable			Overall PI		PI in the molar region		PI on the lingual/palatal surfaces of anterior teeth		GI	
			mean difference	*p*-value	mean difference	*p*-value	mean difference	*p*-value	mean difference	*p*-value
15-day pre–post change	group A	group B	–0.21	0.001*	–0.35	0.000*	–0.29	0.000*	–0.05	0.216
		group C	–0.71	0.000*	–0.38	0.000*	–0.36	0.000*	–0.61	0.000*
	group B	group C	–0.50	0.000*	–0.03	0.050	–0.07	0.050	–0.56	0.000*
30-day pre–post change	group A	group B	–0.21	0.004*	–0.28	0.000*	–0.23	0.000*	–0.12	0.001*
		group C	–0.88	0.000*	–0.31	0.000*	–0.32	0.000*	–0.68	0.000*
	group B	group C	–0.67	0.000*	–0.03	0.050	–0.09	0.050	–0.56	0.000*

Groups: A – straight handle toothbrush; B – flexible handle toothbrush; C – rotatable shank toothbrush; * statistically significant (Tukey's post hoc test).

at day 15 between groups A and B, groups A and C, and groups B and C exhibited a reduction, with the rotatable shank toothbrush group demonstrating the greatest reduction, followed by the flexible handle toothbrush group and the straight handle toothbrush group. The differences were found to be statistically significant (–0.35, –0.38 and –0.03, respectively) ($p \leq 0.05$).

The pre–post 15-day pairwise comparisons between the groups showed that PI on the lingual/palatal surfaces of anterior teeth was reduced to the greatest extent by the rotatable shank toothbrush, followed by the flexible handle toothbrush and the straight handle toothbrush. The differences between the groups were statistically significant (–0.29, –0.36 and –0.07, respectively) ($p \leq 0.05$).

Tukey's post hoc comparison of GI with regard to different types of toothbrushes revealed a significant reduction in the GI scores for the rotatable shank toothbrush group, followed by the flexible handle toothbrush group and the straight handle toothbrush group. However, despite the superiority of the flexible handle toothbrush over the straight handle toothbrush in reducing GI, the difference in mean was not statistically significant ($p > 0.05$). Conversely, the comparison between groups A and C, as well as between groups B and C, demonstrated that the rotatable shank toothbrush exhibited a significantly elevated performance in GI reduction (mean difference: –0.61 and –0.56, respectively) ($p < 0.05$).

The pre–post 30-day pairwise comparisons between groups A and B, groups A and C, and groups B and C revealed a mean difference in the overall PI of –0.21, –0.88 and –0.67, respectively. With regard to PI in the molar region, the mean differences were –0.28, –0.31 and –0.03, respectively. In the case of PI on the lingual/palatal surfaces of anterior teeth, the mean differences between the toothbrush groups were –0.23, –0.32 and –0.09, respectively. As far as GI is concerned, the mean difference values for the respective toothbrush groups were –0.12, –0.68 and –0.56. Tukey's post hoc analysis demonstrated that at 30 days, the PI and GI scores exhibited a superior reduction with the rotatable shank toothbrush, followed by the flexible handle toothbrush ($p \leq 0.05$).

Discussion

The major rationale for daily oral hygiene is to maintain teeth devoid of plaque, which is essential for preserving healthy periodontal tissues. As suggested by Armitage, the presence of supragingival plaque is not a good predictor of disease progression; however, its absence has a good negative predictive value.[21] A toothbrush represents the most common tool for daily plaque removal and it plays a pivotal role in plaque control.

A wide variety of toothbrushes is available on the market. Most of them are capable of adequately removing plaque from the accessible surfaces of the teeth. However, they face limitations in reaching areas that are difficult to access.[22,23] Interventional studies have explicitly demonstrated the persistence of plaque despite good levels of plaque control, underscoring the importance of developing tools that can assist individuals in maintaining a higher level of oral hygiene.[24,25]

A scientifically and ergonomically designed toothbrush with a rotatable shank was developed to address the limitations of a generic toothbrush in reaching all areas of the mouth. The design aims to provide patients with optimal cleaning regardless of the brushing technique, and to maximize user comfort and acceptability, thereby fostering compliance with the recommended brushing time and frequency during normal home use. Once the product had been developed and validated, a multicenter clinical study was conducted to ascertain the clinical benefits of its usage.

The present RCT compared the efficacy of the rotatable shank toothbrush with that of the flexible handle toothbrush and the straight handle toothbrush in the elimination of supragingival plaque at all sites of the oral cavity and in the development of gingival health.

The results of the present study should be interpreted in light of the toothbrush design, as the study aimed to verify its efficacy. They should be understood as an indication of the efficacy of the toothbrushes in removing dental plaque. The study outcomes – plaque and gingivitis resolution – were measured by means of TMQHPI and GI (Löe and Silness), similar to previous studies.[26–30]

The strength of these indices lies in their application in clinical trials of preventive and therapeutic agents.

Plaque was assessed on the facial and lingual/palatal surfaces of all teeth after the application of a disclosing agent. The whole-mouth plaque scores were calculated, as well as the scores for inaccessible areas (in the molar region and on the lingual/palatal surfaces of anterior teeth). Plaque examinations were conducted at 3 experimental time points (baseline, 15 days and 30 days) to assess whether a learning curve affected the results.

The objective of toothbrushing is to eliminate dental plaque from all surfaces of the teeth, including the gingival crevice, while minimizing damage to the teeth and the surrounding structures. The American Dental Association (ADA) recommends positioning the toothbrush against the buccal and palatal surfaces of the teeth so that the bristles are at a 45-degree angle to the gingiva, and performing a slight vibratory motion for thorough plaque removal.[31] Vibratory action has been proposed to contribute to a gingival massage effect at the gingival sulcus, potentially alleviating gingivitis. Thus, in the present study, the patients were instructed to position the toothbrush at a 45-degree angle relative to the gingiva. The plaque and gingivitis scores were evaluated and compared across all surfaces of all teeth at baseline, and at 15 and 30 days.

At baseline, the highest plaque scores were observed in the molar region and on the lingual/palatal surfaces of anterior teeth, probably due to the difficulty in accessing these sites during toothbrushing. In contrast, the lowest plaque accumulation was observed on the facial surfaces of anterior maxillary teeth, presumably due to better and easier access during toothbrushing.

The null hypothesis, which stated that there would be no difference in the efficacy in plaque removal between the examined toothbrushes, was rejected in the analysis.

The mean PI and GI scores, evaluated for groups A, B and C at baseline, and at 15 and 30 days, demonstrated a statistically significant reduction. A comparable statistically significant reduction in the PI and GI scores was observed in the post-hoc analysis from baseline to day 15, from baseline to day 30, and from day 15 to day 30, thereby proving the efficacy of all toothbrushes in plaque removal.

The comparison of the pre–post reduction in the PI and GI values from baseline to day 15 and from baseline to day 30 revealed that group C (the rotatable shank toothbrush) exhibited the greatest reduction in the PI and GI scores ($p < 0.05$).

The pairwise comparison of the pre–post change in the PI and GI scores from baseline to 15 days and from baseline to 30 days between the 3 types of toothbrushes demonstrated the highest reduction in overall plaque, and also in plaque in inaccessible areas for the rotary handle toothbrush, followed by the flexible handle toothbrush and the straight handle toothbrush. This difference was statistically significant.

The results of our study indicate that the rotatable shank toothbrush is most effective in eliminating plaque from all sites, followed by the flexible handle toothbrush. These findings consequently reject the null hypothesis, which asserts that there is no difference in the efficacy in plaque removal between different types of toothbrushes.

The superior plaque elimination demonstrated by the experimental product (the rotatable shank toothbrush) – overall and in inaccessible areas (in the molar region and on the lingual/palatal surfaces of anterior teeth) – is likely related to the brush design, with the 360-degree rotation of the neck around its axis, along with an inclination up to 15°, allowing reachability to all surfaces of the oral cavity (labial/buccal and lingual/palatal tooth surfaces). This could be considered as a clinically relevant advantage.

The fringe benefit of incorporating a rotatable and inclinable neck into the toothbrush design is a significant reduction in handle stiffness in the neck and head regions. This results in a lesser movement, which allows the placement of the brush bristles at 45° to the gingival surface, and the reduction of the force applied to the oral mucosa and tooth surfaces as compared to other toothbrush types. These modifications result in a more gentle brushing action, which may prevent overbrushing and injury to the gingiva.

The product design enables the manipulation of the device by solely using the thumb and the index finger, which overcomes the limitations of a wrist and arm movement, and offers better reachability. This innovation represents a significant advance in the field of manual toothbrush design.

The superiority of the flexible handle toothbrush over the straight handle toothbrush could be due to the incorporation of a flexible component in the neck of the toothbrush, which increases neck flexibility under typical toothbrushing forces and provides comparatively improved simulated plaque control at all risk areas when compared to their rigid-neck counterpart. Theoretically, this could contribute to the prevention of dental caries and gingivitis, and potentially improve dental health. The results of our study align with those of the clinical trial conducted by Acherkouk et al., who, after comparing flexible and rigid neck designs, suggested that flexible toothbrushes might provide superior plaque removal, even in at-risk sites.[32]

Gingivitis, a non-destructive disease associated with the accumulation of plaque, causes the inflammation of the gingiva and can be reversed through the implementation of good oral hygiene practices. The removal of plaque from sites with gingivitis results in the resolution of soft tissue inflammation.[33] Our investigation divulged that the rotatable shank toothbrush brought pronounced improvement in the gingivitis scores in comparison with flexible and straight handle toothbrushes. The rationale is based on the premise that the device provides superior plaque control at all risk areas. Consequently, this could

contribute to the amelioration of gingivitis and potentially improve dental health.

The assessment of the suitability of each toothbrush was conducted on all subjects who used the tested toothbrush. The number of reactions related to the toothbrush was documented, and the final assessment was subsequently conducted. The rotatable shank toothbrush was found to be well tolerated and received high levels of appreciation. We hypothesize that such a commendation of the new product may facilitate long-term compliance with oral health recommendations and improve global oral wellness.

Limitations

A limitation of the present study was the unequal gender distribution of the participants. Additionally, we could not monitor the brushing habits of the participants. However, this may also be considered a main strength, as the utilization of the rotatable shank toothbrush resulted in a higher reduction in the plaque and gingival scores, irrespective of the surveillance.

Furthermore, the effectiveness of the rotatable shank toothbrush was not evaluated in individuals with special needs or in comparison with powered toothbrushes, due to the high market cost of the latter, which was identified as a potential limitation.

Conclusions

The present investigation provides evidence that the ergonomically designed rotatable shank toothbrush effectively removed plaque from all surfaces of the teeth, including inaccessible areas. This substantiates its potential to improve oral hygiene when compared with flexible and straight handle toothbrushes. The rotatable shank toothbrush is a safe and effective brushing aid that can be used by anyone, irrespective of manual dexterity or training. It is an innovative addition to the field of manual toothbrush design.

Trial registration

The trial was registered with Clinical Trials Registry – India (CTRI) under the identification No. CTRI/2022/02/049838.

Ethics approval and consent to participate

The study was approved by the Ethics Committee at the Pravara Institute of Medical Sciences (Deemed to be University) (PIMS-DU), Ahmednagar, India (approval No. PIMS/DR/RDC/2022/138). The respondents provided written informed consent prior to completing the questionnaire.

Data availability

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

Consent for publication

Not applicable.

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References

1. West N, Chapple I, Claydon N, et al.; British Society of Periodontology and Implant Dentistry Guideline Group Participants. BSP implementation of European S3-level evidence-based treatment guidelines for stage I–III periodontitis in UK clinical practice. *J Dent*. 2021;106:103562. doi:10.1016/j.jdent.2020.103562
2. Chapple IL, Van der Weijden F, Doerfer C, et al. Primary prevention of periodontitis: Managing gingivitis. *J Clin Periodontol*. 2015;42(Suppl 16):S71–S76. doi:10.1111/jcpe.12366
3. Axelsson P, Nyström B, Lindhe J. The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. *J Clin Periodontol*. 2004;31(9):749–757. doi:10.1111/j.1600-051X.2004.00563.x
4. Hirschfeld L, Wasserman B. A long-term survey of tooth loss in 600 treated periodontal patients. *J Periodontol*. 1978;49(5):225–237. doi:10.1902/jop.1978.49.5.225
5. Lindhe J, Nyman S. Long-term maintenance of patients treated for advanced periodontal disease. *J Clin Periodontol*. 1984;11(8):504–514. doi:10.1111/j.1600-051x.1984.tb00902.x
6. Rosling B, Nyman S, Lindhe J, Jern B. The healing potential of the periodontal tissues following different techniques of periodontal surgery in plaque-free dentitions. A 2-year clinical study. *J Clin Periodontol*. 1976;3(4):233–250. doi:10.1111/j.1600-051x.1976.tb00042.x
7. Van der Weijden FA, Slot DE. Efficacy of homecare regimens for mechanical plaque removal in managing gingivitis – a meta review. *J Clin Periodontol*. 2015;42(Suppl 16):S77–S91. doi:10.1111/jcpe.12359
8. Bay I, Kardel KM, Skougaard MR. Quantitative evaluation of the plaque-removing ability of different types of toothbrushes. *J Periodontol*. 1967;38(6):526–533. doi:10.1902/jop.1967.38.6_part1.526
9. Bergenholtz A, Hugoson A, Lundgren D, Ostgren A. The plaque-removing ability of various toothbrushes used with the roll technique. *Sven Tandlak Tidskr*. 1969;62(1):15–25. PMID:5257803.
10. Sripriya N, Shaik Hyder Ali KH. A comparative study of the efficacy of four different bristle designs of tooth brushes in plaque removal. *J Indian Soc Pedod Prev Dent*. 2007;25(2):76–81. doi:10.4103/0970-4388.33452
11. Sial AR, Kashif M, Mujahid U, Mehmood H, Shafiq MM, Iqbal MP. Efficacy of tooth brushes of different bristles design in plaque removal. *J Dent Health Oral Disord Ther*. 2014;1(1):8. doi:10.15406/jdhodt.2015.01.00004
12. Claydon N, Leech K, Addy M, Newcombe RG, Ley F, Scratcher C. Comparison of a double-textured prototype manual toothbrush with 3 branded products. A professional brushing study. *J Clin Periodontol*. 2000;27(10):744–748. doi:10.1034/j.1600-051x.2000.027010744.x
13. Frandsen A. Mechanical oral hygiene practices. In: Löe H, Kleinman DV, eds. *Dental Plaque Control Measures and Oral Hygiene Practices: Proceedings from the State-of-the-Science Workshop*. 1st ed. Oxford, Washington D.C.: IRL Press; 1986:93–116.
14. Macgregor ID, Rugg-Gunn AJ. A survey of toothbrushing frequency in children and young adults. *J Periodontal Res*. 1979;14(3):225–230. doi:10.1111/j.1600-0765.1979.tb00227.x

15. Rugg-Gunn AJ, Macgregor ID. A survey of toothbrushing behaviour in children and young adults. *J Periodontal Res*. 1978;13(4):382–389. doi:10.1111/j.1600-0765.1978.tb00193.x
16. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of Workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol*. 2018;45(Suppl 20):S162–S170. doi:10.1111/jcpe.12946
17. Versteeg PA, Piscaer M, Rosema NA, Timmerman MF, Van der Velden U, Van der Weijden GA. Tapered toothbrush filaments in relation to gingival abrasion, removal of plaque and treatment of gingivitis. *Int J Dent Hyg*. 2008;6(3):174–182. doi:10.1111/j.1601-5037.2008.00284.x
18. Drisko C, Henderson R, Yancy J. A review of current toothbrush bristle endo-rounding studies. *Compend Contin Educ Dent*. 1995;16(7):694,696,698;quiz 708. PMID:8595590.
19. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of vitamine C. *J Periodontol*. 1970;41(1):41–43. doi:10.1902/jop.1970.41.41.41
20. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533–551. doi:10.3109/00016356309011240
21. Armitage GC. Periodontal diseases: Diagnosis. *Ann Periodontol*. 1996;1(1):37–215. doi:10.1902/annals.1996.1.1.37
22. Kinane DF. The role of interdental cleaning in effective plaque control: Need for interdental cleaning in primary and secondary prevention. In: Lang NP, Attström R, Löe H, eds. *Proceedings of the European Workshop on Mechanical Plaque Control: Status of the Art and Science of Dental Plaque Control: Castle of Münchenwiler, Berne, Switzerland, May 9–12, 1998*. Berlin, Germany: Quintessence Publishing Co.; 1998:156–168.
23. Mastroberardino S, Cagetti MG, Cocco F, Campus G, Pizzocri J, Strohmenger L. Vertical brushing versus horizontal brushing: A randomized split-mouth clinical trial. *Quintessence Int*. 2014;45(8):653–661. doi:10.3290/j.qi.a32238
24. Haas AN, de Castro GD, Moreno T, et al. Azithromycin as an adjunctive treatment of aggressive periodontitis: 12-months randomized clinical trial. *J Clin Periodontol*. 2008;35(8):696–704. doi:10.1111/j.1600-051X.2008.01254.x
25. Cunha Moreira CH, Weidlich P, Fiorini T, et al. Periodontal treatment outcomes during pregnancy and postpartum. *Clin Oral Investig*. 2015;19(7):1635–1641. doi:10.1007/s00784-014-1386-z
26. Shibly O, Schifferle RE, Ciancio SG, Tarakji M, Mather ML. A clinical comparison of 2 electric toothbrush designs. *J Clin Periodontol*. 1997;24(4):260–263. doi:10.1111/j.1600-051x.1997.tb01840.x
27. Heasman PA, McCracken GI. Powered toothbrushes: A review of clinical trials. *J Clin Periodontol*. 1999;26(7):407–420. doi:10.1034/j.1600-051x.1999.260701.x
28. Heintze SD, Jost-Brinkmann PG, Loundos J. Effectiveness of three different types of electric toothbrushes compared with a manual technique in orthodontic patients. *Am J Orthod Dentofacial Orthop*. 1996;110(6):630–638. doi:10.1016/s0889-5406(96)80040-0
29. Checchi L, Daprile G, Gatto MR, Pelliccioni GA. Gingival recession and tooth brushing in an Italian School of Dentistry: A pilot study. *J Clin Periodontol*. 1999;26(5):276–280. doi:10.1034/j.1600-051x.1999.260502.x
30. Cancro LP, Fischman SL. The expected effect on oral health of dental plaque control through mechanical removal. *Periodontol 2000*. 1995;8:60–74. doi:10.1111/j.1600-0757.1995.tb00045.x
31. Wainwright J, Sheiham A. An analysis of methods of toothbrushing recommended by dental associations, toothpaste, and toothbrush companies and in dental texts. *Br Dent J*. 2014;217(3):E5. doi:10.1038/sj.bdj.2014.651
32. Acherkouk A, Götze M, Kiesow A, et al. Robot and mechanical testing of a specialist manual toothbrush for cleaning efficacy and improved force control. *BMC Oral Health*. 2022;22(1):225. doi:10.1186/s12903-022-02211-4
33. Van der Weijden F, Slot DE. Oral hygiene in the prevention of periodontal diseases: The evidence. *Periodontol 2000*. 2011;55(1):104–123. doi:10.1111/j.1600-0757.2009.00337.x-

Original paper

Patient satisfaction among subjects with a maxillary single denture, treated with implant-supported telescopic versus ball-and-socket overdentures: A randomized controlled trial

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. The present study was performed to rehabilitate maxillary single denture cases with implant-retained telescopic or ball-and-socket attachments, and to evaluate the validity of two-implant-retained maxillary overdentures as a treatment approach in the maxillary arch.

Objectives. The aim of the present study was to evaluate patient satisfaction in maxillary single denture wearers with 2 different attachment systems (telescopic attachment vs. ball-and-socket attachment).

Material and methods. A total of 18 completely edentulous maxillary ridge patients (45–60 years old; mean age: 53 years) were selected for this study. Maxillary single dentures were constructed for all the patients. Group 1 patients received 2 implants with a telescopic attachment and group 2 patients received 2 implants with a ball-and-socket attachment. Patient satisfaction with the implant-retained maxillary single denture was evaluated after insertion, and 3 months after the delivery of each implant-retained maxillary single overdenture.

Results. All 18 patients completed the study. After 3 months, the telescopic group showed significant improvement in terms of comfort, chewing, handling, and overall satisfaction, and in the ball-and-socket group, significant improvement was recorded for appearance only. When comparing the 2 groups, after insertion, group 1 showed significantly better results for the 'handling' and 'hygiene' parameters, whereas group 2 showed a significantly better mean score for the 'appearance' parameter. After 3 months, group 1 showed significantly better results for the 'comfort', 'handling', 'hygiene', and 'overall satisfaction' parameters, and group 1 proved significantly better in terms of 'appearance' and 'speech' parameters.

Conclusions. Maxillary single dentures with a telescopic attachment showed an advantage over those with a ball-and-socket attachment regarding patient satisfaction. Concerning the implant number, two-implant-retained maxillary overdentures can be considered a promising approach for patients from developing countries.

Keywords: patient satisfaction, telescopic attachment, ball-and-socket attachment, implant overdenture, maxillary single complete denture

Introduction

Patients who are completely edentulous in one jaw, but have all or some of their natural teeth in the other jaw, represent one of the most frequent clinical scenarios. It is difficult, and perhaps nearly impossible, to get an effective complete denture for these people, There are 2 reasons that make the task so challenging. First, the occlusal form of the denture will be greatly influenced by the occlusal shape of the remaining natural teeth, which may be overerupted or slanted. Another issue is how hard and firm the natural teeth are when they are embedded in the bone.[1]

In the literature, maxillary implant overdentures are described with the implant survival rates ranging from 81% to 100% for up to 5 years, and being retained with 2–6 unsplinted implants.[2–4] According to a systematic review, at least 4 implants should be placed, both anteriorly and posteriorly.[5] However, short-term results from a prospective study show that outcomes with 4 unsplinted implants placed in the canine and molar regions were less successful.[6] Later on, Boven et al. in their clinical study found that patients with all implants in the anterior position had marginally higher bone loss around 4 unsplinted implants as compared to splinted implants.[7] In a crossover clinical trial, the placement of 2 and 4 unsplinted implants to retain a maxillary overdenture resulted in comparable clinical outcomes, although patients preferred 4 implants.[8] Moreover, according to the systematic review performed by Klemetti, neither the number of implants nor the type of attachment in the maxilla had any influence on the long-term reliability of the final prosthesis or the satisfaction of the patient.[9]

Patients with edentulous maxillary arches now have new confidence with the advent of dental implant therapy, which improves prosthetic retention, support, stability, and biting force. Different attachment methods are employed to keep an overdenture in place, including studs, telescopes, bars with clips, and magnets. Studs have become very popular in clinical therapy due to their straightforward application. Bars are more difficult to clean and more technique-sensitive than solitary attachments.[10]

Due to the intimate fit between the primary and secondary copes, and the improved force dissemination caused by the axial transmission of the occlusal load, telescopic attachments offer excellent retention. Additionally, the overdentures are self-locating and simple to remove. In order to gage patient satisfaction, dentists should pay close attention to each patient's perception of their degree of comfort, appearance, emotion, function, speech, and confidence. The ultimate objective is to reach a level where edentulous patients are completely satisfied with the therapy, and are more aware of how edentulism affects their quality of life.[11,12]

Consequently, this clinical research was conducted to assess if implant-retained maxillary single dentures with a telescopic attachment are an effective alternative to implant-retained maxillary single dentures with a ball-and-socket attachment. The PICOT question addressed here was: In patients with maxillary single dentures (P), will implant-retained maxillary single overdentures with a telescopic attachment (I) result in the equivalence of patient satisfaction (O) in comparison with implant-retained maxillary single overdentures with a ball-and-socket attachment (C) after 3 months of follow-up (T) This trial was done following the CONSORT (Consolidated Standards of Reporting Trials) statement.

Material and methods

Trial design and setting

A parallel-group, 1:1 allocation ratio, randomized clinical trial (RCT) was intended for the investigation. A total of 18 Egyptian patients, 13 males and 5 females, aged 45–60 years (mean age: 53 years), with a fully edentulous maxillary arch opposite a fully or partially dentate mandibular arch, were chosen for this clinical trial from the outpatient clinic of the Department of Prosthodontics at Ahram Canadian University, 6th of October City, Egypt, and the dental clinic of the National Research Center, Cairo, Egypt. The chosen patients had either totally or partially dentate mandibular arches, as well as fully edentulous maxillary ridges. The patients were randomly assigned to the following groups: group 1 (intervention) – the patients received maxillary single dentures retained with a telescopic attachment (a resilient, custom-made telescopic crown); or group 2 (control) – the patients received maxillary single dentures retained with a ball-and-socket attachment (Dentis Implant System Attachment; Dentis Co., Ltd., Daegu, South Korea).

Randomization process

Using a research randomizer (https://www.randomizer.org), the 18 patients were randomly assigned to 2 groups (intervention or control), each containing 9 patients, after the manufacture of maxillary full dentures.

Allocation concealment

Only one investigator (M.H.M.), who took no part in patient selection or treatment, was aware of the randomization sequence and had access to the randomization lists kept on a portable computer that was password-protected. The randomly generated codes were placed in identical, sequentially numbered, sealed, opaque envelopes. The patients were asked to choose one of the envelopes. The investigator who was aware of the randomization procedure was then asked to designate the group, and the patient was treated appropriately.

Blinding

Apparently, neither the participants nor the care providers could be blinded, as the 2 attachment systems were obviously different, but the care providers were instructed to avoid commenting about treatment possibilities to the subjects. Patient satisfaction was assessed by an independent assessor, who was not aware of the type of intervention. The statistician was blinded.

Eligibility criteria for the selected cases

The inclusion criteria were as follows: patients having a completely edentulous maxilla; aged 45–60 years; free from any systemic diseases that may affect bone metabolism; non-smokers; with sufficient interarch space (not less than 15 mm); with maxillary ridges well-formed and covered with firm and healthy mucosa, free from inflammation, ulceration and flappy tissues; with angle Class I maxillomandibular relationship; at least 6–12 months having elapsed since the last tooth extraction; and no previous denture experience.

The exclusion criteria were as follows: patients with the dysfunction of the temporomandibular joint (TMJ), which would impair the prosthetic outcomes; medically compromised patients (an impact on the surgical placement of implants); subjects with a partially edentulous maxillary arch (it could affect the value of the study); uncooperative patients, who might not return for the follow-up, examinations or evaluation; and patients with a history of a surgical operation in the maxillofacial region.

All patients were requested to sign an informed consent form, which was translated into Arabic to be understood. The trial was conducted in accordance with the Declaration of Helsinki (2008).

Preparations

All the required mouth preparations were carried out for the lower natural teeth, including periodontal, surgical, restorative, and prosthetic procedures, as well as occlusal corrections. A conventional single maxillary denture was constructed after the rehabilitation and occlusal adjustment of the mandibular teeth. The patients were asked to wear their denture after it was modified by adding gutta-percha as a radiopaque detector for achieving dual scanning.

The cone-beam computed tomography (CBCT) radiograph of the maxillary arch was evaluated to determine the available bone height, using the Blue Sky Plan® software, v. 3.29.28 (https://blueskybio.com). Virtual implants (Dentis Implant System; Dentis Co., Ltd.) were placed and checked to be at least 2 mm away from any of the vital structures. Also, 1.5 mm of bone was checked to be present, both labially and lingually. Two virtual implants were placed in each maxillary edentulous arch, and the parallelism between them was checked. Once the positions of the implants were accepted in the virtual guide, 2 holes were designed in the software, corresponding to the prefabricated metal sleeves.

This trial was carried out using CBCT imaging and the flapless surgical technique to place 2 implants in the maxillary arch, utilizing tissue-supported computer-aided surgical guides. The 2 implants were then prosthetically restored with either telescopic or ball-and-socket attachments.

Surgical procedures

Root-formed, tapered, threaded dental implants, having dimensions of 4 mm in diameter and 12 mm in length (for canine regions) were prepared before surgery. Two implants were installed according to the flapless delayed implant placement protocol in the canine areas.

Prosthetic procedures

Once the implant osseointegration appeared satisfactory, patients were randomly divided into 2 equal groups according to the attachments used for retaining the maxillary overdenture.

The principles of clinical and laboratory procedures were followed for the construction of a single denture.

In group 2 (control), the ball-and-socket attachments were placed, and the direct pick-up technique was applied, as conventionally as possible.

For group 1, permanent transmucosal titanium (Ti) abutments (Octa®; Dentis Co. Ltd.) were fastened into the implant fixtures with an Octa driver until resistance was achieved, and then torqued up to 35 N·cm, using torque ratchets. Impression copings were placed over the abutments, and then splinted at the abutment level (the open-tray impression technique). The centric jaw relation was recorded using the wax wafer technique. The mounting and and setting up of cross-linked acrylic teeth (Vertex™ Quint Teeth; Vertex Dental, Soesterberg, the Netherlands) was performed like in conventional denture construction. The putty index was obtained using a rubber base (Labor Plus; Dental Line LTD, Inofita Viotias, Greece), and applied over the labial and occlusal surfaces to estimate the ideal size for primary and secondary copings. Then, an anti-rotational plastic cap was screwed to the analog and a resilient telescopic crown was fabricated. The wax pattern of the primary coping was built up with the use of milling wax. The primary copings of the telescopic attachments were cast into chrome-cobalt (Cr-Co) alloy. The primary crown was designed with a taper of 6° and a height of 5 mm. Next, secondary metal copings were fabricated over the primary copings. The secondary crown was designed with an occlusal free space of 0.4 mm between the primary

and secondary crowns, and a small amount of circumferential space of 0.04 mm between the 2 crowns, so that vertical movement between the 2 crowns could be allowed. After waxing up, the try-in with artificial teeth in the patient's mouth was done. Then, the laboratory procedures continued in the usual manner. The insertion of the final denture in the patient's mouth was done following the same conventional steps.

Examination procedures – patient satisfaction

A valid version of the visual analog scale (VAS) questionnaire was used and translated to Arabic for recording patient satisfaction in terms of comfort, appearance, speech, retention, stability, chewing, handling, hygiene, and overall satisfaction. The patients were instructed to rate the denture parameters on a 100-point scale. Most patients were unsatisfied with their conventional single dentures and were looking for an implant therapy option.

The records were taken after implant insertion and 3 months post-insertion.

Sample size and power analysis

Taking into account the *t* test results based on the overall satisfaction scores at 3 months postoperatively (group 1: 90.5 ±2.56 vs. group 2: 86.8 ±1.48), the standardized mean difference two-tail effect size (d) amounted to 1.769 and the power (1β error probability) was 94.06%. A sample size of 9 patients per group (2 groups in total) was sufficient to conduct the study with a power >80%. The post hoc calculations were made using G*Power, v. 3.1.9.2 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower).[13]

The study sample comprised 18 patients with a completely edentulous maxillary arch opposing a fully or partially dentate mandibular arch. A total of 36 implants were placed. The follow-up was completed by all patients. No implant failed in any of the included patients, giving a final follow-up success rate of 100% (Fig. 1).

Statistical analysis

The mean and standard deviation ($M \pm SD$) values were used to represent numerical data. To check for normality, the Shapiro–Wilk test was applied. The Mann–Whitney *U* test for intergroup comparisons and the Wilcoxon signed-rank test for intragroup comparisons were used to assess the non-parametric data. For all tests, the Bonferroni adjustment was used to adjust *p*-values for multiple comparisons. The significance level was set at $p < 0.05$. The R program, v. 4.1.3 (https://www.r-project.org), was used to conduct the statistical analysis.

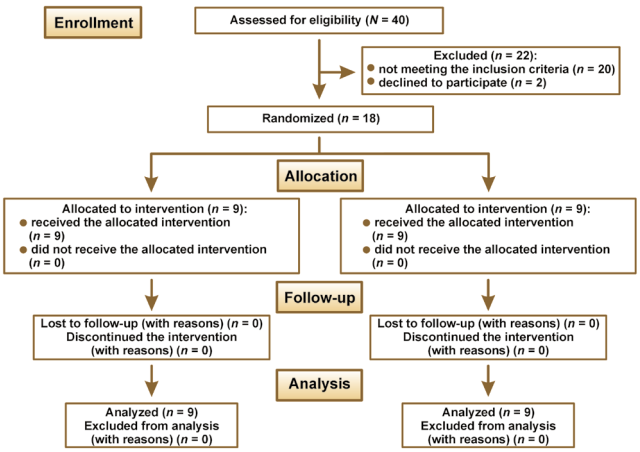

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flowchart

Results

The results of intragroup comparisons for the satisfaction scores are presented in Table 1. They showed that in group 1 there was a significant increase in the measured scores after 3 months for the 'comfort', 'chewing', 'handling', and 'overall satisfaction' categories ($p < 0.05$), while in group 2 there was a significant increase in the 'appearance' parameter only ($p < 0.05$); for other parameters, the change was not statistically significant ($p > 0.05$). The mean satisfaction scores for the telescopic and ball-and-socket groups are presented in Fig. 2 and 3.

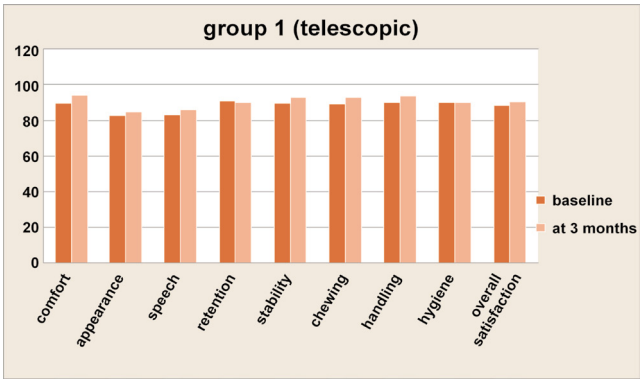

Fig. 2. Bar chart showing the visual analog scale (VAS) satisfaction scores for group 1 (telescopic attachment)

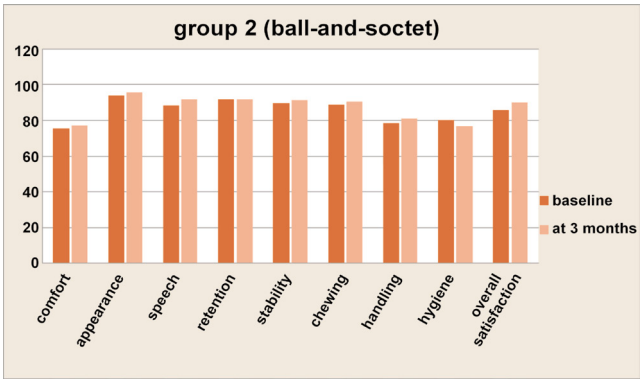

Fig. 3. Bar chart showing the visual analog scale (VAS) satisfaction scores for group 2 (ball-and-socket attachment)

Table 1. Intragroup comparisons of the satisfaction scores for the study groups

Group	Parameter	Satisfaction score		*u*-value	*p*-value
		after insertion	at 3 months		
Group 1 (telescopic)	comfort	89.6 ±3.80	94.0 ±2.69	13.00	0.008*
	appearance	82.8 ±6.45	84.6 ±5.31	30.50	0.200
	speech	83.1 ±4.56	85.8 ±3.21	23.50	0.070
	retention	90.7 ±5.14	90.2 ±4.68	47.00	0.700
	stability	89.8 ±5.88	92.7 ±4.35	25.50	0.090
	chewing	89.2 ±4.91	93.0 ±3.67	18.50	0.020*
	handling	90.3 ±3.67	93.6 ±2.73	18.50	0.020*
	hygiene	90.1 ±3.95	90.0 ±3.50	7.50	0.420
	overall satisfaction	88.2 ±3.49	90.5 ±2.56	19.50	0.030*
Group 2 (ball-and socket)	comfort	75.3 ±3.46	76.8 ±5.88	35.00	0.320
	appearance	93.6 ±2.39	95.6 ±1.41	21.00	0.040*
	speech	88.0 ±4.87	91.7 ±3.23	22.00	0.056
	retention	91.7 ±1.78	91.5 ±1.81	44.50	0.620
	stability	89.6 ±2.78	91.0 ±2.17	28.00	0.140
	chewing	88.8 ±2.75	90.4 ±2.65	26.50	0.110
	handling	78.1 ±5.84	80.7 ±5.97	28.50	0.150
	hygiene	80.0 ±4.33	76.7 ±3.70	58.00	0.930
	overall satisfaction	85.6 ±1.66	86.8 ±1.48	27.50	0.130

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

The results of intergroup comparisons for the satisfaction scores are presented in Table 2. After insertion, group 1 showed significantly better results for the 'handling' and 'hygiene' parameters, whereas group 2 showed a significantly better mean score for the 'appearance' parameter ($p < 0.05$). After 3 months, group 1 showed significantly better results for the 'comfort', 'handling', 'hygiene', and 'overall satisfaction' parameters, and group 1 proved significantly better in terms of 'appearance' and 'speech' parameters ($p < 0.05$).

Table 2. Intergroup comparisons of the satisfaction scores for the study groups

Time point	Parameter	Satisfaction score		*u*-value	*p*-value
		group 1 (telescopic)	group 2 (ball-and socket)		
After insertion	comfort	89.6 ±3.80	75.3 ±3.46	81.00	0.990
	appearance	82.8 ±6.45	93.6 ±2.39	5.50	0.002*
	speech	83.1 ±4.56	88.0 ±4.87	18.50	0.060
	retention	90.7 ±5.14	91.7 ±1.78	44.50	0.620
	stability	89.8 ±5.88	89.6 ±2.78	52.00	0.830
	chewing	89.2 ±4.91	88.8 ±2.75	47.00	0.700
	handling	90.3 ±3.67	78.1 ±5.84	78.00	0.009*
	hygiene	90.1 ±3.95	80.0 ±4.33	79.00	0.007*
	overall satisfaction	88.2 ±3.49	85.6 ±1.66	62.50	0.970
At 3 months	comfort	94.0 ±2.69	76.8 ±5.88	81.00	0.003*
	appearance	84.6 ±5.31	95.6 ±1.41	0.00	0.003*
	speech	85.8 ±3.21	91.7 ±3.23	8.50	0.002*
	retention	90.2 ±4.68	91.5 ±1.81	39.00	0.920
	stability	92.7 ±4.35	91.0 ±2.17	60.50	0.950
	chewing	93.0 ±3.67	90.4 ±2.65	65.00	0.980
	handling	93.6 ±2.73	80.7 ±5.97	80.50	0.000*
	hygiene	90.0 ±3.50	76.7 ±3.70	81.00	0.003*
	overall satisfaction	90.5 ±2.56	86.8 ±1.48	71.00	0.008*

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

Discussion

Our trial evaluated 2 different attachment systems – telescopic and ball-and-socket – in terms of their effect on patient satisfaction in the wearers of implant-retained maxillary single overdentures. At the end of the follow-up, the telescopic group in our study displayed a significantly higher level of comfort, handling, cleanliness, and general satisfaction as compared to the control group, whereas the ball-and-socket group displayed a significantly higher satisfaction level with regard to appearance and speech. The levels of patient satisfaction were measured using VAS.

With regard to the 1st assessment, our study found that the telescopic group showed significantly better results for the 'handling' and 'hygiene' satisfaction categories as compared to the ball-and-socket group. This could be due to a proper path of insertion and more freedom while placing the telescopic attachments as compared to ball attachments; which is in agreement with a previous study.[14]

Furthermore, there was a significant difference between the study groups concerning the 'appearance' parameter in the initial period, in favor of the ball-and-socket group. This could be explained by the presence of the primary and secondary copings of the telescopic attachment, which increased the bulk of the prosthesis and decreased the satisfaction level regarding appearance.

After 3 months, the telescopic group showed a significant increase in comfort, handling, hygiene, and overall satisfaction as compared to the ball-and-socket group, which confirms the fact that ball-and-socket attachments make it more challenging to position the denture in the mouth when compared to other attachments. Even with 2 such attachments, the patient still requires some additional ability to properly place a denture.[15]

As far as the telescopic attachments are concerned, our findings are in conformity with those of a previous study by Krennmair et al.[16] The patients had no trouble with cleaning the overdenture, as evidenced by the periodic recall visits, which implied no signs of irritation of the tissues around the implants.[16] The absence of the undercut stagnation areas in telescopic attachments is the reason for considering them as hygienic attachments, which allow easy access for cleaning.[14,17] This may explain the significant increase in satisfaction with the cleaning of the prosthesis.

After 3 months, the ball-and-socket group showed significant improvement in appearance and speech. On the other side, the telescopic group showed a decrease in the scores for appearance and speech due to the increased bulk of the prosthesis labially and palatally, affecting articulation and increasing lip fullness. Improvement in speech occurred also in the telescopic group at 3 months; yet, the intergroup comparison revealed a significant difference between the groups in this regard.

Despite the fact that the use of telescopes to retain conventional overdentures on the natural teeth is a well-known treatment method, there is a scarcity of information on the use of telescopic crowns with implant-supported overdentures. Thus, the findings far suggest that this approach can result in predictable long-term therapeutic effects.[18]

Telescopic crowns are utilized as abutments, as they have several advantages over other types of attachments where cost-effectiveness is a major priority. Despite costing more than simple ball-and-socket attachments, they might give the prosthesis additional stability and retention due to their design. The requirement for continuing maintenance may be lessened by the fact that telescopic crowns last longer than conventional attachments. Some dentists may also choose telescopic crowns due to their versatility in being employed as a component of a fixed prosthesis or as a support for a removable partial denture.[16]

According to a study that examined a resilient telescopic connection over a 10-year period, non-rigid telescopic connectors with 2 interforaminal implants appeared to be a long-term treatment option that was efficient and successful.[19] This idea may offer benefits in terms of handling, cleanliness and long-term satisfaction, particularly in the care of senior patients. In our study, we used a resilient telescopic attachment to enable straightforward manipulation, reduce the stresses reflected on the implant and permit some degree of vertical movement, comparable to that observed for a ball-and-socket attachment.

Patients with severe maxillary resorption, who do not want to have reconstructive surgery, may benefit from two-implant-supported maxillary overdentures. In a previous study, the efficiency of maxillary overdentures supported by 2 implants in individuals with an atrophic maxilla was examined.[20] The findings demonstrated positive implant and overdenture survival rates, while the masticatory function and patient satisfaction considerably improved with regard to baseline. The patients had a comparably significant chance of implant loss. Maxillary implant overdentures on 2 or 4 implants were both suggested, within the limitations of the study.[20]

A computerized stent can provide a high degree of parallelism among the dental implants needed for telescopic attachments, improving the passive fit of the superstructure, preventing determinant lateral forces on the implant fixture, and minimizing tipping forces on the implants during prosthesis placement and removal.[21]

Our results agree with the results of other studies, which suggested that implant-retained telescopic attachments may be an appropriate alternative for the standard ball-and-socket attachments. In comparison with other types of implant-supported solitary attachments, the application of a double crown over dentures provides a long-term therapeutic effect.[22]

Due to rotational stability and the presence of a frictional fit, the use of telescopic attachments may offer an acceptable and prosthetically straightforward retention modality. This kind of retention functions similarly as in other single attachments, including one-piece abutments, like a ball-and-socket attachment, because the abutment and the inner telescope form an integral unit. Nevertheless, it definitely offers broad prosthetic, technological, economic, and clinical benefits.[23,24]

On the other hand, experimental investigations on implant overdentures that evaluated denture-bearing areas, using 4 types of attachments found that telescopic crowns showed similar results as bars, balls and magnets; some studies revealed that there was a low dropout rate in both the ball-and-socket group and the telescopic group throughout the whole follow-up period.[25,26]

Additionally, a study comparing the retention of Ti and biocompatible high-performance polymer (BioHPP) telescopic abutments found that after simulating a year of overdenture use, the telescopic overdenture supported by abutments at a 2-degree angle demonstrated a significant decrease in the retentive force values. Titanium and BioHPP were both regarded as appropriate materials to hold telescopic implant overdentures in place.[27]

Patient satisfaction with a newly constructed implant-retained maxillary single overdenture was assessed using VAS, which is frequently employed as a guide for an agreed-upon or refused treatment outcome. Patients' pleasure with their implant-retained maxillary single overdenture is often recognized as vital for adaptation to newly constructed overdentures. The VAS is considered a reliable and efficient tool for evaluating the main variances between different countries and cultures.[28,29]

According to our study results, the telescopic attachment group showed a significant increase in overall satisfaction as compared to the ball-and-socket group; this is in agreement with a recent systematic review performed to compare the effects of different attachment systems.[30]

Due to higher expenses and the fact that more implants necessitate more expensive treatment, many patients decide not to receive four-implant overdenture therapy. Thus, the 2-implant maxillary overdenture with full palatal coverage acts as a bridge between the conventional complete denture and the four-implant overdenture.

The suggested treatment option is most successful in patients who have an atrophic maxilla, a shallow palatal vault or xerostomia, without an anterior maxillary undercut. In order to address the limitations of the current study (a short follow-up period and a small sample size), longer follow-up times and a larger sample size are required.

Conclusions

Within the constraints of this investigation, the following conclusion can be drawn:

Implant-retained maxillary single overdentures with a telescopic attachment showed an advantage over implant-retained maxillary single overdentures with a ball-and-socket attachment regarding comfort, handling, hygiene, and overall patient satisfaction.

Recommendation

It is advisable to do larger, expertly conducted RCTs, with extended follow-up times, and a range of functional, prosthodontic and patient-reported outcome measures.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee at the Faculty of Oral and Dental Medicine, Ahram Canadian University, 6th of October City, Egypt (approval No. IRB00012891#37). All patients provided written informed consent prior to the commencement of the trial.

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Bhandari S. Outcome of single maxillary complete dentures opposing mandibular teeth: A need to introspect on the prosthodontic treatment protocol. *J Indian Prosthodont Soc*. 2016;16(1):15–19. doi:10.4103/0972-4052.167941
2. Kern JS, Kern T, Wolfart S, Heussen N. A systematic review and meta-analysis of removable and fixed implant-supported prostheses in edentulous jaws: Post-loading implant loss. *Clin Oral Implants Res*. 2016;27(2):174–195. doi:10.1111/clr.12531
3. Anadioti E, Gates WD 3rd, Elpers J, De Kok IJ, Cooper LF. Retrospective cohort analysis of maxillary overdentures retained by unsplinted implants. *J Prosthet Dent*. 2019;122(3):301–308.e1. doi:10.1016/j.prosdent.2018.10.014
4. Wang F, Monje A, Huang W, Zhang Z, Wang G, Wu Y. Maxillary four implant-retained overdentures via Locator® attachment: Intermediate-term results from a retrospective study. *Clin Implant Dent Relat Res*. 2016;18(3):571–579. doi:10.1111/cid.12335
5. Messias A, Nicolau P, Guerra F. Different interventions for rehabilitation of the edentulous maxilla with implant-supported prostheses: An overview of systematic reviews. *Int J Prosthodont*. 2021;34:s63–s84. doi:10.11607/ijp.7162

6. Bouhy A, Rompen E, Lamy M, Legros C, Lecloux G, Lambert F. Maxillary implant overdenture retained by four unsplinted attachments and opposed by a natural or fixed dentition: One-year clinical outcomes. *Clin Oral Implants Res*. 2020;31(8):747–767. doi:10.1111/clr.13623
7. Boven GC, Meijer HJ, Vissink A, Raghoebar GM. Maxillary implant overdentures retained by use of bars or locator attachments: 1-year findings from a randomized controlled trial. *J Prosthodont Res*. 2020;64(1):26–33. doi:10.1016/j.jpor.2019.04.013
8. Kappel S, Klotz AL, Eberhard L, Bermejo JL, Rammelsberg P, Giannakopoulos NN. Maxillary implant overdentures on two or four implants. A prospective randomized cross-over clinical trial of implant and denture success and survival. *Clin Oral Implants Res*. 2021;32(9):1061–1071. doi:10.1111/clr.13800
9. Klemetti E. Is there a certain number of implants needed to retain an overdenture? *J Oral Rehabil*. 2008;35(Suppl 1):80–84. doi:10.1111/j.1365-2842.2007.01825.x
10. Dede DÖ, Durmuşlar MC, Şahın O, Köroğlu A, İşısağ Ö. Telescopic overdenture and implant supported fixed partial denture: A pragmatic treatment approach. *Case Rep Dent*. 2015;2015:392397. doi:10.1155/2015/392397
11. Pan YH, Lin TM, Liang CH. Comparison of patient's satisfaction with implant-supported mandibular overdentures and complete dentures. *Biomed J*. 2014;37(3):156–162. doi:10.4103/2319-4170.131384
12. Krennmair G, Krainhöfner M, Waldenberger O, Piehslinger E. Dental implants as strategic supplementary abutments for implant-tooth-supported telescopic crown-retained maxillary dentures: A retrospective follow-up study for up to 9 years. *Int J Prosthodont*. 2007;20(6):617–622. PMID:18069371.
13. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175–191. doi:10.3758/bf03193146
14. Frisch E, Ziebolz D, Ratka-Krüger P, Rinke S. Double crown-retained maxillary overdentures: 5-year follow-up. *Clin Implant Dent Relat Res*. 2015;17(1):22–31. doi:10.1111/cid.12087
15. Cakarer S, Can T, Yaltirik M, Keskin C. Complications associated with the ball, bar and Locator attachments for implant-supported overdentures. *Med Oral Patol Oral Cir Bucal*. 2011;16(7):e953–959. doi:10.4317/medoral.17312
16. Krennmair G, Seemann R, Weinländer M, Piehslinger E. Comparison of ball and telescopic crown attachments in implant-retained mandibular overdentures: A 5-year prospective study. *Int J Oral Maxillofac Implants*. 2011;26(3):598–606. PMID:21691608.
17. Mengel R, Kreuzer G, Lehmann KM, Flores-de-Jacoby L. A telescopic crown concept for the restoration of partially edentulous patients with aggressive generalized periodontitis: A 3-year prospective longitudinal study. *Int J Periodontics Restorative Dent*. 2007;27(3):231–239. PMID:17694946.
18. Prakash V, Parkash H, Gupta R. Fixed removable prosthesis employing Marburg double crown system. *J Indian Prosthodont Soc*. 2008;8(1):59–62. doi:10.4103/0972-4052.43258
19. Heckmann SM, Schrott A, Graef F, Wichmann MG, Weber HP. Mandibular two-implant telescopic overdentures. *Clin Oral Implants Res*. 2004;15(5):560–569. doi:10.1111/j.1600-0501.2004.01064.x
20. Rutkunas V, Mizutani H, Peciuliene V, Bendinskaite R, Linkevicius T. Maxillary complete denture outcome with two-implant supported mandibular overdentures. A systematic review. *Stomatologija*. 2008;10(1):10–15. PMID:18493160.
21. Srinivasan M, Schimmel M, Badoud I, Ammann P, Herrmann FR, Müller F. Influence of implant angulation and cyclic dislodging on the retentive force of two different overdenture attachments – an in vitro study. *Clin Oral Implants Res*. 2016;27(5):604–611. doi:10.1111/clr.12643
22. Krennmair G, Weinländer M, Krainhöfner M, Piehslinger E. Implant-supported mandibular overdentures retained with ball or telescopic crown attachments: A 3-year prospective study. *Int J Prosthodont*. 2006;19(2):164–170. PMID:16602365.
23. Attard NJ, Zarb GA, Laporte A. Long-term treatment costs associated with implant-supported mandibular prostheses in edentulous patients. *Int J Prosthodont*. 2005;18(2):117–123. PMID:15889659.
24. Zitzmann NU, Sendi P, Marinello CP. An economic evaluation of implant treatment in edentulous patients – preliminary results. *Int J Prosthodont*. 2005;18(1):20–27. PMID:15754888.
25. Cune M, van Kampen F, van der Bilt A, Bosman F. Patient satisfaction and preference with magnet, bar-clip, and ball-socket retained mandibular implant overdentures: A cross-over clinical trial. *Int J Prosthodont*. 2005;18(2):99–105. PMID:15889656.
26. Payne AG, Solomons YF. Mandibular implant-supported overdentures: A prospective evaluation of the burden of prosthodontic maintenance with 3 different attachment systems. *Int J Prosthodont*. 2000;13(3):246–253. PMID:11203640.
27. Ramadan R, Elsherbeeny Y, Thabet Y, Kandil B, Ghali R. Retention of a telescopic overdenture on customized abutments after the simulation of 1 year in function. *Dent Med Probl*. 2021;58(2):201–206. doi:10.17219/dmp/127963
28. Badr WE, Abo Helal OAE, Baraka OA. Evaluation of patient satisfaction and masticatory efficiency of single implant-retained mandibular overdenture using two types of matrices. *Al-Azhar J Dent Sci*. 2022;25(4):409–416. doi:10.21608/AJDSM.2021.62004.1162
29. Kashyap S, Mittal S, Sharma N, Sukhija U, Kamra M, Gupta N. Evaluation of patient satisfaction and masticatory performance in mandible single implant-assisted overdenture. *Tzu Chi Med J*. 2021;33(4):406–411. doi:10.4103/tcmj.tcmj_242_20
30. Sutariya PV, Shah HM, Patel SD, Upadhyay HH, Pathan MR, Shah RP. Mandibular implant-supported overdenture: A systematic review and meta-analysis for optimum selection of attachment system. *J Indian Prosthodont Soc*. 2021;21(4):319–327. doi:10.4103/jips.jips_158_21

Original paper

Investigating the relationship between the type of occlusion and mandibular radiomorphometric indices in patients with normal facial height

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. The ability to predict the extent and direction of horizontal growth of the patient's face at an early age allows clinicians to timely identify cases that require treatment during the growth period and to ensure that appropriate treatment can be administered.

Objectives. The aim of this study was to investigate the role of the antegonial angle, gonial angle, antegonial notch depth, and the type of the antegonial notch on predicting horizontal facial growth in individuals with normal facial height.

Material and methods. In this descriptive analytic study, lateral and panoramic radiographs of 180 patients aged 17–30 years with normal facial height who were referred to the School of Dentistry of Qazvin University of Medical Sciences and private dental clinics were investigated. The indices of the gonial angle, antegonial angle, type of antegonial notch, and antegonial notch depth were plotted bilaterally on tracing paper and subsequently measured using panoramic radiographs. Additionally, the relationship between the desired radiomorphometric indices and the occlusion class in the samples was investigated.

Results. There was a statistically significant relationship between the occlusion class and the antegonial notch depth (analysis of variance (ANOVA), $p = 0.048$), as well as the antegonial angle (Kruskal–Wallis test, $p = 0.002$).

Conclusions. The results of this study indicate that the antegonial notch depth and angle indices can be clinically valuable in predicting the process of mandibular growth and developing appropriate treatment plans.

Keywords: mandible, radiomorphometric index, skeletal relationship

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Introduction

The variability of occlusion, which can range from a desired state to a severe malocclusion in different individuals, is attributed to the interplay of bone growth, dental development and neuromuscular maturation. A better understanding of the relationship between occlusion, dental development and skeletal structure will facilitate the orthodontic diagnosis.[1] Orthodontists must be able to discern the relationship between the major functional facial parts, namely the cranial base, jaw and teeth.[2] The correlation between occlusion and craniofacial skeletal structure is well established within the field of orthodontics.[1,3]

A thorough diagnosis and treatment plan for malocclusions require a clear understanding of both the dentoalveolar and skeletal components in anterior–posterior and vertical directions.[4]

The capacity to predict a patient's growth pattern is valuable for orthodontists, as it enables the implementation of corrective measures at critical time periods to address skeletal discrepancies and avoid or decrease the necessity for surgical or orthodontic treatment. The direction of the growth of the mandible can significantly affect a person's occlusion, which in turn has an influence on their function and aesthetics. The type of facial growth is primarily determined based on radiographic imaging.[5]

Mandibular stimuli are present from the developmental stage to adulthood and can change the growth of the mandible and its bone remodeling.[6] Some of the most notable changes that have been suggested include alterations in the antegonial, gonial, condylar, and ramus regions.[7] Panoramic radiography is the preferred method in the assessment of mandibular morphology, including vertical and angular measurements.[6]

Panoramic radiography is a valuable tool for evaluating mandibular indices.[8] Dental panoramic radiography is a reliable method for diagnosing and evaluating the mandibular position from the dentist's perspective.[9] The majority of authors who have conducted research into mandibular remodeling have performed morphometric evaluations using panoramic radiographs.[7] The morphological changes of the mandible have been investigated mainly through the analysis of radiomorphometric measurements.[6]

Skieller et al. used multivariate statistical methods to identify 4 morphological variables that, when evaluated in lateral cephalograms, may predict future mandibular rotation. However, their sample included individuals with highly abnormal morphological patterns.[10] Further research using this method conducted by Lee et al. and Leslie et al. did not yield promising results.[11,12] Halazonetis et al. discovered that the overall rotation of the mandible during growth was not related to the mandibular shape.[13] However, the authors observed a correlation between the Fourier coefficient, which explained most of the variability in the ramus slope, and gonial and antegonial angles.[13] Two studies examined the possibility that the mandibular antegonial notch morphology may predict the mandibular growth.[14,15]

Given the paucity of studies in this field that have considered sample matching and the ongoing debate surrounding the study of gonial angle and antegonial notch area changes in mandibular growth, this study aimed to investigate the crucial role of the antegonial angle, gonial angle, antegonial notch depth, and the type of the antegonial notch in predicting horizontal facial growth in individuals with normal facial height.

Material and methods

This descriptive analytic study evaluated 180 individuals between the ages of 17 and 30 who were candidates for orthodontic treatment and referred to the School of Dentistry of Qazvin University of Medical Sciences and Qazvin private dental clinics between 2011 and 2016. The participants were divided into 3 groups of 60 individuals each, according to the occlusion class (Angle's Class I, Class II and Class III occlusion).

The samples were selected from patients who had been referred to orthodontic clinics using simple random sampling and following the conclusion of a clinical study, based on established inclusion and exclusion criteria. The study included patients with normal facial height.

One of the criteria for evaluating and detecting the vertical facial growth pattern is the Jaraback index, which was calculated with the help of natural head position (NHP) cephalometric imaging using the following formula (Equation 1)[16]:

$$\text{Jaraback index} = \text{S–Go/N–Me} \times 100 \quad (1)$$

where:

S–Go – linear distance between Sella (S) and Gonion (Go) (posterior facial height) [mm];

N–Me – linear distance between Nasion (N) and Menton (Me) (anterior facial height) [mm].

The Jaraback index falls within the normal range of 62–65%.[17]

The exclusion criteria were as follows: a mismatch between the ANB and Wits indices in the patient; a discrepancy between the occlusion of the both sides of the patient; patients with mixed dentition; individuals with long or short faces; and individuals with retrusion or severe maxillary protrusion based on the cephalometric index.

All certified digital panoramic radiographs were attached to the parchment paper of the Ortho Organizer type. Tracing was performed by a trained senior-year student, while radiomorphometric indices were determined by a maxillofacial radiologist on digital panoramic radiographs.

The radiomorphologic and radiomorphometric indices evaluated in this study are as follows:

– gonial angle: a line is drawn tangent to the inferior border of the mandibular body, and another line is drawn tangent to the posterior border of the ramus and condyle. The intersection of these 2 lines forms the gonial angle (Fig. 1)[18];

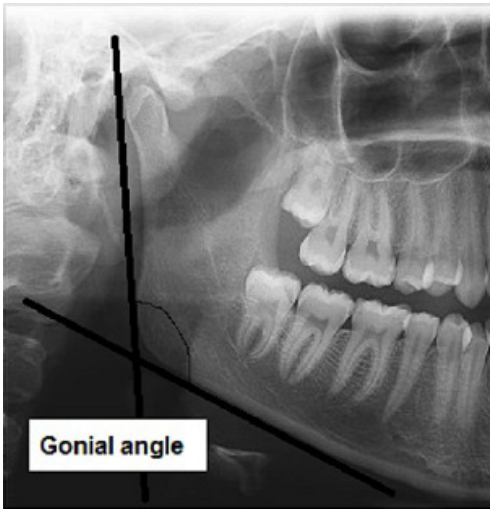

Fig. 1. Gonial angle formed by the junction of the posterior and lower borders of the mandible

– antegonial angle: 2 lines are drawn tangent to the edge of the inferior border of the mandible that intersect each other at the deepest part of the antegonial notch. The angle between these 2 intersecting lines is referred to as the antegonial angle (Fig. 2)[19];

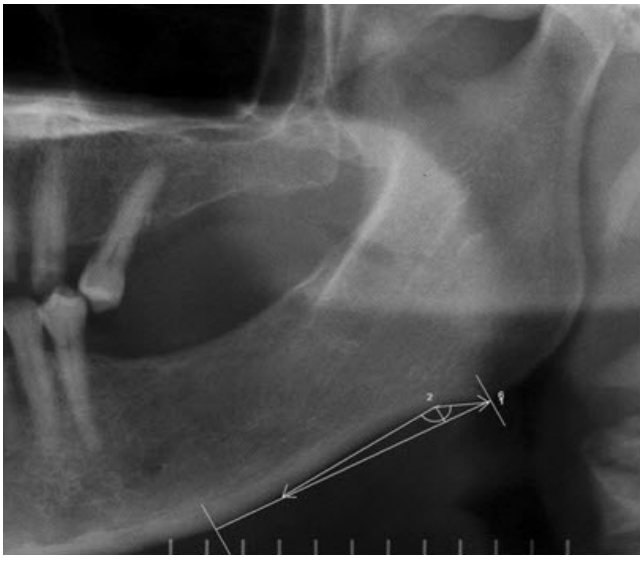

Fig. 2. Antegonial notch depth and the antegonial angle in panoramic radiography

– antegonial notch depth: the distance of a perpendicular line from the deepest point of the antegonial notch concavity to a line tangent to the inferior border of the mandible (Fig. 2–4)[19];

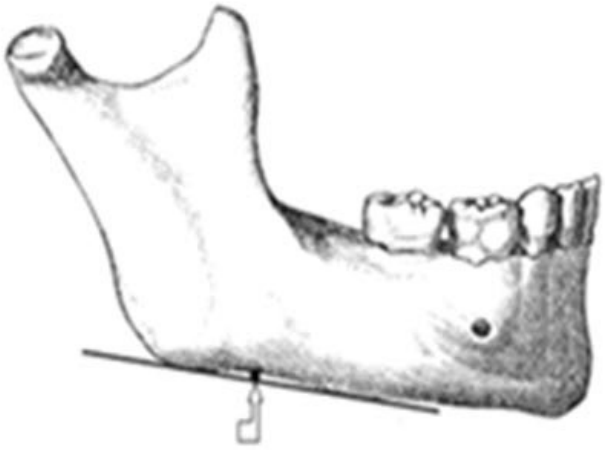

Fig. 3. Antegonial notch depth

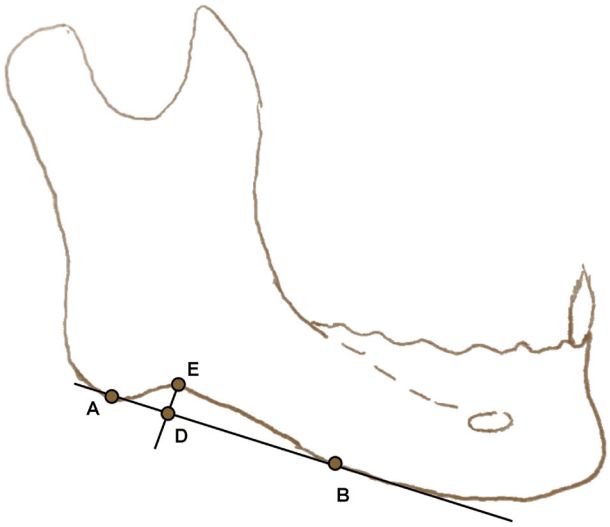

Fig. 4. Shape of the antegonial notch drawn by connecting 3 points (A, B and E) and the antegonial notch depth (E and D)

A – distal border of the antegonial notch; B – proximal border of the antegonial notch; E – fundus of the antegonial notch; D – extrapolated point of the position of the antegonial notch fundus at its basis.

– type of antegonial notch: the triangular shape of the antegonial notch is drawn by connecting 3 points (A, B and E) (Fig. 4); antegonial notch is classified into 3 types, including asymmetrical posterior notch (type 1), symmetrical notch (type 2), and asymmetrical anterior notch (type 3) (Fig. 4,5).[15]

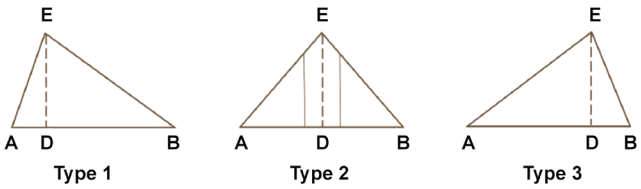

Fig. 5. Types of the antegonial notch

type 1 – asymmetrical posterior notch; type 2 – symmetrical notch; type 3 – asymmetrical anterior notch.

The antegonial notch depth was measured with the use of a digital caliper (Guanglu Instruments Co., Ltd., Guilin, China). The measurements included the gonial angle and the antegonial angle. The obtained data was recorded in the patient form. In instances where the indices were bilaterally visible, two-way measurements were performed and the mean values were recorded. In cases where the index was not visible on one side, only the index of the side that was clearly visible was used in the measurements.

Statistical analysis

The data was analyzed using analysis of variance (ANOVA), and Tukey's post hoc test was employed to facilitate the comparison between the 3 groups. The χ^2 test was utilized to investigate the relationship between qualitative traits. A paired *t*-test was employed to compare the left and right indices, while Wilcoxon test and the Kruskal–Wallis test were utilized to compare indices with a non-normal distribution. The data was analyzed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). In this study, a *p*-value of less than 0.05 was considered statistically significant.

Results

The present study was conducted on a total of 180 subjects aged 17–30 years, who were candidates for orthodontic treatment at the School of Dentistry of Qazvin University of Medical Sciences and Qazvin private dental clinics between 2011 and 2016. Of the total number of subjects, 47 (26.1%) were male and 133 (73.9%) were female. The results demonstrated no statistically significant relationship between sex and the occlusion class.

The mean and standard deviation of the right and left gonial angles were 123.0 ±6.7° and 123.1 ±7.9° for Class I, 124.1 ±5.8° and 122.9 ±6.5° for Class II, and 123.3 ±7.1° and 122.9 ±6.3° for Class III of occlusion. The statistical analysis revealed no statistically significant differences between the mean values of the gonial angle on both sides across the 3 occlusion classes (Table 1).

Table 1. Comparison of the mean gonial angle across 3 occlusion classes

Occlusion class	Side	Gonial angle [°] *M* ±*SD*	*p*-value
Class I	right	123.0 ±6.7	0.860
	left	123.1 ±7.9	
Class II	right	124.1 ±5.8	0.110
	left	122.9 ±6.5	
Class III	right	123.3 ±7.1	0.470
	left	122.9 ±6.3	

M – mean; *SD* – standard deviation.

The results of the statistical analysis indicated a statistically significant difference in the mean value of the antegonial notch depth between the 3 occlusion classes on the right side and in the total sample (Fig. 6).

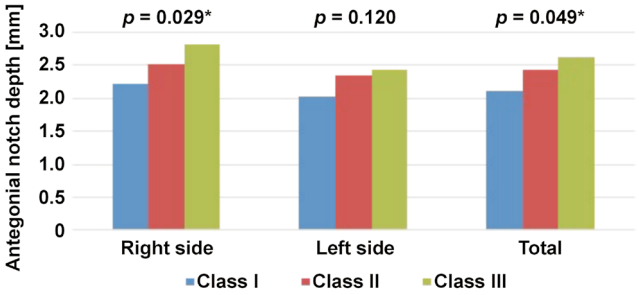

Fig. 6. Comparison of the mean antegonial notch depth by occlusion class
* statistically significant ($p < 0.05$, Dunn's post hoc test).

The data revealed that in Class I, 1.7% of the antegonial notches were tangible, 28.3% were type 1, 45.0% were type 2, and 25.0% were type 3. In Class II, 2.5% of the antegonial notches were tangible, 29.2% were type 1, 50.8% were type 2, and 17.5% were type 3. In Class III, 0.8% of the antegonial notches were tangible, 26.7% were type 1, 42.5% were type 2, and 30.0% were type 3 (Table 2).

Table 2. Relative frequency distribution of the occlusion class and the type of the antegonial notch in the total sample (*N* = 360)

Antegonial notch type	Occlusion class *n* (%)		
	Class I	Class II	Class III
Tangible	2 (1.7)	3 (2.5)	1 (0.8)
Type 1	34 (28.3)	35 (29.2)	32 (26.7)
Type 2	54 (45.0)	61 (50.8)	51 (42.5)
Type 3	30 (25.0)	21 (17.5)	36 (30.0)

The statistical analysis demonstrated statistically significant differences between the right and left sides of the antegonial notch in 3 occlusion classes ($p < 0.001$) (Table 3).

Table 3. Comparison of the type of the antegonial notch on both sides across 3 occlusion classes

Occlusion class	Side	Antegonial notch type (1, 2 or 3)		*p*-value
		Me	*IQR*	
Class I	right	1	1	<0.001*
	left	2	1	
Class II	right	1	1	<0.001*
	left	2	1	
Class III	right	1	1	<0.001*
	left	3	1	

Me – median; *IQR* – interquartile range; * statistically significant ($p < 0.05$, Wilcoxon test).

The results of the statistical analysis revealed no statistically significant difference between the occlusion groups on the right side of the antegonial notch and in total. However, a statistically significant difference was observed between the 3 groups on the left side ($p = 0.007$) (Table 4).

Table 4. Comparison of the type of the antegonial notch between 3 occlusion classes on the right and left sides, and in the total sample

Side	Occlusion class	Antegonial notch type (1, 2 or 3)		Average rank	*p*-value
		Me	*IQR*		
Total	Class I	2	0.5	90.6	0.120
	Class II	2	0.5	81.7	
	Class III	2	0	99.1	
Right	Class I	1	1	92.8	0.890
	Class II	1	1	89.3	
	Class III	1	1	89.3	
Left	Class I	2	1	88.7	0.007*
	Class II	2	1	77.3	
	Class III	2	1	103.6	

* statistically significant ($p < 0.05$, Dunn's post hoc test).

In evaluating the antegonial angle across 3 occlusion groups, the median value of the left antegonial angle was observed to be greater than that of the right angle. However, no statistically significant difference was identified between the 2 sides ($p > 0.001$) (Table 5).

Table 5. Comparison of the antegonial angle on both sides across 3 occlusion classes

Occlusion class	Side	Antegonial angle [°]	
		Me	*IQR*
Class I	right	166.0	7.6
	left	168.0	6.5
Class II	right	162.8	5.8
	left	164.5	9.6
Class III	right	165.0	6.8
	left	165.5	8.8

The statistical analysis revealed a statistically significant difference in the antegonial angle values between the 3 occlusion classes on the right ($p = 0.002$) and left sides ($p = 0.040$) of the body, as well as in the total sample ($p = 0.002$) (Table 6).

Table 6. Comparison of the antegonial angle by occlusion class in the total sample

Side	Occlusion class	Antegonial angle [°]		Average rank	*p*-value
		Me	*IQR*		
Total	Class I	167.0	7.3	109.9	0.002*
	Class II	164.1	7.5	80.4	
	Class III	164.6	7.9	81.7	

* statistically significant ($p < 0.05$, Kruskal–Wallis test).

Discussion

The study investigated the relationship between 4 radiomorphometric indices, including the gonial angle, the antegonial angle, antegonial notch depth, and the type of antegonial notch. A statistically significant relationship was identified between the antegonial angle, antegonial notch depth and the occlusion class. There was no significant relationship between the gonial angle and the type of antegonial notch in relation to the occlusion class.

Dental panoramic radiography is a reliable method for diagnosing and evaluating the mandibular position from the dentist's perspective.[9] Since panoramic radiography is a routine procedure, it can be used effectively to evaluate mandibular indices.[8]

Some studies have reported a significant difference between individuals with very deep and those with very shallow antegonial notches with regard to mandibular growth indices.[14,20] Singer et al. performed a 4-year longitudinal study on 25 patients undergoing orthodontic treatment with a deep antegonial notch, along with a similar group with a shallow antegonial notch.[14] They used lateral cephalometric radiographs and found that cases with a deep antegonial notch exhibited greater retrusion of the mandible, a shorter body length, a shorter ramus, and greater gonial angles than those with a shallow antegonial notch.[14] During the study period, patients with a deeper antegonial notch demonstrated a smaller increase in the mandibular body length and less chin displacement in the horizontal direction (resulting in greater retrusion of the mandible) than subjects with a shallow antegonial notch. The results of this study indicate that the presence of a deep antegonial notch is associated with reduced mandibular growth potential and a distinct vertical growth pattern.[14] Although the present study and the study by Singer et al.[14] are consistent in terms of the orientation of the antegonial notch and its impact on mandibular growth, the pattern of mandibular growth differs. Based on the present study, cases with a higher antegonial angle and a deep antegonial notch are prone to mandibular protrusion. Moreover, no statistically significant difference was identified between the gonial angle and the occlusion class, in contrast to the findings of the study by Singer et al.[14]

The differences between the results of the present study and those of the study by Singer et al. include different sampling methods, different implementation methods, a low sample size, a lack of attention to maxillary horizontal growth, sample mismatch in terms of maxillary horizontal growth, and racial differences.[14] The study sample employed by Singer et al. consisted of patients who had undergone orthodontic treatment, which represents a confounding factor in the study. Additionally, the subjects of the study were individuals with deep and shallow antegonial notches.

Lambrechts et al. investigated the craniofacial features in lateral cephalometric radiography of 40 patients with a shallow antegonial notch and 40 patients with a deep

antegonial notch in order to evaluate different dimensions in craniofacial morphology between the 2 groups.[20] Their findings indicated that, on average, subjects with a deep mandibular antegonial notch had a larger gonial angle, a deeper posterior ramus notch, and a steeper occlusal plane in examination than those with a shallow antegonial notch, whose mandible exhibited a more anterior position than subjects with a deep antegonial notch. The anterior face height of individuals with a shallow antegonial notch was significantly lower than the anterior face height of those with a deep antegonial notch. Also, the maxillary position in relation to the cranial base was more protruding in patients with a deep antegonial notch than in those with a shallow antegonial notch.[20] While the present study and the study by Lambrechts et al. are consistent in terms of the efficacy of the antegonial notch in mandibular growth, the growth pattern of the mandible presented in the study by Lambrechts et al.[20] is inconsistent with the results of our study. Also, in contrast to the findings of Lambrechts et al.,[20] the present study did not identify a statistically significant relationship between the gonial angle and the occlusion class. This discrepancy can be attributed to different sampling methods, racial differences, different implementation techniques, a small sample size, a lack of attention to maxillary horizontal growth, and sample mismatch in terms of maxillary horizontal growth. Moreover, the study by Lambrechts et al. included individuals with deep and shallow antegonial notches, which is indicative of non-random sampling.[20] Furthermore, previous studies[14,20] have not addressed the issue of vertical growth matching, which may be a confounding factor in the study.

The study by Ghosh et al. revealed no significant relationship between mean angles and the depth of the antegonial notch in relation to age.[21] However, antegonial angles decreased with age, while the antegonial notch depth demonstrated an increase with age.[21] The findings of our study are consistent with those of Dutra et al., who found no significant correlations between age and the antegonial angle and depth change.[19] The results indicate that the 17–30 age range is not considered as a confounding factor in the study.

In the study by Ghosh et al., a comparison of the absolute values of the right and left antegonial angles revealed that the values on the left side were significantly higher than those on the right side in each group.[21] This result is consistent with the findings of our study. It supports the theory that even in the absence of malformations and skull base asymmetry, the mandible may be physiologically asymmetric.

In the study by Ghosh et al., a significant decrease in the values of the antegonial angle and a significant increase in the values of the antegonial notch depth were observed as the dentition status changed from completely dentulous to partially dentulous and from partially dentulous to completely edentulous on both the right and left sides and in both sexes.[21] Therefore, the dental position can be considered a confounding factor in the evaluation of the antegonial area. In the present study, all samples had at least 1 interdental position in each side of the jaw, thereby eliminating the confounding role of dental position as a variable.

In a longitudinal study conducted by Kolodziej et al., 40 subjects were examined in 3 age groups (8.5, 12 and >17 years) with no history of treatment.[22] The study concluded that the antegonial notch depth was not a reliable indicator for investigating the potential of facial growth.[22] This result is inconsistent with the findings of our study. The discrepancy between the 2 studies may be attributed to the long-term nature of the study conducted by Kolodziej et al., a small sample size, the use of different implementation methods, racial differences, inadequate consideration of maxillary horizontal growth, and sample mismatch in terms of maxillary horizontal growth.[22]

Conclusions

In evaluating the indices, it was observed that changes based on the occlusion class, the antegonial angle and the type of antegonial notch were affected by the growth pattern in patients with normal facial height. Individuals with a deep antegonial notch and a greater antegonial angle were more prone to mandibular protrusion. Therefore, it is recommended that timely therapeutic measures be taken prior to the growth spurt in patients with a deeper antegonial notch and a wider angle in order to control the growth of the mandible. However, further research is required to determine a suitable cut-off point for these indices.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Qazvin University of Medical Sciences (approval No. IR.QUMS.REC.1395.292).

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.

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References

1. Moyers RE, Wainright R. Skeletal contributions to occlusal development. In: McNamara JA, Ribbens KA, eds. *The Biology of Occlusal Development*. Michigan, MI: University of Michigan; 1977:89–111.
2. Proffit WR, Fields H, Larson B, Sarver DM. *Contemporary Orthodontics*. 6th ed. Elsevier Health Sciences; 2018.
3. Angle EH. *Treatment of Malocclusion of the Teeth: Angle's System*. 7th ed. Philadelphia, PA: White Dental Manufacturing Company; 1907:21–24.
4. Freudenthaler J, Čelar A, Ritt C, Mitteröcker P. Geometric morphometrics of different malocclusions in lateral skull radiographs. *J Orofac Orthop*. 2017;78(1):11–20. doi:10.1007/s00056-016-0057-x
5. Tung K, Lagravère MO. Skeletal and dental relationships in vertical/non-vertical growers using CBCT. *Int Orthod*. 2019;17(1):123–129. doi:10.1016/j.ortho.2019.01.007
6. Bozdag G, Sener S. The evaluation of MCI, MI, PMI and GT on both genders with different age and dental status. *Dentomaxillofac Radiol*. 2015;44(9):20140435. doi:10.1259/dmfr.20140435
7. Mağat G, Özcan Şener S. The morphological changes in the mandible bone: The effects of age, gender and dental status. *Meandros Med Dent J*. 2018;19:111–120. doi:10.4274/meandros.02886
8. Moradi M, Tofangchiha M, Soltanmohammadi E, Golshahi H, Mojtahedi N. The influence of age, gender and dental status on the mandibular radiomorphometric and morphological indices. *Ann Dent Spec*. 2017;5:63–67. https://annalsofdentalspecialty.net.in/article/the-influence-of-age-gender-and-dental-status-on-the-mandibular-radiomorphometric-and-morphological-indices. Accessed January 13, 2020.
9. Wang M. *Antegonial Notching and the Pattern of Vertical Facial Growth* [master's thesis]. Seattle: University of Washington; 2012.
10. Skieller V, Björk A, Linde-Hansen T. Prediction of mandibular growth rotation evaluated from a longitudinal implant sample. *Am J Orthod*. 1984;86(5):359–370. doi:10.1016/s0002-9416(84)90028-9
11. Lee RS, Daniel FJ, Swartz M, Baumrind S, Korn EL. Assessment of a method for the prediction of mandibular rotation. *Am J Orthod Dentofacial Orthop*. 1987;91(5):395–402. doi:10.1016/0889-5406(87)90392-1
12. Leslie LR, Southard TE, Southard KA, et al. Prediction of mandibular growth rotation: Assessment of the Skieller, Björk, and Linde-Hansen method. *Am J Orthod Dentofacial Orthop*. 1998;114(6):659–667. doi:10.1016/s0889-5406(98)70198-2
13. Halazonetis DJ, Shapiro E, Gheewalla RK, Clark RE. Quantitative description of the shape of the mandible. *Am J Orthodont Dentofac Orthop*. 1991;99(1):49–56. doi:10.1016/s0889-5406(05)81680-4
14. Singer CP, Mamandras AH, Hunter WS. The depth of the mandibular antegonial notch as an indicator of mandibular growth potential. *Am J Orthodont Dentofac Orthop*. 1987;91(2):117–124. doi:10.1016/0889-5406(87)90468-9
15. Porwolik M, Porwolik K, Domagała ZA, et al. Typology of the antegonial notch in the human mandible. *Folia Morphol (Warsz)*. 2015;74(3):365–371. doi:10.5603/FM.2015.0055
16. Benedicto EN, Kairalla SA, Oliveira GMS, Junior LRM, Rosário HD, Paranhos LR. Determination of vertical characteristics with different cephalometric measurements. *Eur J Dent*. 2016;10(1):116–120. doi:10.4103/1305-7456.175694
17. Emami Meibodi S, Tehranchi A, Khakbiz M, Habibzadeh Omran S. Evaluation of buccolingual inclinations of posterior teeth in vertical and normal growth pattern [in Persian]. *J Dent Sch*. 2009;27(1):1–6. https://www.researchgate.net/publication/277112549_Evaluation_of_buccolingual_inclinations_of_posterior_teeth_in_vertical_and_normal_growth_pattern. Accessed January 13, 2020.
18. Dutra V, Devlin H, Susin C, Yang J, Horner K, Fernandes ARC. Mandibular morphological changes in low bone mass edentulous females: Evaluation of panoramic radiographs. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;102(5):663–668. doi:10.1016/j.tripleo.2006.02.023
19. Dutra V, Yang J, Devlin H, Susin C. Mandibular bone remodelling in adults: Evaluation of panoramic radiographs. *Dentomaxillofac Radiol*. 2004;33(5):323–328. doi:10.1259/dmfr/17685970
20. Lambrechts AH, Harris AM, Rossouw PE, Stander I. Dimensional differences in the craniofacial morphologies of groups with deep and shallow mandibular antegonial notching. *Angle Orthod*. 1996;66(4):265–272. doi:10.1043/0003-3219(1996)066<0265:DDITCM>2.3.CO;2
21. Ghosh S, Vengal M, Pai KM, Abhishek K. Remodeling of the antegonial angle region in the human mandible: A panoramic radiographic cross-sectional study. *Med Oral Patol Oral Cir Bucal*. 2010;15(5):e802–e807. doi:10.4317/medoral.15.e802
22. Kolodziej RP, Southard TE, Southard KA, Casko JS, Jakobsen JR. Evaluation of antegonial notch depth for growth prediction. *Am J Orthodont Dentofacial Orthop*. 2002;121(4):357–363. doi:10.1067/mod.2002.121561

Original paper

Association between *Porphyromonas gingivalis* in subgingival plaque and coronary artery disease: A case–control study

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Conflict of interest
None declared

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Abstract

Background. Periodontitis is a chronic inflammatory disease of the supporting tissue surrounding the teeth. The disease is caused by specific bacteria, such as *Porphyromonas gingivalis*, which lead to the destruction of periodontal ligaments and alveolar bone.

Objectives. The study aimed to evaluate the relationship between the prevalence of *P. gingivalis* in subgingival plaque and coronary artery disease (CAD).

Material and methods. Fifty patients with CAD and 50 healthy controls (non-CAD) participated in this case–control study. The periodontal health in the groups was evaluated through the assessment of the pocket depth (PD), clinical attachment loss (CAL) and bleeding on probing (BoP). The presence of *P. gingivalis* in subgingival plaque samples was determined through real-time polymerase chain reaction (RT-PCR). The data was analyzed using the χ^2 test and the Mann–Whitney *U* test.

Results. The mean PD was 3.30 ±1.55 mm and 3.56 ±0.97 mm in CAD patients and non-CAD subjects, respectively ($p = 0.028$). No significant differences were observed in the CAL ($p = 0.858$) and BoP ($p = 1.000$) between the groups. The RT-PCR results revealed the presence of *P. gingivalis* 16S rDNA in 32% and 22% of the subgingival plaque of patients with CAD and non-CAD, respectively, with a mean concentration of 7.7×10^6. No statistically significant association was observed between the prevalence of *P. gingivalis* and CAD ($p = 0.260$). The results of the multiple logistic regression analysis showed an association between CAD and male sex ($p = 0.004$, odds ratio (*OR*): 4.163), as well as age ($p = 0.011$, *OR*: 1.067).

Conclusions. The findings of this study indicated that there is no statistically significant correlation between the prevalence of *P. gingivalis* in subgingival plaque and CAD.

Keywords: periodontitis, *Porphyromonas gingivalis*, coronary artery disease

Introduction

Periodontitis is a highly prevalent multifactorial chronic inflammatory disease of the teeth-supporting tissue.[1] It is caused by the activity of dental plaque bacteria in the oral cavity and is the 6th most common human disease, with an overall prevalence of 45–50%. The most severe form of periodontitis affects 11.2% of the global population.[2]

There is an association between severe periodontitis and several non-communicable diseases (NCDs), including diabetes, chronic kidney disease (CKD), cardiovascular disease (CVD), and chronic obstructive pulmonary disease (COPD).[2] As an inflammatory condition, CVD comprises coronary heart diseases, atherosclerotic, cerebrovascular, and peripheral vascular diseases.[3] Over the last decades, a significant body of evidence has indicated a correlation between chronic periodontitis and an increased risk of developing CVD.[4–6]

It is hypothesized that in patients with periodontitis, an acute inflammatory immune response is involved in the transition from a symbiotic microbiota that is compatible with the host to an incipient dysbiotic microbiota, which supplies bacteria with resources from tissue breakdown and initiates a self-replicating pathogenic cycle. This cyclical interaction can persist for years in non-susceptible individuals, but it can develop quickly in those who are sensitive, leading to overt dysbiosis accompanied by an inefficient, protracted inflammatory or immune response.[7,8]

The tissue destruction caused by periodontitis increases the amount of cytokines involved in the development of cardiovascular diseases. For example, matrix metalloproteinase-8 (MMP-8) has been identified as a key stimulatory and activating factor of pro-inflammatory mediators in the development of both cardiovascular diseases and periodontitis.[9,10]

Indeed, periodontal pathogens or harmful endotoxins and exotoxins may penetrate from the oral cavity into the bloodstream during chewing or eating via damaged periodontal pocket epithelium. Therefore, bacterial dissemination and systemic infection lead to an inflammatory response, establishing a link between periodontitis and CVD.[11–13]

Periodontal pathogens are capable of directly invading the cardiovascular system. Reports indicate the presence of periodontal pathogens in human cardiac tissue, heart valves, pericardial fluids, and atherosclerotic lesions.[1] The periodontal pathogens often identified in subgingival plaque of patients with chronic periodontitis include *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola*.[14] Among these, *P. gingivalis* is the principal pathogen in the initiation and development of chronic periodontitis.[15] Additionally, it may act as a risk factor for several diseases, including CVD.[16] For example, a study by Holmlund et al. demonstrated that the level of immunoglobulin G (IgG) antibodies against *P. gingivalis* increased in patients with myocardial infarction.[17] Thus, assessing the clinical risk of oral infection with *P. gingivalis* is essential in patients with coronary artery disease (CAD). However, the implementation of oral hygiene training and the early diagnosis and treatment of periodontal problems can reduce the prevalence of CAD and its associated consequences. The aim of this study was to investigate the prevalence of *P. gingivalis* in subgingival plaque of patients with periodontitis and CAD, diagnosed by angiography as the gold standard due to the lack of adequate studies on the subject among the Iranian population. The hypothesis of the study was that the prevalence of *P. gingivalis* is greater in patients with CAD.

Material and methods

Study design

A total of 100 individuals were randomly selected from those referred to the Fatemeh Zahra Hospital (Mazandaran Heart Center), Mazandaran University of Medical Sciences, Sari, Iran, for inclusion in this observational study. The patients were divided into 2 groups of 50 individuals each, with one group serving as the case group (CAD group) and the other as the control group (non-CAD group). Only individuals with CAD as their sole systemic condition were included in the case group. Additionally, the study's inclusion criteria encompassed the willingness to participate in the study and the absence of any other systemic illnesses. Individuals with artificial valves, those on immunosuppressive medications, those with infective endocarditis, pregnant women, those who declined to participate in the study, and edentulous patients were excluded from the study.[18] Following the provision of informed consent, subjects were invited to participate in the study. Individuals who underwent angiography were divided into 2 groups based on the results of the procedure: patients without CAD; and patients with CAD involving 1, 2 or 3 arteries. All procedures were conducted in accordance with the ethical standards set forth by the Medical Ethics Committee of Mazandaran University of Medical Sciences and in alignment with the 1964 Helsinki Declaration and its subsequent amendments. The study was approved by the Medical Ethics Committee of Mazandaran University of Medical Sciences (ethics code: IR.MAZUMS.REC.1396.3183).

Sample size

According to the study conducted by Hyvärinen et al., the prevalence of *P. gingivalis* was 65.2% in patients with CAD and 37.5% in patients with normal coronary arteries.[19] Based on a confidence interval (*CI*) of 95%

and a study power of 80%, the requisite sample size for our study was determined to be 100 (i.e., 50 subjects in the CAD group and 50 subjects in the non-CAD group). The sample size was calculated using G*Power software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower).

Periodontal examination

The researchers obtained pertinent information about the patients, including their family history of heart disease, diabetes, age, and smoking status. A trained periodontist performed examinations on the subsequent day following the angiography. The bleeding on probing (BoP), periodontal pocket depth (PD) and clinical attachment loss (CAL) were recorded in 6 teeth based on the method proposed by Ramfjord[20] with a manual UNC-15 periodontal probe (Medisporex Company, Sialkot, Pakistan).

A diagnosis of periodontitis was based on the presence of at least 1 site with a PD ≥ 3 mm and a CAL ≥ 2 mm.[21]

Subgingival plaque samples

Subgingival bacterial samples were obtained from the deepest pockets in the 4 quadrants. After the removal of the supragingival sample, the site was isolated from saliva using cotton rolls. The paper cones were then inserted into the periodontal pocket for 30 s.[18,22] Subsequently, they were placed in a sterile microtube containing phosphate-buffered saline (PBS) for storage at –20°C until analysis for bacterial identification.

DNA extraction

According to the manufacturer's instructions, the bacterial DNA was isolated from the samples using a G-spin™ Total DNA Extraction Mini Kit (iNtRON Biotechnology DR, Seongnam, South Korea). Briefly, 200 µL of PBS was added to the microtube containing the paper cones, vortexed for 10 s, and centrifuged at 19,000 g for 2 min. Finally, the supernatant was discarded. The protocol included treatment with RNase A and proteinase K, which were incubated at 56°C for 10 min. After isolation, the DNA was eluted in 100 µL of elution buffer. The quality, quantity and integrity of the purified DNA were analyzed using a nanospectrophotometer (NanoDrop Spectrophotometer; DNA Technologies Core, Davis, USA) and 1% agarose gel.

Polymerase chain reaction for the detection of *P. gingivalis*

This study employed real-time polymerase chain reaction (RT-PCR) for the detection of *P. gingivalis*. The DNA samples were analyzed to determine the presence of *P. gingivalis* by means of a 16S rRNA-based RT-PCR detection method.[23] The sequences of the 16S rRNA-specific primers were as follows: forward 5'-ACCTTCAACCAATTCTCCTTA-3'; and reverse 5'-GGTAATAATCGGCGTCTGA-3'. Amplifications were conducted in a final volume of 25 µL, containing 0.2 µL of Taq DNA polymerase, 1 µL of deoxynucleotide triphosphate (dNTP), 1 µL of each primer, 1 µL of template DNA, 1.7 µL of $MgCl_2$, 2.5 µL of PCR buffer, and 16.6 µL of H_2O. The PCR temperature profile was as follows: an initial denaturation at 94°C for 4 min; annealing at 60°C for 60 s; and extension at 72°C for 45 s. After 38 cycles, the PCR products underwent electrophoresis through 1% agarose gel in Tris/acetic acid–ethylenediaminetetraacetic acid (EDTA) buffer. The gel was stained with a green viewer and visualized under ultraviolet (UV) light.

Quantitative measurement of *P. gingivalis* by RT-PCR

The RT-PCR assay was performed using the PrimeScript™ RT Master Mix (Takara Bio Inc., Shiga, Japan). The specific primers were designed based on the 149bp sequence of the *16S rRNA* gene. The primer sets comprised the forward primer (5'-GGGCGATACGAGTATTGCAT-3') and the reverse primer (5'-TTCACCGCTGACTTACCG-3'). The amplification of the samples was conducted in duplicate using the ABI StepOne RT-PCR system (Applied Biosystems, Foster City, USA). A master mix without isolated DNA was used as a negative control. The absolute quantification of *P. gingivalis* was performed using the cycle threshold (Ct) of the samples and its comparison with the Ct of the standard samples.

Statistical analysis

The collected data was analyzed using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA). The χ^2 test was applied to assess the prevalence of *P. gingivalis* in grouped variables. The Mann–Whitney *U* test was used to ascertain the differences between the CAD and non-CAD groups. The mean and standard deviation (*M* ±*SD*) were employed for quantitative data. Finally, a multiple logistic regression analysis was utilized to analyze the relationship between CAD and periodontal disease. The calculations were based on a 95% *CI* and a *p*-value of less than 0.05.

Results

In this case–control study, 100 subjects (50 CAD and 50 non-CAD patients) with a mean age of 54.86 ±9.59 years were analyzed. Of these, 47 were female and 53 were male. The prevalence of CAD differed significantly between male and female patients ($p = 0.028$). Specifically, 32 males (60.4%) and 18 females (38.2%) were diagnosed with CAD.

As illustrated in Table 1, a significant difference was observed in the mean age between CAD patients (57.46 ±10.18 years) and non-CAD individuals (52.26 ±8.27 years), with a p-value of 0.006. Moreover, the mean PD was 3.30 ±1.55 mm in CAD patients and 3.56 ±0.97 mm in non-CAD individuals, indicating a significant difference between these 2 groups (p = 0.028). On the other hand, no significant difference was observed in the mean CAL between the CAD group (4.16 ±1.93 mm) and the non-CAD group (4.02 ±1.37 mm) (p = 0.858).

The prevalence of periodontitis in the CAD patients was 34%, while this proportion was 32% in the non-CAD patients, indicating that there was no significant relationship between periodontal problems and CAD (p = 0.674). The 16S rDNA of *P. gingivalis* was identified in 16 (32%) and 11 (22%) subgingival plaque samples obtained from patients with CAD and non-CAD individuals, respectively (Table 2). Moreover, 27 patients exhibited the presence of *P. gingivalis*, with a mean concentration of 7.7×10^6, as identified by RT-PCR. Therefore, no statistically significant association was observed between the prevalence of *P. gingivalis* and CAD (p = 0.260).

Table 1. Comparative analysis of age and periodontal status between patients with and without coronary artery disease (CAD)

Variable	CAD patients (n = 50) $M \pm SD$	Non-CAD patients (n = 50) $M \pm SD$	p-value
Age [years]	57.46 ±10.18	52.26 ±8.27	0.006*
PD [mm]	3.30 ±1.55	3.56 ±0.97	0.028*
CAL [mm]	4.16 ±1.93	4.02 ±1.37	0.858

PD – pocket depth; CAL – clinical attachment loss; *M* – mean; *SD* – standard deviation; * statistically significant ($p < 0.05$, Mann–Whitney *U* test).

Table 2. Comparative analysis of the prevalence of *P. gingivalis* in subgingival plaque of patients with and without coronary artery disease (CAD)

Group	*P. gingivalis*, *n* (%)	p-value
CAD patients (n = 50)	16 (32)	0.260
Non-CAD patients (n = 50)	11 (22)	

Table 3. Results of the multiple logistic regression analysis between coronary artery disease (CAD) and periodontitis

Variable	p-value	*OR* (95% *CI*)
Age	0.011*	1.067 (1.015–1.122)
Sex (male)	0.004*	4.163 (1.585–10.934)
Periodontal problems	0.137	7.089 (0.536–93.758)
PD	0.082	0.609 (0.349–1.065)
CAL	0.302	1.234 (0.828–1.840)
BoP	0.171	0.151 (0.01–2.266)
P. gingivalis	0.168	2.131 (0.727–6.248)

BoP – bleeding on probing; *OR* – odds ratio; *CI* – confidence interval; * statistically significant ($p < 0.05$).

Finally, multiple logistic regression analyses were conducted to examine the association between CAD and periodontitis, along with some potential risk factors (i.e., age and sex). There was a statistically significant association between CAD and male sex (p = 0.004, odds ratio (*OR*): 4.163), as well as age (p = 0.011, *OR*: 1.067) (Table 3).

Discussion

Cardiovascular disease is responsible for 17.9 million deaths and accounts for 45% of non-communicable disease-induced mortality worldwide.[2] Several risk factors have been identified for CVD, including smoking, dyslipidemia, altered glucose metabolism, and hypertension.[2]

In recent decades, many studies have focused on the role of chronic infections, such as periodontitis, in the pathogenesis of CVD. Cumulative evidence from several studies has supported the role of periodontitis as an independent risk factor for CVD.[1] Two meta-analyses have investigated the potential association between periodontal disease and CVD.[24,25] The studies concluded that periodontal disease is associated with an increased risk of cardiovascular events, including stroke and coronary heart disease.[1]

The purpose of this study was to determine the association between periodontitis and the prevalence of *P. gingivalis* in patients with CAD diagnosed by angiography. A significant difference in PD was observed between the groups. The mean CAL of patients with CAD was observed to be higher in comparison to non-CAD patients; however, this difference was not statistically significant. These results indicate that CAD patients exhibited poor oral and periodontal health.

In line with our report, Akbari et al. identified a significant difference only in PD between CAD and non-CAD cases.[26] They reported that individuals with CAD had poorer periodontal health than those with normal angiography.[26] Bateni et al. presented similar results, indicating that CAL and PD were elevated in the CVD group compared to the control group.[27]

Saliva plays a significant role in sustaining oral health by maintaining the integrity of dental tissues and preventing caries due to its biological functions, such as lubrication of oral tissue, antimicrobial and cleansing activities, removal of food debris and sugars, buffering capacity, control of plaque pH, remineralization of enamel with calcium and phosphates, and tissue repair. Moreover, oral hygiene is severely affected by a decrease in the salivary flow rate, dilution capacity, self-cleansing, and pH.[28] Multiple factors can affect the salivary flow rate, including aging, pharmacological agents, certain health conditions, and stress.[29] Therefore, poor periodontal status in patients with CAD may be attributed to the use of medications for the treatment of CAD.[30] The use of statins and angiotensin II receptor blockers (ARBs) has been observed to result in a higher prevalence of periodontal disease.[30]

In addition, periodontium tissue changes and immunological alterations due to the aging process contribute to the causation and perpetuation of periodontal disease.[31]

This study revealed a prevalence rate of 32% for *P. gingivalis* in CAD patients and 22% in non-CAD cases, with no statistically significant differences between these groups. Similarly, Ardakani et al. have indicated that there is no meaningful relationship between the prevalence of *P. gingivalis* in subgingival plaque and the incidence and severity of atherosclerosis in experimental groups.[18] The results demonstrated the presence of rDNA of *P. gingivalis* in 71.4% of Iranian patients diagnosed with periodontitis and atherosclerosis.[18]

In another study, the prevalence rate of *P. gingivalis* was reported at 61% in gingival sulcus plaque of patients with CAD and chronic periodontitis in the Iranian population.[32] The contradictory results can be due to differences in the severity of periodontitis, sampling methods and racial differences.

The results of our study indicate a significant association between CAD and sex and age, which aligns with the findings of Bazile et al.[33] The multiple logistic regression analysis demonstrates that age and male sex are comparable to other CAD-associated coronary risk factors. This study presents an *OR* of 1.067 (95% *CI*: 1.015–1.122, $p = 0.011$) and 4.163 (95% *CI*: 1.585–10.934, $p = 0.004$) between age and male sex, respectively, with CVD. Thus, it can be concluded that there is a close relationship between these variables.

Aging is recognized as a potential independent risk factor for CVD. Additional factors, such as obesity, diabetes and frailty, complicate and increase the cardiovascular risk factors associated with advanced age.[34] The study performed by Mosca et al. indicates that the prevalence of coronary heart disease is higher in males within every age group until after 75 years of age compared to females.[35] The estrogen hormone plays a cardioprotective role in females against coronary heart disease by regulating several metabolic factors, including lipids, the coagulation system and inflammation markers.[35]

The limitation of this study was its sample size. It is recommended that further research be conducted with a larger number of samples.

Conclusions

The results of this study indicated that there was no statistically significant association between the prevalence of *P. gingivalis* in subgingival plaque of patients with CAD compared to healthy subjects. However, the PD and CAL in the CAD patients were greater than those of the healthy participants. Consequently, it can be concluded that patients with CAD exhibit a more adverse periodontal status. It is therefore imperative that patients with CVD receive more comprehensive oral health instruction.

Ethics approval and consent to participate

All procedures were conducted in accordance with the ethical standards set forth by the Medical Ethics Committee of Mazandaran University of Medical Sciences and in alignment with the 1964 Helsinki Declaration and its subsequent amendments. The study was approved by the Medical Ethics Committee of Mazandaran University of Medical Sciences (ethics code: IR.MAZUMS.REC.1396.3183) and written informed consent was obtained from all patients.

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.

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References

1. Liccardo D, Cannavo A, Spagnuolo G, et al. Periodontal disease: A risk factor for diabetes and cardiovascular disease. *Int J Mol Sci*. 2019;20(6):1414. doi:10.3390/ijms20061414
2. Sanz M, Marco del Castillo A, Jepsen S, et al. Periodontitis and cardiovascular diseases: Consensus report. *J Clin Periodontol*. 2020;47(3):268–288. doi:10.1111/jcpe.13189.
3. Sjögren B, Bigert C, Gustavsson P. Cardiovascular disease. In: Nordberg GF, Fowler BA, Nordberg M, eds. *Handbook on the Toxicology of Metals*. 4th ed. Elsevier; 2015:313–331.
4. Zanella SM, de Souza LV, Suzigan BH, Saba-Chujfi E, Barbisan JN. The association between oral health and atherosclerotic coronary artery disease in patients submitted to coronary angiography: A controlled cross-sectional study. *Rev Bras Cardiol Invasiva*. 2012;20(2):178–183. doi:10.1016/S2214-1235(15)30049-1
5. Tezuka D, Suzuki JI, Kosuge H, et al. Deteriorated clinical outcome in coronary artery disease patients with a high prevalence of *Porphyromonas gingivalis* infection. *IJC Metabolic & Endocrine*. 2016;11:7–12. doi:10.1016/j.ijcme.2016.05.006
6. Szulc M, Kustrzycki W, Janczak D, Michalowska D, Baczynska D, Radwan-Oczko M. Presence of periodontopathic bacteria DNA in atheromatous plaques from coronary and carotid arteries. *BioMed Res Int*. 2015;2015:825397. doi:10.1155/2015/825397
7. Ghorbani P, Esfahrood ZR, Foroughi M. Paraoxonase-1, a novel link between periodontitis and ischemic heart disease: A case–control study. *Dent Med Probl*. 2023;60(1):55–59. doi:10.17219/dmp/152181
8. Guarnieri R, Reda R, Zanza A, Miccoli G, Di Nardo D, Testarelli L. Can peri-implant marginal bone loss progression and a-MMP-8 be considered indicators of the subsequent onset of peri-implantitis? A 5-year study. *Diagnostics (Basel)*. 2022;12(11):2599. doi:10.3390/diagnostics12112599

9. Furuholm J, Sorsa T, Qvarnström M, et al. Salivary matrix metalloproteinase-8 in patients with and without coronary heart disease may indicate an increased susceptibility to periodontal disease. *J Periodont Res*. 2006;41(5):486–489. doi:10.1111/j.1600-0765.2006.00900.x
10. 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
11. Dewan M, Pandit AK, Goyal L. Association of periodontitis and gingivitis with stroke: A systematic review and meta-analysis. *Dent Med Probl*. 2024;61(3):407–415. doi:10.17219/dmp/158793
12. Narendran N, Shenoy S, Kodangala S, Vamsi AR, Kamath V. Assessment of myocardial strain in hypertensive patients with periodontitis. *Dent Med Probl*. 2023;60(1):61–69. doi:10.17219/dmp/152707
13. Sudhakara P, Gupta A, Bhardwaj A, Wilson A. Oral dysbiotic communities and their implications in systemic diseases. *Dent J (Basel)*. 2018;6(2):10. doi:10.3390/dj6020010
14. Patini R, Staderini E, Lajolo C, et al. Relationship between oral microbiota and periodontal disease: A systematic review. *Eur Rev Med Pharmacol Sci*. 2018;22(18):5775–5788. doi:10.26355/eurrev_201809_15903
15. Fiorillo L, Cervino G, Laino L, et al. *Porphyromonas gingivalis*, periodontal and systemic implications: A systematic review. *Dent J (Basel)*. 2019;7(4):114. doi:10.3390/dj7040114
16. Maboudi A, Hajifathalian K, Negahban Z, et al. Correlation of *Porphyromonas gingivalis* with esophageal squamous cell carcinoma: A systematic review. *Clin Oral Investig*. 2024;29(1):1. doi:10.1007/s00784-024-06094-3
17. Holmlund A, Hedin M, Pussinen PJ, Lerner UH, Lind L. *Porphyromonas gingivalis* (Pg) a possible link between impaired oral health and acute myocardial infarction. *Int J Cardiol*. 2011;148(2):148–153. doi:10.1016/j.ijcard.2009.10.034
18. Ardakani MRT, Nejad AE, Kazemi B, et al. Prevalence of *Porphyromonas gingivalis* fimbriae A genotypes II and IV in patients with chronic periodontitis and atherosclerosis. *J Adv Periodontol Implant Dent*. 2018;10(2):50–57. doi:10.15171/japid.2018.009
19. Hyvärinen K, Mäntylä P, Buhlin K, et al. A common periodontal pathogen has an adverse association with both acute and stable coronary artery disease. *Atherosclerosis*. 2012;223(2):478–484. doi:10.1016/j.atherosclerosis.2012.05.021
20. Ramfjord SP. The periodontal disease index (PDI). *J Periodontol*. 1967;38(6):602–610. doi:10.1902/jop.1967.38.6.602
21. Maboudi A, Ahmadi A, Heidari M, et al. Association between *Porphyromonas gingivalis* bacteria in infra-gingival plaque and premature labor with low birth weight. *J Nurs Midwifery Sci*. 2022;9:237–240. doi:10.4103/jnms.jnms_93_21
22. Walker C, Sedlacek MJ. An in vitro biofilm model of subgingival plaque. *Oral Microbiol Immunol*. 2007;22(3):152–161. doi:10.1111/j.1399-302X.2007.00336.x
23. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol*. 1996;11(4):266–273. doi:10.1111/j.1399-302x.1996.tb00180.x
24. Guo X, Li X, Liao C, Feng X, He T. Periodontal disease and subsequent risk of cardiovascular outcome and all-cause mortality: A meta-analysis of prospective studies. *PLoS One*. 2023;18(9):e0290545. doi:10.1371/journal.pone.0290545
25. Larvin H, Kang J, Aggarwal VR, Pavitt S, Wu J. Risk of incident cardiovascular disease in people with periodontal disease: A systematic review and meta-analysis. *Clin Exp Dent Res*. 2021;7(1):109–122. doi:10.1002/cre2.336
26. Akbari S, Molla R, Namayandeh M, Ahmadi S, Rostaeizadeh Z, Hameiil Z. Investigating the periodontal health in patients with coronary heart diseases. *JSSU*. 2013;21(3):336–343. https://jssu.ssu.ac.ir/article-1-1902-en.html. Accessed April 1, 2023.
27. Bateni E, Rabiei A, Sabzikari N, Ghanbarzadegan A. Comparison of periodontal parameters among cardiovascular patients and healthy controls. *J Occup Health Epidemiol*. 2016;5(3):129–134. doi:10.18869/acadpub.johe.5.3.129
28. Dodds M, Roland S, Edgar M, Thornhill M. Saliva A review of its role in maintaining oral health and preventing dental disease. *BDJ Team*. 2015;2:15123. doi:10.1038/bdjteam.2015.123
29. Dawes C. Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. *J Dent Res*. 1987;66:648–653. doi:10.1177/00220345870660S107
30. Pająk-Łysek E, Polak M, Kopeć G, et al. Associations between pharmacotherapy for cardiovascular diseases and periodontitis. *Int J Environ Res Public Health*. 2021;18(2):770. doi:10.3390/ijerph18020770
31. Bhadbhade S. Aging and periodontium. *Int J Dentistry Oral Sci*. 2015;2:79–83. doi:10.19070/2377-8075-1500017
32. Talebi Ardakani M, Sobouti F, Alizadeh Ghavidel A, Gholampour Dehaki M, Shariati M, Kazemi B. Association between periodontal pathogens and coronary artery disease: A case-control study. 2011;12(2):34–40. https://journal.iha.org.ir/article_83898_9068e5f0533761ec4616af8b0446d809.pdf. Accessed April 1, 2023.
33. Bazile A, Bissada NF, Nair R, Siegel BP. Periodontal assessment of patients undergoing angioplasty for treatment of coronary artery disease. *J Periodontol*. 2002;73(6):631–636. doi:10.1902/jop.2002.73.6.631
34. Rodgers JL, Jones J, Bolleddu SI, et al. Cardiovascular risks associated with gender and aging. *J Cardiovasc Dev Dis*. 2019;6(2):19. doi:10.3390/jcdd6020019
35. Mosca L, Barrett-Connor E, Kass Wenger N. Sex/gender differences in cardiovascular disease prevention: What a difference a decade makes. *Circulation*. 2011;124(19):2145–2154. doi:10.1161/CIRCULATIONAHA.110.968792

Original paper

Evaluation of the association between periodontal disease and total cancer risk: A cross-sectional study

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Abstract

Background. The body is in a continuous state of inflammatory burden due to local and systemic inflammation, which is triggered in periodontal tissues in response to microorganisms. A number of studies have linked periodontitis to systemic diseases such as diabetes mellitus, cardiovascular disease and respiratory disease. Periodontal inflammation acts as a focus of infection, which can cause detrimental effects on distant target organs. In some cases, it may lead to tumor progression in various cancers.

Objectives. The aim of the study was to evaluate the correlation between periodontal disease (PD) and its potential role as a risk factor for the development of systemic cancer and its metastasis.

Material and methods. A single-center, cross-sectional study was conducted, including 66 patients with different systemic malignancies (group 1, the test group) and 66 healthy individuals (group 2, the control group). Group 1 was further subdivided into 2 categories: M0, comprising patients without metastases ($n = 34$); and M1, comprising patients with distant metastases ($n = 32$). The number of missing teeth, Greene and Vermilion's simplified oral hygiene index (OHI-S), probing pocket depth (PPD), Löe and Silness' bleeding on probing (BoP), clinical attachment loss (CAL), and Ramfjord's periodontal disease index (PDI) were recorded. Appropriate statistical tests were performed to analyze the data. A p-value <0.05 was considered statistically significant.

Results. The differences between the PDI, CAL and PPD values in both group 1 and group 2 were found to be statistically significant ($p = 0.000$). In M0 and M1, no statistically significant differences were observed between any of the parameters. The odds ratios (*OR*s) between group 1 and group 2 for CAL and PDI were 3.986 and 4.286, respectively. The *OR*s for M0 and M1 with regard to CAL and the mean number of teeth lost were 0.373 and 0.188, respectively.

Conclusions. The findings of the study indicate a significant association between the overall risk of cancer and PD. In cases of known systemic malignancies, no significant correlation has been identified between PD and the risk of metastasis.

Keywords: periodontitis, metastasis, attachment loss, total cancer risk, periodontal inflammation

Introduction

Cancer, or malignant neoplasm, is characterized by uncontrolled cell growth, tissue invasion and metastasis to various organs via the hematological and lymphatic systems.[1] Inflammation plays a critical role in tumor progression, enhancing cellular proliferation and mutagenesis, among others.[2] As stated in a report by Papapanou et al., "periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilm and characterized by progressive destruction of the tooth-supporting apparatus."[3] This is clinically detectable as periodontal pockets and alveolar bone loss.[3] The inflammatory response to periodontal infection extends beyond the oral cavity and leads to elevated levels of circulating inflammatory markers. Periodontal infection has been linked to various organs and systemic diseases such as cardiovascular disease, diabetes mellitus or adverse pregnancy outcomes.[4]

Chronic diseases, including type 2 diabetes, heart disease, stroke, hypertension, and cancer, can affect morbidity and quality of life of patients. While the majority of these diseases are not life-threatening, cancer is a notable exception, as it requires immediate attention. In a study by Michaud et al., it was found that after adjustment for known risk factors such as smoking and diet, patients with a history of periodontal disease (PD) had an increased risk of overall cancer compared to those without PD.[5] However, there is limited literature on the association between PD and overall cancer risk. The rationale of the study was to assess the potential role of PD as a risk factor for the development of systemic cancer and its metastasis. The aim of the present study was to evaluate the association between PD and overall cancer risk, as well as the risk of cancer metastasis in individuals with systemic malignancies. The novelty of this study lies in its evaluation of the role of periodontitis in the metastasis of cancer, which has not been extensively investigated to date.

Material and methods

This cross-sectional, case–control study has been approved by the ethics committee of Modern Dental College and Research Centre (approval No. IEC/MDCRC/2011-2012/S). The study included 132 individuals, who were divided into 2 groups. Group 1, the test group, consisted of 66 patients with known systemic malignancies, while group 2, the control group, consisted of 66 healthy individuals. Group 1 was further divided into 2 subgroups: M0 (34 patients with no distant metastases); and M1 (32 patients with distant metastases). Both the test and control groups were evaluated for PD.

The inclusion criteria were as follows: patients with systemic malignancies, including ovarian, breast, lung, rectal, cervical, and esophageal cancer; cancer patients who had not undergone more than 1 module of chemotherapy; individuals between the ages of 25 and 70; and family members of cancer patients who were from the same socioeconomic strata and had at least 14 teeth present in the oral cavity. Patients with oral cancer, those who have recently undergone tracheotomy, those who had received more than 1 module of anticancer drugs, as well as individuals with blood cancer (leukemia) and those suffering from other systemic diseases, including diabetes mellitus, hypertension, hypothyroidism, and hyperthyroidism were excluded from the study. The diagnosis of cancer and metastasis was made by experts on the basis of a histopathological report, computerized tomography scans and magnetic resonance imaging scans.

Prior to the commencement of the study, informed consent was obtained from all the patients and their demographic data was recorded. A dichotomous scoring system was employed to record the habit history, including both tobacco chewing and smoking. The periodontal examination consisted of the number of missing teeth, Greene and Vermilion's simplified oral hygiene index (OHI-S), probing pocket depth (PPD), Löe and Silness' bleeding on probing (BoP), clinical attachment loss (CAL), and Ramfjord's periodontal disease index (PDI) on 4 sites of 6 Ramfjord's teeth (14, 11, 16, 36, 31, 44).

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). The unpaired *t*-test, odds ratio (*OR*), Mann–Whitney *U* test, and multiple regression analysis were applied to obtain the results. A *p*-value of less than 0.05 was considered statistically significant.

Results

The unpaired *t*-test was used to evaluate the difference in means between the test and control groups. No statistically significant differences were observed between the test and control groups with respect to the number of teeth lost, mean age and the OHI-S. The mean PDI was 3.56 ±1.48 for the test group and 2.54 ±1.45 for the control group. The mean PPD was found to be 2.43 ±0.65 mm for the test group and 0.84 ±0.89 mm for the control group. The mean CAL was 3.40 ±2.17 mm and 1.59 ±1.60 mm for the test and control groups, respectively. The difference between these 3 parameters in both groups was found to be statistically highly significant (p = 0.000) (Table 1). The mean difference in age,

Table 1. Clinical parameters of cancer and non-cancer patients

Parameter	Test group ($n = 66$) $M \pm SD$	Control group ($n = 66$) $M \pm SD$	*p*-value
Age [years]	49.00 ±3.73	52.70 ±9.74	0.077
Teeth lost, *n*	2.33 ±4.95	2.38 ±3.39	0.951
OHI-S	3.09 ±1.60	2.78 ±1.25	0.214
PDI	3.56 ±1.48	2.54 ±1.45	0.000**
PPD [mm]	2.43 ±0.65	0.84 ±0.89	0.000**
CAL [mm]	3.40 ±2.17	1.59 ±1.60	0.000**

M – mean; *SD* – standard deviation; OHI-S – simplified oral hygiene index; PDI – periodontal disease index; PPD – probing pocket depth; CAL – clinical attachment loss; ** statistically highly significant ($p < 0.005$, unpaired *t*-test).

Table 2. Clinical parameters of patients with and without metastasis

Parameter	Metastasis patients (M1, $n = 32$) $M \pm SD$	Non-metastasis patients (M0, $n = 34$) $M \pm SD$	*p*-value (unpaired *t*-test)
Age [years]	48.97 ±14.58	49.03 ±13.11	0.986
Teeth lost, *n*	3.12 ±5.46	1.06 ±0.24	0.211
OHI-S	3.20 ±1.57	2.99 ±1.66	0.607
PDI	3.75 ±1.39	3.39 ±1.56	0.313
PPD [mm]	2.56 ±0.64	2.87 ±0.46	0.432
CAL [mm]	3.66 ±2.29	3.17 ±2.07	0.371

the number of teeth lost, as well as the OHI-S, PDI, CAL, and PPD was found to be statistically non-significant between the M0 and M1 groups (Table 2).

The χ^2 test was conducted to assess the differences in habit history (tobacco use) and sex between the test and control groups, as well as between metastasis (M1) and non-metastasis (M0) patients. The analysis revealed a statistically significant difference in sex between the test and control groups ($p = 0.035$) (Table 3).

The Mann–Whitney *U* test was applied to evaluate the mean difference between the test and control groups with respect to the BoP. The difference was found to be statistically significant ($p = 0.019$).

A multiple regression analysis was conducted to ascertain the impact of individual confounding factors. The analysis demonstrated that the total number of teeth lost, CAL, sex, and tobacco use exhibited statistically significant correlations with the incidence of cancer. The adjusted *OR* at the 95% confidence interval (*CI*) was found to be 30.947 and 27.286 for tobacco use and the CAL, respectively (Table 4). The elevated *OR* can be attributed to the smaller sample size of the study.

Table 5 illustrates the *OR*s for the CAL and PDI in group 1 and group 2, which were found to be 3.986 and 4.286, respectively. Additionally, the *OR* for the CAL and the mean number of teeth lost for the M0 and M1 groups was 0.373 and 0.188, respectively.

Table 3. Differences in habit history and sex between the study groups

Group	Habit history (tobacco use)				Sex (male)			
	present	absent	χ^2 value	*p*-value	present	absent	χ^2 value	*p*-value
Test group ($n = 66$)	29 (43.9%)	37 (56.1%)	3.674	0.055	22 (33.3%)	44 (66.7%)	4.466	0.035*
Control group ($n = 66$)	40 (60.6%)	26 (39.4%)			34 (51.5%)	32 (48.5%)		
Metastasis patients (M1, $n = 32$)	17 (53.1%)	15 (46.9%)	0.504	0.478	10 (31.2%)	22 (68.8%)	0.121	0.728
Non-metastasis patients (M0, $n = 34$)	21 (61.8%)	13 (38.2%)			12 (35.3%)	22 (64.7%)		

* statistically significant ($p < 0.05$).

Table 4. Results of the multiple regression analysis for the test and control groups

Variable	B	*SE*	Wald	*df*	*p*-value	Exp(B)/*OR*
Age	–0.089	0.046	3.783	1	0.052	0.914
Sex (male)	–5.038	1.649	9.333	1	0.002**	0.006
Habit history (tobacco use)	3.432	1.541	4.969	1	0.026*	30.947
Number of teeth lost	–0.679	0.184	13.677	1	0.000**	0.507
PPD	1.176	0.88	1.785	1	0.182	3.241
OHI-S	–0.779	0.532	2.142	1	0.143	0.459
BoP	2.176	1.168	3.466	1	0.062	8.815
PDI	–0.198	0.682	0.085	1	0.771	0.823
CAL	3.306	0.882	14.041	1	0.000**	27.286
Constant	1.182	2.186	0.291	1	0.594	3.253

B – estimated coefficient; *SE* – standard error; *df* – degrees of freedom; Exp(B) – exponential value of B; *OR* – odds ratio; BoP – bleeding on probing; * statistically significant ($p < 0.05$); ** statistically highly significant ($p < 0.005$).

Table 5. Odds ratios for the selected variables in the study groups

Parameter		*OR*	95% *CI*	
			lower	upper
CAL (test/control) *n* = 132	CAL (1/2)	3.986	1.808	8.787
	cohort cancer = 1 (≤3 mm)	2.174	1.307	3.616
	cohort cancer = 2 (>3 mm)	0.545	0.397	0.750
PDI (test/control) *n* = 132	PDI (1/2)	4.286	2.069	8.877
	cohort cancer = 1 (≤3 mm)	2.079	1.408	3.070
	cohort cancer = 2 (>3 mm)	0.485	0.331	0.710
CAL (M1/M0) *n* = 66	CAL (1/2)	0.373	0.138	1.011
	cohort metastasis = 1 (≤3 mm)	0.606	0.363	1.013
	cohort metastasis = 2 (>3 mm)	1.624	0.974	2.707
Number of teeth lost (M1/M0) *n* = 66	teeth lost (1/2)	0.188	0.036	0.964
	cohort metastasis = 1 (≤3 mm)	0.536	0.347	0.826
	cohort metastasis = 2 (>3 mm)	2.857	0.810	10.074

CI – confidence interval; 1/2 – analyzed study groups.

Discussion

The present study was conducted to establish the association between PD and cancer and to further substantiate the relationship between periodontitis and its systemic effects. The mean age and socioeconomic strata of the test and control groups were similar, minimizing the confounding effect of age, genetics, environmental factors, and socioeconomic status. The present study excluded patients with oral cancer because of the difficulty in establishing whether PD was the cause or effect of oral cancer, given the poor and compromised oral hygiene often observed in these patients. The inclusion of oral cancer patients could have led to a biased approach and affected the overall results in a significant manner, potentially leading to a type I error.

The effect of tobacco was nullified in both the test and control groups, as evidenced by a non-significant difference. An earlier study had estimated that the risk of lung cancer increases by 20-fold in smokers compared to non-smokers.[6] In our study, all types of systemic cancers were included, suggesting that concerns about residual confounding by smoking may not be applicable to all cancers.

The present study revealed that the mean number of teeth lost was statistically similar in both cancer and non-cancer patients. Tu et al. reported no significant increase in the risk of cancer-related mortality with increased number of missing teeth.[7] They considered the association between lung cancer and tooth loss to be evidence of residual confounding due to smoking.[7] Cabrera et al. also found no significant association between cancer-related mortality and an increased number of missing teeth.[8] Michaud et al. did not find any association between esophageal cancer and PD, which was verified by radiographic bone loss or missing teeth.[5] This is in contrast to the study conducted by Abnet et al., who reported a stronger association between tooth loss and upper gastrointestinal (GI) cancer, which might be attributed to changes in dietary habits.[9] They also reported age as an effect modifier, with the greatest increase in risk observed among participants under 50 years of age.[9] Watabe et al. observed a significant dose–response relationship between the odds of developing gastric cancer and the number of teeth lost.[10] In a large-scale case–control study including 5,240 cancer patients, the association between tooth loss and 14 common cancers was evaluated. After adjusting for potential confounding factors, a significant positive association was observed between tooth loss and an increased risk of head and neck, esophageal, and lung cancers.[11] It was therefore proposed that the preservation of teeth may decrease the risk of these cancers.[11] Shi et al. performed a dose–response meta-analysis, including 25 studies, to clarify and quantify the correlation between tooth loss and the risk of cancer.[12] They concluded that with each additional 10 teeth lost, there was a 9% increment in cancer risk. They also estimated a 3–31% increase in the risk of various cancers, including head and neck, esophageal, gastric, colorectal, pancreatic, lung, hematopoietic and bladder cancers, with tooth loss in a dose–response manner.[12] The dissimilarities in these results can be attributed to the variation in the consideration of the cause of tooth loss in the abovementioned studies. In most of the aforementioned studies, the reasons for tooth loss were not taken into consideration and were largely self-reported. Similarly, in our study, we had to rely on the information provided by patients regarding the cause of tooth loss.

In the present study, no statistically significant difference was identified in the OHI-S between the test group and the control group. This study included patients who had not undergone more than 1 module of chemotherapy because, in such patients, the effect of chemotherapeutic drugs on periodontal health, quality of life and dexterity might not have been considered. Additionally, factors with a substantial impact on oral hygiene, such as age, socioeconomic background and tobacco history, were comparable between the groups. In another study, a significant correlation was demonstrated between an increased risk of esophageal cancer and poor oral hygiene, as well as an increased number of tooth loss (in patients controlled for smoking).[13]

Demirer et al. found that patients with stomach cancer brushed their teeth less often and had a higher prevalence of deficient teeth compared to controls.[14]

Statistically significant differences were identified between cancer patients and healthy subjects with respect to the PDI, PPD and CAL. Two studies evaluated periodontal health using Russell's periodontal index and self-reported history of PD, which was validated by radiographic examination.[5,15] Both studies found an increased risk of overall cancer-related mortality in patients with periodontitis.[5,15] In the present study, the *OR* was 4.286 for PDI, whereas in other studies, the *OR* was reported to be 1.14[5] and 1.55.[15]

In a retrospective study by Chung and Chan, the evidence of an association between all-cause, all-cancer and specific-cancer mortality related to lung and prostate cancer and PD was inconclusive.[16] Ding et al. conducted an inverse-variance weighted Mendelian randomization analysis and did not establish any significant causal relationship between breast cancer and periodontitis.[17] Meurman et al. performed a 30-year follow-up for the investigation into the association between periodontal inflammation (PI), including gingivitis and periodontitis, and all types of malignancies.[18] The authors reported that the probability of developing cancer increased by approx. 38% in periodontitis patients, whereas no association was identified in those with gingivitis.[18] Cheng et al. proposed that interleukin (IL)-1β plays a role in breast cancer and its metastatic progression.[19] It was hypothesized that PI promotes metastasis of breast cancer by recruiting myeloid-derived suppressor cells (MDSCs). Pyroptosis-induced IL-1β generation was detected in patients with PI, which resulted in the downstream signaling of monocyte chemoattractant protein-1 (CCL2), chemokine ligand 5 (CCL5) and C-X-C motif chemokine 5 (CXCL5). This caused the formation of pre-metastatic niches within the inflammatory site during the initial stages of metastasis. The authors thus emphasized the necessity of controlling PI.[19] Hwang et al. evaluated the impact of PD treatment in a cohort of 38,902 patients and subsequent cancer risk.[20] They concluded that PD treatment resulted in a significant reduction in overall cancer risk, with the greatest impact observed in patients with GI, lung, gynecological, and brain malignancies.[20] As the present study is a non-interventional study, the effects of periodontal therapy on inflammation, cancer and metastasis could not be assessed.

A meta-analysis conducted by Verma et al., which included 194,850 participants from observational studies, concluded that there is an increased risk of lung cancer occurrence in patients with chronic periodontitis, with a risk ratio of 1.41 for lung neoplasm.[21] Similar findings were reported by Kesharani et al. in a pooled analysis of 12 studies, after controlling for age and smoking.[22] The researchers observed that the incidence of lung cancer was twice as high in PD patients and proposed that periodontitis may be a potential risk factor for the development of lung cancer.[22] A meta-analysis by Ma et al. revealed a potential link between periodontitis and various cancers, including esophageal, prostate, hematological, and melanoma of the skin.[23] However, a recent meta-analysis by Corbella et al. did not establish any considerable correlation between periodontitis and cancer.[24]

A comparative analysis of cancer patients with and without metastasis revealed no statistically significant differences in any of the examined parameters. These findings can be attributed to the small sample size (M0 = 34, M1 = 32). Patients with cancer and a CAL of more than 3 mm exhibited a 1.624-fold increased risk of developing metastasis, while those with a greater number of missing teeth demonstrated a 2.857-fold elevated risk of metastasis. To the best of our knowledge, no studies have compared the periodontal status of metastatic and non-metastatic groups.

A number of potential mechanisms may account for the observed correlation between PD and systemic cancer (Fig. 1).

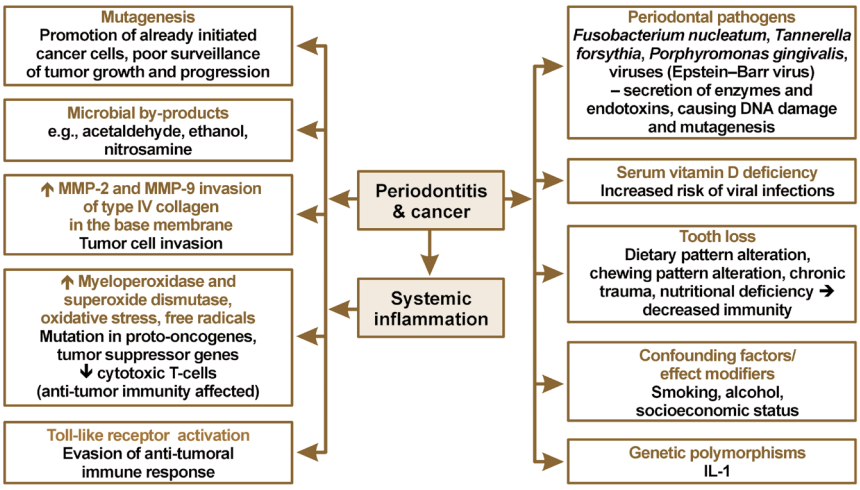

Fig. 1. Proposed pathogenic mechanisms explaining the correlation between periodontal disease and systemic cancer
MMP – matrix metalloproteinase; IL – interleukin.

Inflammation plays an important role in the pathophysiology of both PD and cancer. Chronic inflammation induced by periodontal pathogens may serve to promote and initiate cells, leading to a breakdown of normal growth and the potential promotion of carcinogenesis.[25] Chronic inflammation may also prevent apoptosis.[26] The following are the possible mechanisms explaining the correlation between PD and cancer:

– chronic inflammation may be a sign of poor surveillance of tumor growth by the body.[27] Periodontitis may act as a marker of immune dysfunction, which has implications for tumor growth and progression;
– formation of endogenous nitrosamine by the nitrate-producing bacteria is promoted by inadequate oral hygiene, PD, tobacco, and dietary factors.[28–30] Tooth loss resulting from poor oral hygiene may contribute to greater nitrosamine production[31];
– increased production of ethanol by-products, such as acetaldehyde, by oral microbes[32,33];
– matrix metalloproteinase (MMP)-2 and MMP-9 cleave type IV collagen of epithelium and vascular basement membranes. There is compelling evidence to support the role of MMPs that degrade type IV collagen in tumor cell invasion.[34] It has been demonstrated that MMP-9 levels are elevated in periodontitis subjects[35];
– inflammation may enhance cellular proliferation, mutagenesis, reduce adaptation to oxidative stress, promote angiogenesis, inhibit apoptosis, and increase secretion of inflammatory mediators.[36] All these factors may promote carcinogenesis;
– the enzymatic activity of antioxidant enzymes is reduced in periodontitis.[37] Myeloperoxidase and superoxide dismutase help to regulate inflammation and have been observed to be elevated in periodontitis. A polymorphism in the genes encoding these enzymes has been found to be associated with an increased risk of pancreatic cancer.[38] In a study, the level of glutathione peroxidase 1 (GPX1) transcript levels increased with the severity of PD. Its activity in the salivary and gingival crevicular fluid (GCF) proteome is affected by oxidative stress related to the inflammatory changes observed in PD[37];
– the inflammatory processes generate free radicals and active intermediates, which cause oxidative or nitrosative stress. This may lead to DNA mutations in cells or interfere with DNA repair mechanisms[39];
– serum 25-hydroxyvitamin D has been demonstrated to reduce the risk of PD and tooth loss with cancer by inducing human cathelicidin and LL-37. This reduces the risk of several cancers by lowering the risk of viral infections, such as the Epstein–Barr virus.[40]

The vast majority of cancers are the result of abnormalities in the genetic material of the transformed cells. These abnormalities can be caused by carcinogens such as tobacco, radiation, chemicals, or infectious agents.[25] As viruses are implicated in the etiology of PD, this can indirectly link to the etiology of cancer.[41] The enzymes, metabolic by-products and endotoxins secreted by oral bacteria are toxic to human tissues, causing direct DNA damage to neighboring epithelial cells. They can induce mutations in proto-oncogenes and tumor suppressor genes or interfere with the molecular pathways of cell proliferation or survival.[42]

Confounding represents a bias that the investigator hopes to prevent or remove from the effect estimate. In contrast, effect modification is a property of the effect under study. Tobacco and other risk factors, such as alcohol intake, age and socioeconomic factors, can act as confounding factors or effect modifiers.[9,25] The possible association between PD and lung cancer may be confounded. Periodontitis serves as a surrogate for the unmeasured aspects of smoking and mimics the effects of smoking.[25] Thus, effect modification is a finding that should be reported, rather than a bias to be avoided.[43]

Various mechanisms may explain the relationship between tooth loss and the causation of cancer:

– chronic trauma and irritation of the oral mucosa may play a role in carcinogenesis[10];
– tooth loss is a marker of poor general health and a risk factor[44];
– loss of teeth leads to alterations in dietary patterns, which can elevate the risk of cancer[44];
– tooth loss reduces chewing and affects swallowing, which can contribute to esophageal cancer by causing irritation or damage to the esophageal epithelium[44];
– tooth loss can increase the risk of upper GI cancer through alterations in oral bacterial flora, leading to increased exposure and in vivo production of nitrosamines.[44]

Increased consumption of fruits, vegetables and vitamin C may reduce the risk of oral and gastric cancers. A reduction in fruit and vegetable intake may result from tooth loss.[45]

Genetic factors may increase susceptibility to PD and cancer or may modify the relationship between environmental factors. Genetic polymorphism in IL-1 has been associated with an increased risk of gastric cancer and PD.[46,47] Indirectly, this association may strengthen or facilitate the link between PD and cancer.

Toll-like receptor-5 (TLR-5) is activated by oral bacteria, which results in the evasion of an anti-tumor immune response.[48]

Periodontal pathogens (*Fusobacterium nucleatum*, *Tannerella forsythia*, *Porphyromonas gingivalis*) have been isolated from various cancer lesions. These pathogens modulate the anti-tumor myeloid cell immune system, attract tumor-infiltrating myeloid cells, and create a pro-inflammatory milieu that promotes carcinogenesis.[48]

The epithelial–mesenchymal transition (EMT) barrier can be breached by EMT-predisposing factors like

cytokines and bacterial products. A correlation has been observed between the severity of periodontitis and the expression of the EMT process markers (transforming growth factor-beta 1 (TGF-β1) and vimentin).[49] This suggests that EMT plays an important role in inflammation and cancer metastasis.

Limitations

A key limitation of the current study is its focus on the overall risk of cancer, rather than on specific cancer types. Though nearly all the patients belonged to a lower socioeconomic background, it was not possible to control for other confounding factors, such as malnutrition. Tobacco chewing and smoking were grouped together as habit history, precluding the assessment of the separate outcomes of each behavior.

Conclusions

Although it is not possible to establish a cause-and-effect relationship between PD and the occurrence and metastasis of systemic malignancies using a cross-sectional study design, the findings of this study indicate a significant association between total cancer risk and PD. This study also demonstrates a two-way relationship between PD and the overall risk of cancer. However, no significant association has been identified between PD and the risk of metastasis in known systemic malignancies. Therefore, long-term longitudinal studies with a larger sample size are necessary to establish PD as a risk factor for the occurrence and spread of cancers.

Ethics approval and consent to participate

The study was approved by the ethics committee of Modern Dental College and Research Centre (approval No. IEC/MDCRC/2011-2012/S)

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.

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References

1. Cotran RS, Kumar V, Collins T. *Robbins Pathologic Basis of Disease*. 6th ed. Philadelphia, PA: WB Saunders Company; 1999:260–327.
2. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420(6917):860–867. doi:10.1038/nature01322
3. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89 Suppl 1:S173–S182. doi:10.1002/JPER.17-0721
4. Martínez-García M, Hernández-Lemus E. Periodontal inflammation and systemic diseases: An overview. *Front Physiol*. 2021;12:709438. doi:10.3389/fphys.2021.709438
5. Michaud DS, Liu Y, Meyer M, Giovannucci E, Joshipura K. Periodontal disease, tooth loss, and cancer risk in male health professionals: A prospective cohort study. *Lancet Oncol*. 2008;9(6):550–558. doi:10.1016/S1470-2045(08)70106-2
6. Alberg AJ, Samet JM. Epidemiology of lung cancer. *Chest*. 2003;123(Suppl 1):21S–49S. doi:10.1378/chest.123.1_suppl.21s
7. Tu YK, Galobardes B, Smith GD, McCarron P, Jeffreys M, Gilthorpe MS. Associations between tooth loss and mortality patterns in the Glasgow Alumni Cohort. *Heart*. 2007;93(9):1098–1103. doi:10.1136/hrt.2006.097410
8. Cabrera C, Hakeberg M, Ahlqwist M, et al. Can the relation between tooth loss and chronic disease be explained by socio-economic status? A 24-year follow-up from the population study of women in Gothenburg, Sweden. *Eur J Epidemiol*. 2005;20(3):229–236. doi:10.1007/s10654-004-5961-5
9. Abnet CC, Qiao YL, Dawsey SM, Dong ZW, Taylor PR, Mark SD. Tooth loss is associated with increased risk of total death and death from upper gastrointestinal cancer, heart disease, and stroke in a Chinese population-based cohort. *Int J Epidemiol*. 2005;34(2):467–474. doi:10.1093/ije/dyh375
10. Watabe K, Nishi M, Miyake H, Hirata K. Lifestyle and gastric cancer: A case-control study. *Oncol Rep*. 1998;5(5):1191–1194. doi:10.3892/or.5.5.1191
11. Hiraki A, Matsuo K, Suzuki T, Kawase T, Tajima K. Teeth loss and risk of cancer at 14 common sites in Japanese. *Cancer Epidemiol Biomarkers Prev*. 2008;17(5):1222–1227. doi:10.1158/1055-9965.EPI-07-2761
12. Shi J, Leng W, Zhao L, et al. Tooth loss and cancer risk: A dose-response meta analysis of prospective cohort studies. *Oncotarget*. 2017;9(19):15090–15100. doi:10.18632/oncotarget.23850
13. Abnet CC, Kamangar F, Islami F, et al. Tooth loss and lack of regular oral hygiene are associated with higher risk of esophageal squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*. 2008;17(11):3062–3068. doi:10.1158/1055-9965.EPI-08-0558
14. Demirer T, Icli F, Uzunalimoglu O, Kucuk O. Diet and stomach cancer incidence. A case-control study in Turkey. *Cancer*. 1990;65(10):2344–2348. doi:10.1002/1097-0142(19900515)65:10<2344::aid-cncr2820651030>3.0.co;2-h
15. Hujoel PP, Drangsholt M, Spiekerman C, Weiss NS. An exploration of the periodontitis-cancer association. *Ann Epidemiol*. 2003;13(5):312–316. doi:10.1016/s1047-2797(02)00425-8
16. Chung PC, Chan TC. Association between periodontitis and all-cause and cancer mortality: Retrospective elderly community cohort study. *BMC Oral Health*. 2020;20(1):168. doi:10.1186/s12903-020-01156-w
17. Ding M, Zhang Z, Chen Z, Song J, Wang B, Jin F. Association between periodontitis and breast cancer: Two-sample Mendelian randomization study. *Clin Oral Investig*. 2023;27(6):2843–2849. doi:10.1007/s00784-023-04874-x
18. Meurman JH, Källmén H, Andersson LC, Yucel-Lindberg T, Söder B. Prevalence of cancer in relation to signs of periodontal inflammation. *PLoS One*. 2022;17(10):e0276375. doi:10.1371/journal.pone.0276375
19. Cheng R, Billet S, Liu C, et al. Periodontal inflammation recruits distant metastatic breast cancer cells by increasing myeloid-derived suppressor cells. *Oncogene*. 2020;39(7):1543–1556. doi:10.1038/s41388-019-1084-z
20. Hwang IM, Sun LM, Lin CL, Lee CF, Kao CH. Periodontal disease with treatment reduces subsequent cancer risks. *QJM*. 2014;107(10):805–812. doi:10.1093/qjmed/hcu078

21. Verma UP, Singh P, Verma AK. Correlation between chronic periodontitis and lung cancer: A systematic review with meta-analysis. *Cureus*. 2023;15(3):e36476. doi:10.7759/cureus.36476
22. Kesharani P, Kansara P, Kansara T, et al. Is periodontitis a risk factor for lung cancer? A meta-analysis and detailed review of mechanisms of association. *Contemp Clin Dent*. 2022;13(4):297–306. doi:10.4103/ccd.ccd_117_22
23. Ma H, Zheng J, Li X. Potential risk of certain cancers among patients with periodontitis: A supplementary meta-analysis of a large-scale population. *Int J Med Sci*. 2020;17(16):2531–2543. doi:10.7150/ijms.46812
24. Corbella S, Veronesi P, Galimberti V, Weinstein R, Del Fabbro M, Francetti L. Is periodontitis a risk indicator for cancer? A meta-analysis. *PLoS One*. 2018;13(4):e0195683. doi:10.1371/journal.pone.0195683
25. Yao SG, Fine JB. Periodontitis and cancer...a link? A review of the recent literature. *Compend Contin Educ Dent*. 2010;31(6):436–444. PMID:20712107.
26. Michaud DS, Joshipura K, Giovannucci E, Fuchs CS. A prospective study of periodontal disease and pancreatic cancer in US male health professionals. *J Natl Cancer Inst*. 2007;99(2):171–175. doi:10.1093/jnci/djk021
27. Meyer MS, Joshipura K, Giovannucci E, Michaud DS. A review of the relationship between tooth loss, periodontal disease, and cancer. *Cancer Causes Control*. 2008;19(9):895–907. doi:10.1007/s10552-008-9163-4
28. Nair J, Ohshima H, Nair UJ, Bartsch H. Endogenous formation of nitrosamines and oxidative DNA-damaging agents in tobacco users. *Crit Rev Toxicol*. 1996;26(2):149–161. doi:10.3109/10408449609017928
29. Shapiro KB, Hotchkiss JH, Roe DA. Quantitative relationship between oral nitrate-reducing activity and the endogenous formation of N-nitrosoamino acids in humans. *Food Chem Toxicol*. 1991;29(11):751–755. doi:10.1016/0278-6915(91)90183-8
30. Risch HA. Etiology of pancreatic cancer, with a hypothesis concerning the role of N-nitroso compounds and excess gastric acidity. *J Natl Cancer Inst*. 2003;95(13):948–960. doi:10.1093/jnci/95.13.948
31. Abnet CC, Kamangar F, Dawsey SM, et al. Tooth loss is associated with increased risk of gastric non-cardia adenocarcinoma in a cohort of Finnish smokers. *Scand J Gastroenterol*. 2005;40(6):681–687. doi:10.1080/00365520510015430
32. Salaspuro MP. Acetaldehyde, microbes, and cancer of the digestive tract. *Crit Rev Clin Lab Sci*. 2003;40(2):183–208. doi:10.1080/713609333
33. Homann N, Tillonen J, Rintamäki H, Salaspuro M, Lindqvist C, Meurman JH. Poor dental status increases acetaldehyde production from ethanol in saliva: A possible link to increased oral cancer risk among heavy drinkers. *Oral Oncol*. 2001;37(2):153–158. doi:10.1016/s1368-8375(00)00076-2
34. Zeng ZS, Cohen AM, Guillem JG. Loss of basement membrane type IV collagen is associated with increased expression of metalloproteinases 2 and 9 (MMP-2 and MMP-9) during human colorectal tumorigenesis. *Carcinogenesis*. 1999;20(5):749–755. doi:10.1093/carcin/20.5.749
35. Luchian I, Goriuc A, Sandu D, Covasa M. The role of matrix metalloproteinases (MMP-8, MMP-9, MMP-13) in periodontal and peri-implant pathological processes. *Int J Mol Sci*. 2022;23(3):1806. doi:10.3390/ijms23031806
36. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? *Free Radic Biol Med*. 2010;49(11):1603–1616. doi:10.1016/j.freeradbiomed.2010.09.006
37. Toczewska J, Baczyńska D, Zalewska A, Maciejczyk M, Konopka T. The mRNA expression of genes encoding selected antioxidant enzymes and thioredoxin, and the concentrations of their protein products in gingival crevicular fluid and saliva during periodontitis. *Dent Med Probl*. 2023;60(2):255–265. doi:10.17219/dmp/150888
38. Wheatley-Price P, Asomaning K, Reid A, et al. Myeloperoxidase and superoxide dismutase polymorphisms are associated with an increased risk of developing pancreatic adenocarcinoma. *Cancer*. 2008;112(5):1037–1042. doi:10.1002/cncr.23267
39. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. *Int J Cancer*. 2007;121(11):2381–2386. doi:10.1002/ijc.23192
40. Grant WB. Vitamin D, periodontal disease, tooth loss, and cancer risk. *Lancet Oncol*. 2008;9(7):612–613. doi:10.1016/S1470-2045(08)70164-5
41. Rajendran P, Anand M. A comprehensive review on role of viruses in gingival and periodontal diseases. *J Datta Meghe Inst Med Sci Univ*. 2022;17:479–486. doi:10.4103/jdmimsu.jdmimsu_309_20
42. Anil S, Varma SV, Preethanath RS, Anand PS, Farraj Aldosari AA. The emerging concepts on the impact of periodontitis on systemic health [Internet]. In: Manakil J, ed. *Periodontal Diseases – A Clinician's Guide*. IntechOpen; 2012:131–164. doi:10.5772/26457
43. Hyman JJ, Reid BC. Cigarette smoking, periodontal disease: And chronic obstructive pulmonary disease. *J Periodontol*. 2004;75(1):9–15. doi:10.1902/jop.2004.75.1.9
44. Abnet CC, Qiao YL, Mark SD, Dong ZW, Taylor PR, Dawsey SM. Prospective study of tooth loss and incident esophageal and gastric cancers in China. *Cancer Causes Control*. 2001;12(9):847–854. doi:10.1023/a:1012290009545
45. Hung HC, Colditz G, Joshipura KJ. The association between tooth loss and the self-reported intake of selected CVD-related nutrients and foods among US women. *Community Dent Oral Epidemiol*. 2005;33(3):167–173. doi:10.1111/j.1600-0528.2005.00200.x
46. El-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*. 2000;404(6776):398–402. doi:10.1038/35006081
47. Kornman KS, di Giovine FS. Genetic variations in cytokine expression: A risk factor for severity of adult periodontitis. *Ann Periodontol*. 1998;3(1):327–338. doi:10.1902/annals.1998.3.1.327
48. Nwizu N, Wactawski-Wende J, Genco RJ. Periodontal disease and cancer: Epidemiologic studies and possible mechanisms. *Periodontol 2000*. 2020;83(1):213–233. doi:10.1111/prd.12329
49. Wadie KW, Bashir MH, Abbass MMS. Epithelial–mesenchymal transition in gingival tissues from chronic periodontitis patients: A case–control study. *Dent Med Probl*. 2021;58(3):311–319. doi:10.17219/dmp/133514

Original paper

Is malocclusion a predictor of pain in patients suffering from TMD pain?

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Abstract

Background. Temporomandibular disorders (TMD) affect the masticatory muscles, temporomandibular joints (TMJs) and associated structures. The relationship between occlusion and TMD is a contentious issue in the dental field.

Objectives. Although there is a strong argument against invasive and irreversible therapeutic TMD procedures, the TMD biopsychosocial model is still not accepted by some clinicians. Hence, this study aimed to verify whether malocclusions are related to TMD pain.

Material and methods. The study included 49 adult patients with one or multiple TMD diagnoses and without any other diseases that could mimic TMD. A reliable investigator diagnosed the patients using the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) protocol. The sample was divided into pain and non-pain TMD groups, and the predictor of malocclusion was categorized as the dependent variable. There were 33 patients in the TMD pain group and 16 patients in the non-pain TMD group. Analyses were conducted at a significance level of 0.05. The χ^2 test (with Yates' correction for 2×2 matrix) was used to compare qualitative variables between the groups.

Results. Malocclusion was present in 13 patients in the pain group and 7 patients in the non-pain group.

Conclusions. According to our study, there is no correlation between malocclusion and TMD pain.

Keywords: pain, TMD, TMJ, RDC/TMD, malocclusion

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Introduction

Temporomandibular disorders (TMD) affect the masticatory muscles, temporomandibular joints (TMJs) and associated structures.[1] Patients with TMD commonly report fluctuating and dull pain in the masticatory muscles and the temporomandibular region. Other TMD symptoms include limitations in the mandibular range of motion and TMJ sounds such as clicking and crepitation during jaw function, which may cause discomfort on a daily basis. However, the etiology, diagnostics and treatment of TMD remain unclear.

The relationship between occlusion and TMD is a contentious topic in dentistry, often leading to controversy.[2] In 1934, the otolaryngologist James Costen diagnosed 11 patients and concluded that missing teeth or malocclusion could cause hearing loss, blocked ears, tinnitus, sinus problems, dizziness, TMD, and headaches.[3] In the following decades, patients underwent irreversible and invasive dental, orthodontic, and sometimes even surgical procedures.[4–7] Evidence-based medicine is required to link malocclusion and TMD with scientific research, given the invasive nature of such therapies. This approach has correctly explained the correlation between these 2 disorders. Recent literature does not support the prominent role of malocclusion as a primary risk factor in the onset of TMD symptoms. The role of occlusion as a risk factor for TMD remains controversial in the field of dentistry.[8–14]

The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) and Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), an improved and updated version of the former test representing the current reference for standardizing TMD diagnoses for research purposes, have been used to assess diagnosis frequency.[15,16] Axis I disease has been reported as the most common within the muscles in both European and non-European countries. Research conducted among non-patient and patient populations in Poland confirmed that myofascial pain was the most frequent diagnosis.[17,18]

According to Manfredini et al., the epidemiology of different TMD should be studied separately due to specific risk factors and age-related features identified in research samples.[19] It is a shortcoming in the literature that most risk assessment studies on TMD are based on non-patient populations, while studies on TMD patient populations focus on specific TMD subpopulations. Additional studies conducted by Schiffman et al. and Osiewicz et al. have shown that the primary Axis I diagnoses of the RDC/TMD can be distinguished based on the presence or absence of pain, resulting in 2 distinct groups. However, malocclusion was not considered a risk factor.[16,20]

Although there is a strong body of evidence against invasive and irreversible therapeutic TMD procedures, the biopsychosocial model of TMD is still not accepted by some clinicians. Therefore, this study aimed to verify whether malocclusions are related to TMD pain.

Material and methods

Participants

The study included 49 adult patients treated at the University Dental Clinic of Jagiellonian University in Krakow, Poland. The study period was 6 months and the inclusion criteria were:

1. the presence of one or multiple TMD, diagnosed according to the RDC/TMD;
2. the absence of symptoms of systemic or local diseases that may mimic TMD (e.g., fibromyalgia, hypothyroidism, lupus erythematosus, scleroderma, Parkinson's disease, Lyme disease, and dystonia). When in doubt, patients consulted a specialist who was authorized to exclude the patient from the study;
3. the absence of other orofacial disorders that may mimic TMD-like signs and/or symptoms, as determined by the patient's previous treatment history and relevant consultant's report. These symptoms included, but were not limited to, neuropathic pain, tension-type headaches, autonomic cephalalgias, migraines, psychogenic pain, myositis, infections, or any injuries.

All patients signed a consent form. The study adhered to the principles of the Helsinki Declaration and was approved by the Bioethics Committee of Jagiellonian University (approval No. KBET/90/B/2010).

Study design

This cross-sectional study involved examination of all patients by a reliable investigator who had undertaken specific training on RDC/TMD evaluation from a gold-standard examiner within the framework of a three-year specialty program in TMD and Orofacial Pain, and was rated as excellent.[21] All participants underwent a thorough assessment in accordance with the RDC/TMD guidelines to receive both Axis I and Axis II diagnoses based on the Polish official adaptation of the RDC/TMD.[16,22]

Axis I assessment involves a questionnaire and a clinical examination that can assign an individual to one or more of the 3 diagnosis groups. Group I comprises 2 muscle disorder subgroups: myofascial pain (IA); and myofascial pain with limited opening (IB). The only difference between IB and IA is that IB exhibits limited movement and stiffness of the muscle during stretching in the presence of myofascial pain. Group II disc displacements consist of disc displacement with reduction (IIA), disc displacement without reduction or limited mouth opening (IIB), and disc displacement without reduction or limited opening (IIC). Group III was represented by 3 subgroups: arthralgia with pain and tenderness in the joint capsule and/or the synovial lining of the TMJ (IIIA); osteoarthritis with pain and tenderness in the joint capsule and/or the synovial lining of the TMJ and the appearance of coarse crepitus in the TMJ and/or tomograms showing pathology in the TMJ

(IIIB); and osteoarthrosis with no pain but coarse crepitus in the TMJ and/or tomograms showing pathology in the TMJ (IIIC). All patients received a diagnosis based on the RDC/TMD and a malocclusion diagnosis.

The clinical research classified a malocclusion when the diagnosis was far from the correct class based on Angle's classification, which refers to the posterior-anterior jaw position. An anterior crossbite was diagnosed when 2 or more mandibular front teeth overlapped the maxillary teeth. A posterior crossbite was diagnosed when 2 or more back teeth overlapped the mandibular teeth. An open bite was registered when overbite was negative, and a deep bite was noted when the top front teeth covered more than half the length of the bottom front crown. Finally, an overjet was registered when the distance between the lip surface of the top foretooth was more than 4 mm larger than the lip surface of the bottom foretooth. All of the above cases were classified as malocclusion.[23]

Statistical analysis

The study sample consisted of 2 groups based on the RDC/TMD diagnosis: a pain TMD group; and a non-pain TMD group. The predictor of malocclusion was identified as the dependent variable. Analyses were conducted at a significance level of 0.05, and the χ^2 test (with Yates' correction for 2 × 2 matrix) was used to compare qualitative variables in both groups. All analyses employed R v. 3.6.1 software (https://cran-archive.r-project.org/bin/windows/base/old/3.6.1).

Results

Data analysis was performed on 49 patients (81.7% females, mean age: 33.2 ±14.7 years, min = 18, max = 72, range = 54). The study sample consisted of a pain TMD group, which included patients with myofascial pain/myofascial pain with limited opening (IA/IB) and arthralgia/osteoarthritis (IIIA/IIIB), and a non-pain TMD group including patients with disc displacement with reduction (IIA), disc displacement without reduction and with limited opening/disc displacement without reduction or limited opening (IIB/IIC), and osteoarthritis (IIIC). The study included 33 patients in the pain TMD group and 16 patients in the non-pain TMD group. The number of patients diagnosed with malocclusion in the pain TMD group was 13, and 7 patients were diagnosed in the non-pain TMD group (Table 1). Malocclusion had no significant effect on pain in both groups ($p > 0.05$).

Table 1. Frequency of malocclusion in TMD groups with and without pain

Study group	Malocclusion ($n = 20$)	No malocclusion ($n = 29$)	p-value (χ^2 test)
Pain TMD	13 (65.00)	20 (68.97)	1.000
Non-pain TMD	7 (35.00)	9 (31.03)	

Data presented as frequency (percentage) (n (%)). TMD – temporomandibular disorders.

Discussion

Based on the results of the present study, the null hypothesis stating that malocclusion is related to painful TMD was rejected. The association between ear symptoms, occlusion and TMD has been hypothesized since the early theories of Costen. Malocclusion is a prevalent oral disorder worldwide.[24] However, according to the multiple regression analysis of 11 common occlusal features conducted by Pullinger et al., occlusion cannot be considered the unique or dominant factor in defining TMD populations.[25] A review by Manfredini clearly indicated that, based on the available literature, the concept of equilibrating the occlusion to treat and/or prevent TMD should not be used. Also, further routine appraisal of biological rationale is not recommended.[26] A study by Amer et al. investigated the dental and skeletal aspects of malocclusion in the antero-posterior and vertical dimensions in a population of TMD patients and found no association between TMD and malocclusion.[27] Similarly, there was no correlation between transverse malocclusion and TMD signs and symptoms.[28] Moreover, Manfredini et al. found no significant association between dental Angle class asymmetry and TMD, despite Angle class being one of the most frequently used factors to determine malocclusion.[29] Our findings suggest that malocclusion does not directly cause TMD pain.

Many disease-related variables across multiple domains (i.e., biological, psychosocial and pain processing) impact TMD pain, making TMD a complex disease.[30] The current study supports the so-called biopsychosocial model of pain, indicating that malocclusion cannot be considered the sole risk factor. Therefore, the evaluation of both Axis I and Axis II is necessary.

Due to the small sample size, we recommend adopting the updated DC/TMD to a larger and more diverse patient population, including individuals from different countries and cultures. Such an approach would enable the potential application of the study findings in cross-cultural comparisons. Another limitation concerns the diagnosis of malocclusion, which should have been more specifically assessed.

Conclusions

The investigation rejected the null hypothesis that malocclusion is related to painful TMD and suggested that the contribution of occlusion to TMD pain is negligible. Furthermore, the study supports a much-diminished role of peripheral anatomical-structural factors in the pathogenesis of TMD pain.

Ethics approval and consent to participate

The study adhered to the principles of the Helsinki Declaration and was approved by the Bioethics Committee of Jagiellonian University (approval No. KBET/90/B/2010). All patients signed a consent form.

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.

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References

1. De Leeuw R, Klasser GD, eds. Differential diagnosis and management of TMDs. In: *Orofacial Pain: Guidelines for Assessment, Diagnosis, and Management (American Academy of Orofacial Pain)*. 6th ed. Hanover Park, IL: Quintessence Publishing; 2018:143–207.
2. Türp JC, Greene CS, Strub JR. Dental occlusion: A critical reflection on past, present and future concepts. *J Oral Rehabil*. 2008;35(6):446–456. doi:10.1111/J.0305-182X.2007.01820.X
3. Costen JB. I. A syndrome of ear and sinus symptoms dependent upon disturbed function of the temporomandibular joint. *Ann Otol Rhinol Laryngol*. 1934;43(1):1–15. doi:10.1177/000348943404300101
4. Schwartz LL. Pain associated with the temporomandibular joint. *J Am Dent Assoc*. 1955;51(4):394–397. doi:10.14219/JADA.ARCHIVE.1955.0223
5. Laskin DM. Etiology of the pain-dysfunction syndrome. *J Am Dent Assoc*. 1969;79(1):147–153. doi:10.14219/JADA.ARCHIVE.1969.0234
6. Goodman P, Greene CS, Laskin DM. Response of patients with myofascial pain-dysfunction syndrome to mock equilibration. *J Am Dent Assoc*. 1976;92(4):755–758. doi:10.14219/JADA.ARCHIVE.1976.0419
7. Laskin DM, Greene CS. Influence of the doctor-patient relationship on placebo therapy for patients with myofascial pain-dysfunction (MPD) syndrome. *J Am Dent Assoc*. 1972;85(4):892–894. doi:10.14219/JADA.ARCHIVE.1972.0416
8. Perrotta S, Bucci R, Simeon V, Martina S, Michelotti A, Valletta R. Prevalence of malocclusion, oral parafunctions and temporomandibular disorder-pain in Italian schoolchildren: An epidemiological study. *J Oral Rehabil*. 2019;46(7):611–616. doi:10.1111/joor.12794
9. Bindayel NA. Occurrence of malocclusion in patients with orofacial pain and temporomandibular disorders. *J Contemp Dent Pract*. 2018;19(5):477–482. PMID:29807955.
10. Boscato N, Nascimento GG, Leite FRM, Horta BL, Svensson P, Demarco FF. Role of occlusal factors on probable bruxism and orofacial pain: Data from the 1982 Pelotas birth cohort study. *J Dent*. 2021;113:103788. doi:10.1016/j.jdent.2021.103788
11. Zúñiga-Herrera ID, Herrera-Atoche JR, Escoffié-Ramírez M, Casanova-Rosado JF, Alonzo-Echeverría ML, Aguilar-Pérez FJ. Malocclusion complexity as an associated factor for temporomandibular disorders. A case-control study. *Cranio*. 2023;41(5):461–466. doi:10.1080/08869634.2020.1868907
12. Uetanabaro LC, Gerber JT, Dos Santos KM, et al. Prevalence and associated factors of myofascial pain in orthognathic patients with skeletal class II malocclusion. *Oral Maxillofac Surg*. 2023;27(1):25–31. doi:10.1007/s10006-022-01046-1
13. Manfredini D, Lombardo L, Siciliani G. Temporomandibular disorders and dental occlusion. A systematic review of association studies: End of an era? *J Oral Rehabil*. 2017;44(11):908–923. doi:10.1111/joor.12531
14. Myllymäki E, Heikinheimo K, Suominen A, et al. Longitudinal trends in temporomandibular joint disorder symptoms, the impact of malocclusion and orthodontic treatment: A 20-year prospective study. *J Oral Rehabil*. 2023;50(9):739–745. doi:10.1111/joor.13471
15. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: Review, criteria, examinations and specifications, critique. *J Craniomandib Disord*. 1992;6(4):301–355. PMID:1298767.
16. 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
17. 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
18. Loster JE, Osiewicz MA, Groch M, Ryniewicz W, Wieczorek A. The prevalence of TMD in Polish young adults. *J Prosthodont*. 2017;26(4):284–288. doi:10.1111/jopr.12414
19. Manfredini D, Castroflorio T, Perinetti G, Guarda-Nardini L. Dental occlusion, body posture and temporomandibular disorders: Where we are now and where we are heading for. *J Oral Rehabil*. 2012;39(6):463–471. doi:10.1111/J.1365-2842.2012.02291.X
20. Osiewicz MA, Lobbezoo F, Loster BW, Loster JE, Manfredini D. Frequency of temporomandibular disorders diagnoses based on RDC/TMD in a Polish patient population. *Cranio*. 2018;36(5):304–310. doi:10.1080/08869634.2017.1361052
21. Lobbezoo F, van der Zaag J, Visscher CM, Naeije M. Oral kinesiology. A new postgraduate programme in the Netherlands. *J Oral Rehabil*. 2004;31(3):192–198. doi:10.1046/J.0305-182X.2003.01244.X
22. Osiewicz MA, Lobbezoo F, Loster BW, Wilkosz M, Naeije M, Ohrbach R. Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD): The Polish version of a dual-axis system for the diagnosis of TMD.* RDC/TMD form. *J Stomatol*. 2013;66(5):576–649. doi:10.5604/00114553.1065048
23. Manfredini D, Visscher CM, Guarda-Nardini L, Lobbezoo F. Occlusal factors are not related with self-reported bruxism. *J Orofac Pain*. 2012;26(3):163–167. PMID:22838000.
24. Kragt L, Dhamo B, Wolvius EB, Ongkosuwito EM. The impact of malocclusions on oral health-related quality of life in children – a systematic review and meta-analysis. *Clin Oral Investig*. 2016;20(8):1881–1894. doi:10.1007/S00784-015-1681-3
25. Pullinger AG, Seligman DA, Gornbein JA. A multiple logistic regression analysis of the risk and relative odds of temporomandibular disorders as a function of common occlusal features. *J Dent Res*. 1993;72(6):968–979. doi:10.1177/00220345930720061301
26. Manfredini D. Occlusal equilibration for the management of temporomandibular disorders. *Oral Maxillofac Surg Clin North Am*. 2018;30(3):257–264. doi:10.1016/j.coms.2018.04.002
27. Amer NM, Aboalnaga AA, Elnahas MO, et al. Malocclusion and temporomandibular disorders: Verification of the controversy. *J Oral Facial Pain Headache*. 2019;33(4):440–450. doi:10.11607/ofph.2260
28. Amer NM, Aboalnaga AA, Fayed MMS, Labib AH. Transverse malocclusion and temporomandibular disorders: Verification of the controversy. *J Oral Facial Pain Headache*. 2019;33(4):355–361. doi:10.11607/ofph.2286
29. Manfredini D, Lombardo L, Siciliani G. Dental angle class asymmetry and temporomandibular disorders. *J Orofac Orthop*. 2017;78(3):253–258. doi:10.1007/S00056-016-0079-4
30. Ohrbach R, Slade GD, Bair E, et al. Premorbid and concurrent predictors of TMD onset and persistence. *Eur J Pain*. 2020;24(1):145–158. doi:10.1002/ejp.1472

Original paper

YouTube videos as an information source about exercises for temporomandibular disorders

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Abstract

Background. Temporomandibular disorders (TMD) are musculoskeletal and/or neuromuscular conditions that affect the muscles, joints and associated structures of the stomatognathic system.

Objectives. This study aimed to evaluate the quality and reliability of publicly available English-language videos on YouTube about TMD exercises, and to examine the video sources and professional groups responsible for the creation of the videos.

Material and methods. The quality and reliability of the YouTube videos related to TMD exercises were evaluated using the DISCERN score, the global quality scale (GQS) and the JAMA (Journal of the American Medical Association) score.

Results. Of the 121 videos evaluated, 30 (24.8%) were uploaded by professional organizations, 49 (40.5%) by health information websites, and 42 (34.7%) were uploaded by independent users. Professional organizations had a significantly higher number of subscribers, likes, comments, and views than healthcare webpages and independent users ($p < 0.001$). The duration of videos uploaded by independent users was significantly longer than that of videos uploaded by healthcare webpages ($p = 0.018$). With regard to the profession of the video narrators, the unspecified group exhibited significantly lower JAMA ($p < 0.001$), GQS ($p = 0.011$) and DISCERN scores ($p = 0.002$) compared to chiropractors, physiotherapists, physicians, and other healthcare professionals. The JAMA scores for physicians, personal trainers and chiropractors were significantly lower than those for other healthcare professionals ($p < 001$). The JAMA score was positively correlated with the GQS ($r = 0.469$, $p < 0.001$) and DISCERN ($r = 0.505$, $p < 0.001$) scores. Similarly, the DISCERN score was positively correlated with the GQS score ($r = 0.924$, $p < 0.001$).

Conclusions. Despite the abundance of video content on YouTube about TMD exercises, the quality of these videos is low, and their reliability is questionable.

Keywords: YouTube, Internet, temporomandibular disorders, health education, exercises

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Introduction

The American Academy of Orofacial Pain (AAOP) defines temporomandibular disorders (TMD) as an umbrella term encompassing the temporomandibular joint, masticatory muscles and related structures.[1] Temporomandibular disorder is the most common musculoskeletal disorder following back pain.[2] The symptoms may include pain in the masticatory muscles or jaw joint, as well as headaches in the temple area.[3] Studies have shown that TMD affect 7–15% of the global population, and the National Institutes of Health (NIH) states that the highest incidence of TMD occurs between the ages of 18 and 43.[4,5] Biomechanical, psychosocial, neurobiological, and neuromuscular factors play a role in the development of TMD.[6] Additionally, trauma, bruxism, hypermobility of the joint, and stress are potentially contributing to their etiology.[6]

Physiotherapy is one of the first interventions used in the management of pain in individuals with TMD. Physiotherapists should address issues such as pain in the masticatory muscles and joints, functional limitations in the temporomandibular joint, and cervical spine dysfunction in individuals with TMD.[7] The most commonly used methods of treating patients with TMD are manual therapy and dry needling.[8] The term "manual therapy" involves numerous applications, including joint mobilization and manipulation, as well as soft tissue interventions such as stretching or trigger point therapy.[9] When orofacial pain is caused by cervical region dysfunction, interventions for the cervical spine should be incorporated into the treatment plan.[10] Two meta-analyses demonstrated the efficacy of manual therapy and exercises for TMD pain symptoms, but there was no consensus on which approach is more effective.[11,12]

The Internet has become an important source of information on health-related topics.[13] Social media platforms such as YouTube, Facebook, Instagram, and Twitter have become an environment for users to obtain information about specific subjects and to share their experiences. YouTube is the most popular video-sharing platform in the world, enabling users to upload and watch videos.[14] The platform boasts over 2 billion users and reaches billions of views.[15] It is an effective tool for obtaining and disseminating health-related information, offering a variety of videos at no cost. In addition, YouTube functions as an educational instrument for both patients and healthcare professionals.[16]

The content of YouTube videos on health-related topics ranges from basic health education to the latest medical treatments.[16] Videos about health problems are becoming increasingly popular, but concerns have been raised about the quality and reliability of videos intended for patient education.[17] In particular, the accuracy of information and the content of the uploaded videos must be verified because there is no quality filter in terms of content accuracy, and these videos can be used for advertising purposes.[18] Since YouTube may have such disadvantages, it may do more harm than good for patients.[19]

In the literature, there are studies that assess the quality and reliability of YouTube videos on a variety of topics, including the rehabilitation of lymphedema,[20] ankylosing spondylitis,[18] fibromyalgia,[21] and piriformis syndrome.[19] However, there is currently no study that evaluates YouTube videos about TMD exercises. In this respect, this study will represent a novel contribution to the existing literature. The primary objective of the study was to evaluate the quality and reliability of publicly available English-language videos on TMD exercises. The second aim was to examine the properties of the videos in terms of the identity of the publishers.

Material and methods

Search strategy

This descriptive study was conducted on July 16, 2022, using the YouTube webpage (https://www.youtube.com). As in previous studies,[21–23] the YouTube search history was entirely deleted prior to the commencement of the study. The researchers selected 4 keywords to identify the videos to be included in the study. These keywords were "TMJ disorder and exercises", "Temporomandibular joint dysfunction and exercises", "Temporomandibular joint syndrome and exercises", and "TMD and exercises". Approximately the first 100 English-language videos for each of these keywords were included, as most users typically view the first few pages of search results.[24] Subsequently, the selected videos were saved to a playlist in the YouTube library for further analysis. A total of 410 videos were watched and evaluated. Similar to previous studies,[21,25] videos in a language other than English, off-topic videos, music videos, videos with no audio or visual content, duplicate videos, and videos longer than 30 min were excluded from the analysis. Finally, 121 videos were selected for inclusion in the study, which were assessed independently by 2 physiotherapists. A flowchart of the screening and selection process of the videos is shown in Fig. 1. All observers participated in a standard training session and received identical instructions regarding the pre-study questionnaires and the scoring criteria. The remaining videos were then viewed and evaluated independently, after which they were combined and their scores were compared. In case of any inconsistency between the physiotherapists' assessments, they re-discussed and re-evaluated the videos in order to reach a consensus. In the event that consensus could not be reached, a third physiotherapist was consulted for a final evaluation.

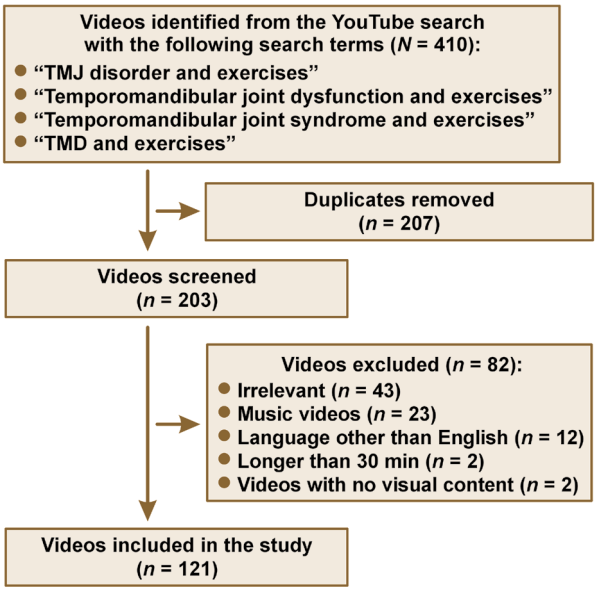

Fig. 1. Flowchart of the screening and selection process

TMJ – temporomandibular joint; TMD – temporomandibular disorders.

Video parameters

The video parameters subjected to analysis included the video upload date, the publisher's subscriber count, the number of likes, the number of comments, the duration of the video (min), the total number of views, and the average number of views per day. The mean daily view count was calculated by dividing the total number of views and comments by the total number of days the video was available on YouTube.[26] All of these parameters were recorded for each video.

Video sources

The sources of the video were classified into 4 categories: professional organizations; health information websites; independent users; and other sources.

Profession of the narrator

The profession of the narrator in the video was classified into 7 categories: physician; physiotherapist; chiropractor; other healthcare professional; personal trainer; and unspecified.

Type of exercise

The type of exercise depicted in the video was classified into the following categories: static stabilization exercises; dynamic stabilization exercises; stretching exercises; joint mobilization; joint manipulation; myofascial release; deep friction massage; trigger point release; posture exercises; TMJ rotation exercises; occipital lift; massage therapy; jaw exercises; and tongue exercises.

Evaluation of quality and reliability

In this study, the global quality scale (GQS) and the JAMA (Journal of the American Medical Association) score were used to assess the quality of the videos.[27–29] The GQS is a tool developed by Bernard et al.[30] The GQS questionnaire has been partially modified to align with the subject matter. The score for each item on the questionnaire ranges from 1 to 5. The survey evaluates the usefulness, flow and quality of the videos, in which a score of 4 or 5 points indicates excellent quality, a score of 3 indicates medium quality, and a score of 1 or 2 indicates low quality. The GQS scoring system used in the present study is outlined below:

– videos with 1 point are of poor quality, exhibiting deficiencies in flow and information content. Therefore, they are not useful for patients with TMD. The exercises depicted in these videos are not suitable for the treatment of TMD;
– videos with 2 points are usually of poor quality. Although they include some exercises, they contain limited information for patients with TMD. While some of the exercises are suitable for the treatment of TMD, the majority are not. The videos contain some useful information, but they also include a considerable amount of misinformation;
– videos with 3 points are of moderate quality. In these videos, the demonstration of some exercises is accurate, whereas the presentation of other exercises is incorrect. The content is a combination of useful and potentially misleading information for patients. However, the videos do not contain a significant amount of misleading information;
– videos with 4 points are of good quality, with a satisfactory flow. They contain useful exercises for patients with TMD. The vast majority of exercises are presented correctly. The videos exhibit only minor flaws and are suitable for patients with TMD;
– videos with 5 points are of superior quality and have excellent flow. They contain very useful exercises for the treatment of TMD patients. All exercises are explained accurately. The number of repetitions and the duration of the exercises are specified in detail. They are an accurate exercise guide for patients with TMD.

Another survey used to determine the quality of videos is the JAMA score. The JAMA score evaluates 4 criteria related to the quality of videos: authorship; description; attribution; and validity. If a criterion is met, 1 point is awarded. A maximum of 4 points can be awarded, with a total of 4 indicating a video of good quality.[31]

Finally, the DISCERN tool, developed by Charnock et al., was used to evaluate the reliability of the videos.[32] The DISCERN tool has previously been used in similar YouTube studies.[21] It consists of 16 questions and an overall quality rating. It is divided into 3 sections. The first part comprises 8 questions and assesses the reliability of the video in

quesiton. The second part consists of 7 questions and evaluates the quality of the information provided regarding treatment options. The third part consists of a single question, which is used to evaluate the overall quality of the information provided, and to assign an overall rating. Each question is assigned a score between 1 and 5. If a criterion is fully met, the highest score is awarded. Conversely, if a criterion is not met at all, it is assigned the lowest score.[32] Detailed information, examples and instructions for each question are described in the DISCERN handbook.[33] The total score is calculated by adding the scores of the first 15 questions.[34] Any score between 63 and 75 points indicates perfect results, between 51 and 62 points indicates good results, between 39 and 50 points indicates intermediate results, between 27 and 38 points indicates poor results, and a score of 27 points or less is indicative of very poor results.[35,36] The DISCERN tool is presented in Table 1.

Ethics statement

The study did not involve the use of human or animal subjects. It exclusively examined publicly accessible videos. Accordingly, ethical approval was not required for this study. A similar approach has been taken in previous YouTube studies.[37–39]

Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). The normality of the distribution was evaluated through the application of the Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous variables are presented as median (interquartile range) (*M* (*IQR*)), while categorical variables are given as number and percentage (*n* (%)). The Mann–Whitney *U* test was used for comparisons between 2 groups, in accordance with the results of the normality tests. In cases where there were more than 2 groups, the Kruskal–Wallis test was utilized. The relationships between JAMA, DISCERN and GQS scores were assessed by Spearman's correlation analysis. A p-value <0.05 was considered statistically significant.

Results

Of the 410 videos analyzed, 121 were included in the study. The general features of the included videos, such as the video duration, the number of likes, comments and views, as well as the distribution of video sources are given in Table 2. Of the 121 videos evaluated, 30 (24.8%) were uploaded by professional organizations, 42 (34.7%) by independent users, and 49 (40.5%) by health information websites. The results revealed a statistically significant difference between groups in terms of the number of subscribers ($p < 0.001$), likes ($p < 0.001$), comments ($p < 0.001$), views ($p < 0.001$), views per day ($p < 0.001$), and video duration ($p = 0.028$) according to the video source. In the pairwise comparisons of professional organizations versus healthcare webpages and professional organizations versus independent users, the number of subscribers, likes, comments, and views was found to be significantly higher for professional organizations than for healthcare webpages and independent users ($p < 0.001$). The videos uploaded by independent users had a significantly longer duration than those uploaded

Table 1. DISCERN reliability tool

Section	Question number	Question	Score range
Section 1	1	Are the aims clear?	1–5
	2	Does it achieve its aims?	1–5
	3	Is it relevant?	1–5
	4	Is it clear what sources of information were used to compile the publication (other than the author or producer)?	1–5
	5	Is it clear when the information used or reported in the publication was produced?	1–5
	6	Is it balanced and unbiased?	1–5
	7	Does it provide details of additional sources of support and information?	1–5
	8	Does it refer to areas of uncertainty?	1–5
Section 2	9	Does it describe how each treatment works?	1–5
	10	Does it describe the benefits of each treatment?	1–5
	11	Does it describe the risks of each treatment?	1–5
	12	Does it describe what would happen if no treatment is used?	1–5
	13	Does it describe how the treatment choices affect overall quality of life?	1–5
	14	Is it clear that there may be more than 1 possible treatment choice?	1–5
	15	Does it provide support for shared decision-making?	1–5
Section 3	16	Based on the answers to all of these questions, rate the overall quality of the publication as a source of information about treatment choices	1–5

Table 2. Characteristics of the analyzed videos according to their source

Variable	Professional organizations (*n* = 30) *M* (*IQR*)	Health information websites (*n* = 49) *M* (*IQR*)	Independent users (*n* = 42) *M* (*IQR*)	*p*-value	*p*-value		
					professional organizations vs health information websites	professional organizations vs independent users	health information websites vs independent users
Time since upload [months]	46 (30–59)	30 (16–48)	24 (17–40)	0.084	0.266	0.200	0.266
Subscribers, *n*	1,760,000 (178,000–4,270,000)	7,740 (706–28,800)	13,800 (1,530–111,000)	<0.001*	<0.001**	<0.001**	0.094
Likes, *n*	2,600 (1,200–8,400)	75 (13–255)	130 (41–893)	<0.001*	<0.001**	<0.001**	0.085
Comments, *n*	179 (68–565)	9 (2–23)	12 (2–146)	<0.001*	<0.001**	<0.001**	0.202
Video duration [s]	365 (250–535)	260 (104–454)	345 (231–685)	0.028*	0.033**	0.805	0.018**
Views, *n*	95,723 (34,592–513,486)	4,389 (1,049–17,269)	6,754 (2,193–44,391)	<0.001*	<0.001**	<0.001**	0.248
Views per day, *n*	130 (52–325)	5 (2–26)	6 (3–75)	<0.001*	<0.001**	<0.001**	0.160
JAMA	3 (2–3)	2 (2–3)	3 (2–3)	0.491	0.235	0.393	0.758
GQS	3 (2–4)	2 (2–3)	3 (2–4)	0.198	0.169	0.931	0.101
DISCERN	38 (30–50)	34 (29–43)	41 (30–51)	0.276	0.448	0.606	0.102

* statistically significant ($p < 0.05$, Kruskal–Wallis test (comparison of the groups)); ** statistically significant ($p < 0.05$, Mann–Whitney *U* test (pairwise comparison of the groups)); *M* – mean; *IQR* – interquartile range; JAMA – Journal of the American Medical Association score; GQS – global quality scale.

by health information websites ($p = 0.018$). Additionally, professional organizations had a significantly longer video duration than healthcare webpages ($p = 0.033$).

The basic characteristics of the videos, according to the profession of the narrator, are given in Table 3. Significant differences were observed in video duration and the JAMA, GQS and DISCERN scores between the professions ($p < 0.05$). The unspecified group had significantly lower JAMA ($p < 0.001$), GQS ($p = 0.011$) and DISCERN ($p = 0.002$) scores compared to chiropractors, physiotherapists, physicians, and other healthcare professionals. Additionally, the JAMA scores of the physicians, personal trainers and chiropractors were significantly lower than those of the other healthcare professionals ($p < 0.001$). The mean video duration of personal trainers was significantly higher than that of chiropractors, physiotherapists and other healthcare professionals ($p = 0.025$).

A review of the video content revealed that massage therapy, static stabilization exercises and jaw exercises were the most prevalent, in descending order. The contents of the analyzed videos are presented in Table 4.

The JAMA score was positively correlated with the GQS and DISCERN scores ($r = 0.469$ and $r = 0.505$, respectively; $p < 0.01$). A positive correlation was observed between the DISCERN score and the GQS score ($r = 0.924$, $p < 0.01$). A comparison of the JAMA, GQS and DISCERN scores is given in Table 5.

Discussion

The aim of this study was to evaluate the quality and reliability of YouTube videos about exercises used in the treatment of temporomandibular joint dysfunction. The results of this study indicate that there was no significant difference in the JAMA, GQS and DISCERN scores according to the video sources. However, a significant difference was found in the JAMA, GQS and DISCERN scores in relation to the profession of the video narrator ($p < 0.05$). A significant difference was observed in the basic features of the videos, including the number of subscribers, likes and comments, as well as the duration of the videos, according to the video sources. However, only a significant difference in video duration was noted between the profession groups.

Many scoring systems can be used to evaluate the quality and reliability of video content.[40,41] One of the main reasons for using the JAMA, DISCERN and GQS scores in the analysis of YouTube videos is that these scores have been previously applied in other studies, thereby facilitating comparisons across different analyses. In our study, a positive correlation was identified between the JAMA, GQS and DISCERN scores. Similar results have been documented in the literature.[42,43] Video content related to numerous conditions, including

Table 3. Characteristics of the analyzed videos according to the profession of the narrator

Variable	Physician (*n* = 16) *M* (*IQR*)	Physiotherapist (*n* = 45) *M* (*IQR*)	Chiropractor (*n* = 23) *M* (*IQR*)	Other healthcare professional (*n* = 16) *M* (*IQR*)	Personal trainer (*n* = 7) *M* (*IQR*)	Unspecified (*n* = 14) *M* (*IQR*)	*p*-value
Video upload date	39 (24–59)	31 (22–58)	23 (15–46)	24 (17–38)	27 (10–36)	24.5 (13–67)	0.313
Subscribers, *n*	229,000 (11,150–5,010,000)	14,000 (776–154,000)	54,300 (1,680–150,000)	11,250 (1,520–15,350)	81,700 (1,350–205,000)	20,250 (381–69,200)	0.096
Likes, *n*	660 (69–3,100)	189 (44–1,100)	506 (43–6,300)	86 (38–910)	418 (135–1,200)	85.5 (31–228)	0.347
Comments, *n*	73 (7–216)	18 (3–85)	25 (2–663)	23 (4–147)	68 (21–112)	5 (2–16)	0.099
Video duration [s]	348 (250–515)	348 (153–535)	461 (174–759)	250 (145–322)	857 (330–1,294)	269 (125–426)	0.025*
Views, *n*	37,506 (4,607–116,622)	6,754 (1,974–63,453)	17,269 (2,053–286,940)	4,884 (1,656–49,256)	15,094 (4,245–35,398)	7,058 (2,577–15,942)	0.603
Views per day, *n*	31 (3–113)	8 (3–64)	13 (4–400)	19 (2–63)	59 (13–137)	8 (3–18)	0.292
JAMA	2 (2–3)	3 (2–3)	2 (2–3)	3 (3–3)	2 (2–3)	1 (1–2)	<0.001*
GQS	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	2 (2–3)	2 (1–2)	0.011*
DISCERN	38 (32–45)	39 (32–50)	40 (28–51)	42 (30–52)	36 (31–43)	26.5 (22–30)	0.002*

* statistically significant ($p < 0.05$, Kruskal–Wallis test (comparison of the groups)).

Table 4. Exercises included in the analyzed YouTube videos (*N* = 121)

Exercise type	Included	Not included
Static stabilization exercises	37 (30.6)	84 (69.4)
Dynamic stabilization exercises	28 (23.1)	93 (76.9)
Stretching exercises	31 (25.6)	90 (74.4)
Joint mobilization	8 (6.6)	113 (93.4)
Joint manipulation	3 (2.5)	118 (97.5)
Myofascial release	7 (5.8)	114 (94.2)
Deep friction massage	5 (4.1)	116 (95.9)
Trigger point release	16 (13.2)	105 (86.8)
Posture exercises	35 (28.9)	86 (71.1)
TMJ rotation exercises	30 (24.8)	91 (75.2)
Occipital lift	5 (4.1)	116 (95.9)
Massage therapy	53 (43.8)	68 (56.2)
Jaw exercises	36 (29.8)	85 (70.2)
Tongue exercises	9 (7.4)	112 (92.6)

Data presented as frequency (percentage) (*n* (%)); TMJ – temporomandibular joint.

Table 5. Comparison of the JAMA (Journal of the American Medical Association), global quality scale (GQS) and DISCERN scores

Score	Spearman's rho	
	GQS	DISCERN
JAMA	0.469*	0.505*
GQS	1.000	0.924*
DISCERN	0.924*	1.000

* statistically significant ($p < 0.01$, Spearman's correlation analysis).

shoulder instability, fibromyalgia, hallux valgus, TMD, and neck pain, has been analyzed in terms of quality and reliability.[21,25,43–45] These studies have revealed that the quality of video content is low and that its reliability is often poor. As social media continue to play an increasingly prominent role in our lives, particularly with the advancement of new technologies, the potential for the dissemination of misinformation is likely to accelerate. It is important that video content producers take measures to prevent the dissemination of misinformation by subjecting the information to rigorous screening.

A comparative analysis revealed that professional organizations had a significantly higher number of views than the other 2 groups. This shows that the videos uploaded by professional organizations attract more attention. YouTube studies have shown that healthcare webpages on osteoporosis and the side effects of biologic therapy have a higher number of views than the videos of professional organizations and independent users on the subject.[23,46] In this study, while the number of subscribers, likes and comments was significantly higher in professional organizations than in the other groups, no significant differences were found between independent users and professional organizations in terms of video duration. It can be posited that the reason for the higher number of subscribers, likes and comments associated with professional organizations is reflected in the interaction between the video and the audience, which is influenced by the number of views. In addition, professional organizations possess the necessary equipment for filming. The incorporation of a variety of enhancements in their videos may attract the attention of the audience.

The results of our study indicated no significant differences in the JAMA, DISCERN and GQS scores based on the video sources. On the contrary, in previous similar studies, the scale scores of professional organizations and health service webpages have exceeded those of individual users.[29,46] Given that the majority of patients do not verify the sources, they may be exposed to videos of low quality and reliability. In this regard, the literature underscores the importance of professional organizations increasing the quantity of their video content.[22]

One of the main results of our study was that the unspecified group of narrators uploaded videos of lower quality and reliability when compared to the videos of chiropractors, physiotherapists, physicians, and other healthcare professionals. Similarly, Ertem et al. found that videos created by independent users for the treatment of piriformis syndrome were less reliable than those created by healthcare professionals and physicians.[19] It is widely accepted that high scores for the DISCERN and GQS indicate high reliability and quality.[23,47] In addition, our study revealed that the reliability of videos created by physicians, personal trainers and chiropractors was lower than that of videos created by other healthcare professionals. Our results indicate that videos uploaded to YouTube may lack reliability, even when uploaded by physicians. It may be difficult for physicians to determine the specific triggers for TMD, given the multifaceted nature of the underlying causes.[48] It is recommended that physicians thoroughly review the current guidelines before creating video content on this topic.[14,49]

Upon examination of the video content included in the study, it was determined that massage therapy, static stabilization exercises and jaw exercises were the most prevalent. Physiotherapy techniques such as active and passive stretching, postural exercises, strengthening of related muscles, and manual therapy are effective in the treatment of TMD.[50] Medlicott and Harris stated that a treatment program consisting of active exercises, manual therapy and relaxation techniques is the most effective treatment method for TMD.[51] Calixtre et al. found that manual therapy and stabilization exercises for the upper cervical spine increased the pain threshold and maximum mouth opening in patients with TMD.[50] Despite the prevalence of stabilization exercises in the videos examined in our study, the number of manual therapy approaches, such as mobilization and manipulation, was limited, which may have resulted in a reduction in the reliability of the videos.

Limitations

The present study had some limitations. Firstly, only English-language videos were included in the study, which restricted the generalizability of the results. As the present study did not review videos in languages other than English, the information and experiences derived from these languages are not available. Given that English is the most widely used language globally,[52] we believe that this limitation does not significantly impact the validity of the study findings. It should not be overlooked that the order in which the videos appear may vary depending on the IP address and the location from which they are searched. This may have introduced a potential minor bias. In addition, the YouTube videos were evaluated at a single point in time. However, it should be noted that YouTube has a dynamic structure, and new videos may be added, potentially influencing the results. It is also possible that the number of views and comments on the videos may change over time.

Conclusions

The results of the research indicated that the quality and reliability of the videos were low when analyzed according to the source of the video and the profession groups. Despite the rapid growth of YouTube, the platform often fails to provide accurate information for patients. It is important for patients to be aware that the control mechanisms designed to ensure the accuracy of information on YouTube are insufficient, and that the information they contain may be incorrect. Therefore, patients should not base their decisions on the content of videos related to the subject, as the information presented in YouTube videos may have a detrimental impact on patients' health. To prevent the potential for adverse effects resulting from inappropriate use, it is recommended that YouTube videos be used only for the purpose of obtaining guidance from qualified medical professionals, such as physicians.

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.

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References

1. de Leeuw R, Klasser GD. Orofacial pain: Guidelines for Assessment, Diagnosis, and Management. Chicago, IL: Quintessence Publishing Co, Inc; 2013.

2. Facial Pain. National Institute of Dental and Craniofacial Research. 2018. https://www.nidcr.nih.gov/research/data-statistics/facial-pain. Accessed May 11, 2023.
3. Hadler-Olsen E, Thon E, Holde GE, Jönsson B, Oscarson N, Tillberg A. Temporomandibular disorders in an adult population in northern Norway: A cross-sectional study. *Clin Exp Dent Res*. 2021;7(6):1144–1153. doi:10.1002/cre2.463
4. List T, Jensen RH. Temporomandibular disorders: Old ideas and new concepts. *Cephalalgia*. 2017;37(7):692–704. doi:10.1177/0333102416686302
5. Department of Health and Human Services (DHHS). Prospective studies on craniofacial pain and dysfunction. RFA Number: RFA-DE-05-007. https://grants.nih.gov/grants/guide/rfa-files/RFA-DE-05-007.html. Accessed May 11, 2023.
6. 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
7. Harrison AL, Thorp JN, Ritzline PD. A proposed diagnostic classification of patients with temporomandibular disorders: Implications for physical therapists. *J Orthop Sports Phys Ther*. 2014;44(3):182–197. doi:10.2519/jospt.2014.4847
8. Fernández-de-Las-Peñas C, Von Piekartz H. Clinical reasoning for the examination and physical therapy treatment of temporomandibular disorders (TMD): A narrative literature review. *J Clin Med*. 2020;9(11):3686. doi:10.3390/jcm9113686
9. American Physical Therapy Association (APTA). *Manipulation Education Manual for Physical Therapist Professional Degree Programs*. https://www.orthopt.org/uploads/content_files/files/manipulation_education_manual.pdf. Accessed May 11, 2023.
10. Kraus S. Temporomandibular disorders, head and orofacial pain: Cervical spine considerations. *Dent Clin North Am*. 2007;51(1):161–193. doi:10.1016/j.cden.2006.10.001
11. Dickerson SM, Weaver JM, Boyson AN, et al. The effectiveness of exercise therapy for temporomandibular dysfunction: A systematic review and meta-analysis. *Clin Rehabil*. 2017;31(8):1039–1048. doi:10.1177/0269215516672275
12. Armijo-Olivo S, Pitance L, Singh V, Neto F, Thie N, Michelotti A. Effectiveness of manual therapy and therapeutic exercise for temporomandibular disorders: Systematic review and meta-analysis. *Phys Ther*. 2016;96(1):9–25. doi:10.2522/ptj.20140548
13. Powell JA, Darvell M, Gray J. The doctor, the patient and the world-wide web: How the Internet is changing healthcare. *J R Soc Med*. 2003;96(2):74–76. doi:10.1258/jrsm.96.2.74
14. Chang MC, Lee BJ, Park D. The quality, reliability, and accuracy of videos regarding exercises and management for dysphagia in pediatric populations uploaded on YouTube. *Children (Basel)*. 2022;9(10):1514. doi:10.3390/children9101514
15. Chatzopoulou G, Sheng C, Faloutsos M. A first step towards understanding popularity in YouTube. *2010 INFOCOM IEEE Conference on Computer Communications Workshops*. 2010:1–6. doi:10.1109/INFCOMW.2010.5466701
16. Pant S, Deshmukh A, Murugiah K, Kumar G, Sachdeva R, Mehta JL. Assessing the credibility of the "YouTube approach" to health information on acute myocardial infarction. *Clin Cardiol*. 2012;35(5):281–285. doi:10.1002/clc.21981
17. Drozd B, Couvillon E, Suarez A. Medical YouTube videos and methods of evaluation: Literature review. *JMIR Med Educ*. 2018;4(1):e3. doi:10.2196/mededu.8527
18. Kocyigit BF, Nacitarhan V, Koca TT, Berk E. YouTube as a source of patient information for ankylosing spondylitis exercises. *Clin Rheumatol*. 2019;38(6):1747–1751. doi:10.1007/s10067-018-04413-0
19. Ertem U, Özçakır S, İrdesel FJ, Günay SM. YouTube as a source of information on piriformis syndrome exercises. *Turkish J Phys Med Rehabil*. 2023;69(1):15–22. doi:10.5606/tftrd.2022.10459
20. Küçükakkaş O, İnce B. Can YouTube be used as an educational tool in lymphedema rehabilitation? *Arch Physiother*. 2022;12(1):5. doi:10.1186/s40945-022-00130-9
21. Ozsoy-Unubol T, Alanbay-Yagci E. YouTube as a source of information on fibromyalgia. *Int J Rheum Dis*. 2021;24(2):197–202. doi:10.1111/1756-185X.14043
22. Onder ME, Zengin O. Quality of healthcare information on YouTube: Psoriatic arthritis. *Z Rheumatol*. 2023;82(Suppl 1):30–37. doi:10.1007/s00393-021-01069-1
23. Onder ME, Onder CE, Zengin O. Quality of English-language videos available on YouTube as a source of information on osteoporosis. *Arch Osteoporos*. 2022;17(1):19. doi:10.1007/s11657-022-01064-2
24. Ng CH, Lim GRS, Fong W. Quality of English-language videos on YouTube as a source of information on systemic lupus erythematosus. *Int J Rheum Dis*. 2020;23(12):1636–1644. doi:10.1111/1756-185X.13852
25. Kim MJ, Kim JR, Jo JH, Kim JS, Park JW. Temporomandibular disorders-related videos on YouTube are unreliable sources of medical information: A cross-sectional analysis of quality and content. *Digit Health*. 2023;9:20552076231154377. doi:10.1177/20552076231154377
26. Koçyiğit BF, Okyay RA, Akaltun MS. YouTube as a source of eHealth: Is it beneficial for lumbar disc herniation exercises? *J PMR Sci*. 2020;23(3):217–223. doi:10.31609/jpmrs.2020-75515
27. Zhang S, Fukunaga T, Oka S, et al. Concerns of quality, utility, and reliability of laparoscopic gastrectomy for gastric cancer in public video sharing platform. *Ann Transl Med*. 2020;8(5):196. doi:10.21037/atm.2020.01.78
28. Starks C, Akkera M, Shalaby M, et al. Evaluation of YouTube videos as a patient education source for novel surgical techniques in thyroid surgery. *Gland Surg*. 2021;10(2):697–705. doi:10.21037/gs-20-734
29. Singh AG, Singh S, Singh PP. YouTube for information on rheumatoid arthritis – a wakeup call? *J Rheumatol*. 2012;39(5):899–903. doi:10.3899/jrheum.111114
30. Bernard A, Langille M, Hughes S, Rose C, Leddin D, Veldhuyzen van Zanten S. A systematic review of patient inflammatory bowel disease information resources on the World Wide Web. *Am J Gastroenterol*. 2007;102(9):2070–2077. doi:10.1111/j.1572-0241.2007.01325.x
31. Silberg WM, Lundberg GD, Musacchio RA. Assessing, controlling, and assuring the quality of medical information on the Internet: Caveant lector et viewor—let the reader and viewer beware. *JAMA*. 1997;277(15):1244–1245. doi:10.1001/jama.1997.03540390074039
32. Charnock D, Shepperd S, Needham G, Gann R. DISCERN: An instrument for judging the quality of written consumer health information on treatment choices. *J Epidemiol Community Health*. 1999;53(2):105–111. doi:10.1136/jech.53.2.105
33. Charnock D. *The DISCERN Handbook. Quality Criteria for Consumer Health Information on Treatment Choices*. Abingdon, UK: Radcliffe Medical Press; 1998:7–51.
34. Kaicker J, Debono VB, Dang W, Buckley N, Thabane L. Assessment of the quality and variability of health information on chronic pain websites using the DISCERN instrument. *BMC Med*. 2010;8(1):59. doi:10.1186/1741-7015-8-59
35. Som R, Gunawardana NP. Internet chemotherapy information is of good quality: Assessment with the DISCERN tool. *Br J Cancer*. 2012;107(2):403. doi:10.1038/bjc.2012.223
36. Weil AG, Bojanowski MW, Jamart J, Gustin T, Lévêque M. Evaluation of the quality of information on the Internet available to patients undergoing cervical spine surgery. *World Neurosurg*. 2014;82(1–2):e31–e39. doi:10.1016/j.wneu.2012.11.003
37. Tolu S, Yurdakul OV, Basaran B, Rezvani A. English-language videos on YouTube as a source of information on self-administer subcutaneous anti-tumour necrosis factor agent injections. *Rheumatol Int*. 2018;38(7):1285–1292. doi:10.1007/s00296-018-4047-8
38. Nason GJ, Kelly P, Kelly ME, et al. YouTube as an educational tool regarding male urethral catheterization. *Scand J Urol*. 2015;49(2):189–192. doi:10.3109/21681805.2014.975837
39. Esen E, Aslan M, Sonbahar BÇ, Kerimoğlu RS. YouTube English videos as a source of information on breast self-examination. *Breast Cancer Res Treat*. 2019;173(3):629–635. doi:10.1007/s10549-018-5044-z
40. Gonzalez-Estrada A, Cuervo-Pardo L, Ghosh B, et al. Popular on YouTube: A critical appraisal of the educational quality of information regarding asthma. *Allergy Asthma Proc*. 2015;36(6):e121–e126. doi:10.2500/aap.2015.36.3890
41. Haymes AT, Harries V. "How to stop a nosebleed": An assessment of the quality of epistaxis treatment advice on YouTube. *J Laryngol Otol*. 2016;130(8):749–754. doi:10.1017/S0022215116008410
42. Yildiz S, Toros SZ. The quality, reliability, and popularity of YouTube education videos for vestibular rehabilitation: A cross-sectional study. *Otol Neurotol*. 2021;42(8):e1077–e1083. doi:10.1097/MAO.0000000000003197
43. Zhang X, Yang Y, Shen YW, et al. Quality of online video resources concerning patient education for neck pain: A YouTube-based quality-control study. *Front Public Health*. 2022;10:972348. doi:10.3389/fpubh.2022.972348

44. Etzel CM, Bokshan SL, Forster TA, Owens BD. A quality assessment of YouTube content on shoulder instability. *Phys Sportsmed*. 2022;50(4):289–294. doi:10.1080/00913847.2021.1942286
45. Tekin SB, Bozgeyik B. Quality and content analysis of hallux valgus videos on YouTube®. *J Foot Ankle Surg*. 2023;62(1):85–90. doi:10.1053/j.jfas.2022.05.003
46. Zengin O, Onder ME. YouTube for information about side effects of biologic therapy: A social media analysis. *Int J Rheum Dis*. 2020;23(12):1645–1650. doi:10.1111/1756-185X.14003
47. Delli K, Livas C, Vissink A, Spijkervet FKL. Is YouTube useful as a source of information for Sjögren's syndrome? *Oral Dis*. 2016;22(3):196–201. doi:10.1111/odi.12404
48. Simoen L, Van den Berghe L, Jacquet W, Marks L. Depression and anxiety levels in patients with temporomandibular disorders: Comparison with the general population. *Clin Oral Investig*. 2020;24(11):3939–3945. doi:10.1007/s00784-020-03260-1
49. Tran C, Ghahreman K, Huppa C, Gallagher JE. Management of temporomandibular disorders: A rapid review of systematic reviews and guidelines. *Int J Oral Maxillofac Surg*. 2022;51(9):1211–1225. doi:10.1016/j.ijom.2021.11.009
50. Calixtre LB, da Silva Grüninger BL, Haik MN, Alburquerque-Sendín F, Oliveira AB. Effects of cervical mobilization and exercise on pain, movement and function in subjects with temporomandibular disorders: A single group pre–post test. *J Appl Oral Sci*. 2016;24(3):188–197. doi:10.1590/1678-775720150240
51. Medlicott MS, Harris SR. A systematic review of the effectiveness of exercise, manual therapy, electrotherapy, relaxation training, and biofeedback in the management of temporomandibular disorder. *Phys Ther*. 2006;86(7):955–973. doi:10.1093/ptj/86.7.955
52. Desai G. How to leverage the language: A guide for medical writers in India. *Perspect Clin Res*. 2019;10(1):37–41. doi:10.4103/picr.PICR_129_18

Original paper

Quality of YouTube videos on botulinum toxin management in bruxism, assessed using the DISCERN instrument

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Abstract

Background. Patients are increasingly turning to Internet platforms for health-related information. An example is YouTube, one of the largest media-sharing networks in the world.

Objectives. The aim of the present study was to assess the informational value of YouTube videos on the treatment of bruxism with botulinum toxin, a procedure that is becoming increasingly popular in the field of dentistry.

Material and methods. After collecting 30 videos for each of the 5 keywords, a total of 150 videos were examined. The following search terms were used: 'bruxism Botox treatment'; 'tooth grinding Botox treatment'; 'jaw clenching Botox treatment'; 'Botox for bruxism'; and 'Botox for masseter reduction'. Two researchers independently assessed the quality of the video content using the DISCERN scoring system. Additionally, the relationships between quantitative variables, such as video duration, the source of upload and video popularity, and the DISCERN scores, were examined.

Results. The mean overall DISCERN score was 32.3. The YouTube videos were divided into the following categories based on their DISCERN scores: very poor (26.3%); poor (61.4%); fair (10.5%); good (1.8%); and excellent (0.0%). Videos that addressed risk factors during therapy, treatment outcomes, bruxism symptoms, and the muscle anatomy had significantly higher overall DISCERN scores.

Conclusions. In general, YouTube videos on botulinum toxin treatment for bruxism had poor informational value. It is important that dentists recognize the significance of YouTube as a source of health-related information, and ensure that the content they provide is of the highest quality, accurate and up-to-date.

Keywords: bruxism, quality, YouTube, DISCERN, Botox

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Introduction

Bruxism is a repeated activity of the jaw muscles, defined as tooth grinding or jaw clenching.[1] It can be manifested in 2 distinct forms: sleep bruxism (SB); and awake bruxism (AB).[2] Bruxism may result in the hypertrophy of the masticatory muscles, the loss of the tooth surface, hypersensitive teeth, the breakage of restorations or teeth, the loss of periodontal support, and arthralgia characteristic of temporomandibular disorders (TMD).[1]

Although etiological factors such as emotional stress, neurological disorders, certain drugs, and occlusal interferences have been proposed,[3,4] the exact etiology and pathophysiology of bruxism remain unknown. However, it appears to have a multifactorial origin that is mediated by the central and autonomous nervous systems.[5,6]

There are numerous methods of treating bruxism, including occlusal splints, drugs such as benzodiazepines or L-DOPA, and cognitive behavioral therapy (CBT). However, their ultimate efficacy has yet to be demonstrated, since they do not seem to address the fundamental cause, and are primarily used for the management of the patient's signs and symptoms, thereby reducing the harmful consequences of bruxism for anatomical structures.[7,8] Botulinum toxin, or onabotulinumtoxinA, represents another highly successful treatment method that has been validated by prior research[9] and will be the focus of this study.

The complexity of bruxism has led to many misconceptions about the behavior. Patients are often interested in their condition and treatment options. A well-informed patient may participate in the decision-making process more actively, and hence feel less anxious. However, healthcare providers may be unable to deliver sufficient information due to the lack of consultation time or by communicating in a manner that the patient cannot comprehend. Consequently, more and more patients are turning to the Internet for easily accessible medical information, with YouTube being one of the most prominent online resources.

YouTube is the world's largest media-sharing network and the second most popular website after Google.[10] Anyone may contribute movies, including non-peer-reviewed medical content.[11] Contradictory advice might undermine the credibility of medical professionals, particularly when addressing alternative treatment options.[12] Therefore, examining the integrity of patient information about bruxism therapy on YouTube is essential.

Prior to the present study, no research had been conducted using the DISCERN scale[13] to evaluate the quality of YouTube videos on bruxism therapy with botulinum toxin. Consequently, we aimed to assess the effectiveness of YouTube videos as a source of patient education. A secondary goal was to investigate the relationships between quantitative data, such as video duration, the source of upload and video popularity, and the quality of the videos.

Material and methods

Study design

This was a cross-sectional analysis of publicly accessible videos that did not involve human or animal subjects, and therefore ethics committee approval was not required.

YouTube search

A YouTube search was conducted on October 30, 2022, using the incognito mode. The following keywords were used in the search: 'bruxism Botox treatment'; 'tooth grinding Botox treatment'; 'jaw clenching Botox treatment'; 'Botox for bruxism'; and 'Botox for masseter reduction'. The video search was performed after erasing all the browsing data from the Google web browser, and with no user account. The YouTube results were sorted using relevance-based ranking, and the first 30 videos for each keyword were evaluated. It has been stated that 95% of YouTube viewers do not watch more than the first 60 videos returned by a search query. Additionally, the majority of prior research has only examined the first 60–200 videos.[14]

Selection criteria

The study included English-language videos that explained the mechanism of treatment with its benefits and risks, as well as the potential treatment options for bruxism. Duplicate or irrelevant videos, where "irrelevant" was defined as offering no information about bruxism treatment, were excluded from the study.

Video quality assessment

A fifth-year and a fourth-year medical dentistry students separately evaluated the video content using the DISCERN scoring system for video quality analysis. The DISCERN instrument is employed to assess the credibility of a publication and the quality of the treatment information provided to patients.[13] The DISCERN tool was also designed for individuals without medical expertise. DISCERN provides a set of guidelines for the evaluation of health information for users, both consumers and professionals, as well as standards for information producers. The DISCERN criteria are used to evaluate the reliability and credibility of the information.[15] The instrument comprises 15 questions, each scored from 1 to 5 points. The first part consists of 8 questions designed to assess the credibility of a publication (in this case, an Internet video), followed by 7 questions that analyze treatment-related details.[16] The 16th question evaluates the overall quality of the video. In accordance with the methodology proposed by Weil et al.,[17] the total DISCERN score was presented as the sum of the scores for the first 15 ques-

tions, ranging from 15 to 75 and grouped into 5 DISCERN categories: excellent (63–75 points); good (51–62 points); fair (39–50 points); poor (28–38 points); or very poor (15–27 points).

Video classification

The type of YouTube channel was a criterion used to classify videos into 5 groups based on the source of upload:
– "hospital/clinic" when the source of upload was a hospital or clinic channel;
– "health" when the source of upload was a health information channel;
– "educational" when the source of upload was an educational channel;
– "news" when the source of upload was an information channel;
– "patient" when the source of upload was the patient.

Video features

In order to evaluate a set of statistics, the YouTube data was utilized. We collected details regarding the name of the YouTube channel, the view count, video duration (converted into seconds), the number of subscribers to the channel, the number of comments, and the number of likes. The last 2 variables may be hidden by the channel. We used the "timeanddate" calculator (https://www.timeanddate.com/date/duration.html) to determine the time elapsed since the video upload (in days). A Google Chrome extension called "return YouTube dislike" (https://returnyoutubedislike.com) was used to calculate the number of dislikes. The functionality of this extension relies upon the visibility of likes and comments on a YouTube channel, which may or may not be hidden.

In addition, the ratio of likes, the ratio of views and the video power index (VPI) were applied in the examination of video popularity.

The ratio of likes was calculated according to the following formula (Equation 1):

$$\text{ratio of likes} = \frac{\text{number of likes}}{\text{number of likes} + \text{number of dislikes}} \times 100 \quad (1)$$

The ratio of views was calculated according to the following formula (Equation 2):

$$\text{ratio of views} = \frac{\text{view count}}{\text{time since upload}} \quad (2)$$

The VPI was calculated as follows (Equation 3):

$$\text{VPI} = \frac{\text{ratio of likes} \times \text{ratio of views}}{100} \quad (3)$$

where:
VPI – video power index.

In the course of the analysis, the qualitative information included in the videos was examined in accordance with the following questions: Were the symptoms of bruxism discussed?; Were the risk factors during treatment explained?; Were the results of treatment presented?; Were the steps of the procedure described?; Was the prognosis discussed?; Was any form of animation incorporated in the video?; Were there diagrams?; Was the muscle anatomy explained?; Was the speaker a doctor?; Was it a patient's experience?

Statistical analysis

The statistical analysis was performed using the R-Studio program (8.9 build 680; R-Tools Technology Inc., Richmond Hill, Canada) and Google Sheets (Google LLC, Mountain View, USA).

The normality of the data was evaluated using the Shapiro–Wilk test. The Mann–Whitney and Kruskal–Wallis tests were employed to determine statistically significant differences with regard to an independent variable between two or more than two groups, respectively. For pairwise comparisons, the Dunn–Bonferroni post hoc test was utilized following a statistically significant outcome in the Kruskal–Wallis test. Spearman's test was used to analyze the correlations between variables. The intraclass correlation coefficient (*ICC*) was employed to establish the degree of inter-rater agreement. The results were interpreted using a 95% confidence interval (*CI*) and a significance threshold of 0.05.

Results

A total of 150 videos were analyzed after collecting 30 videos for each of the 5 keywords. Subsequently, 55 duplicates were removed. After the screening process, which was conducted based on the established inclusion and exclusion criteria, 57 videos were subjected to further analysis (Fig. 1).

Table 1 presents the mean DISCERN score for each inquiry. The question "Is it clear when the information used or reported in the publication was produced?" obtained the lowest mean score (1.0), while the question "Are the aims clear?" obtained the highest score (4.3). The mean total DISCERN score was 32.3 ±7.3. According to the DISCERN categories, 26.3% of the YouTube videos were classified as very poor, 61.4% as poor, 10.5% as fair, 1.8% as good, and 0.0% as excellent (Table 2).

The videos uploaded by patients were the most prevalent (49.1%), followed by educational (26.4%), hospital/clinic (21.1%), and news (3.5%) sources (Table 2). The mean total DISCERN score was the highest for news

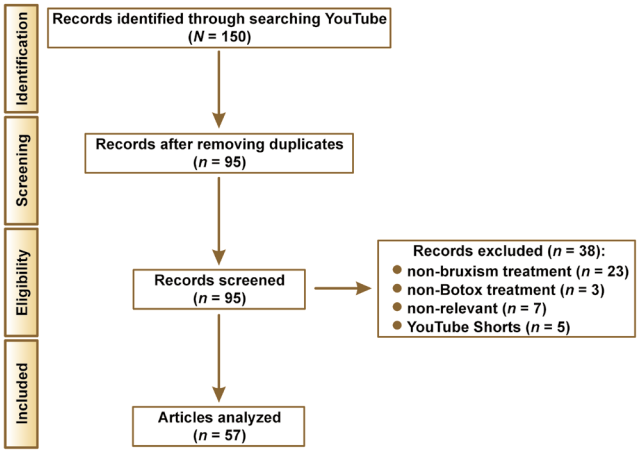

Fig. 1. Flowchart of the study

Table 1. Mean scores for each DISCERN inquiry (score range: 1–5)

Question number	Question	Score
1	Are the aims clear?	4.3 ±1.0
2	Does it achieve its aims?	3.3 ±0.9
3	Is it relevant?	2.9 ±0.8
4	Is it clear what sources of information were used to compile the publication?	1.3 ±0.8
5	Is it clear when the information used or reported in the publication was produced?	1.0 ±1.0
6	Is it balanced and unbiased?	2.6 ±0.8
7	Does it provide details of additional sources of support and information?	1.2 ±0.5
8	Does it refer to areas of uncertainty?	1.4 ±0.7
9	Does it describe how each treatment works?	2.5 ±1.0
10	Does it describe the benefits of each treatment?	2.4 ±0.9
11	Does it describe the risks of each treatment?	2.0 ±1.3
12	Does it describe what would happen if no treatment is used?	1.2 ±0.6
13	Does it describe how the treatment choices affect the overall quality of life?	2.3 ±1.1
14	Is it clear that there may be more than one possible treatment choice?	1.6 ±1.0
15	Does it provide support for shared decision making?	2.3 ±0.9

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

sources (37.0 ±19.8), followed by the videos uploaded by patients (33.0 ±5.5), hospital or clinic channels (32.5 ±9.8), and educational channels (30.3 ±6.5) (Table 3).

The average number of views per video was 32,587 ±58,515, with the mean length of 561 ±1,156 s or 9.4 ±19.3 min. Each video had an average of 448 ±947 likes and 29 ±69 dislikes (Table 4).

Table 2. Characteristics of the YouTube videos (*N* = 57)

Characteristic		*n* (%)
Video quality (DISCERN)	very poor	15 (26.3)
	poor	35 (61.4)
	fair	6 (10.5)
	good	1 (1.8)
	excellent	0 (0.0)
Source of upload	hospital/clinic	12 (21.1)
	educational	15 (26.3)
	news	2 (3.5)
	patient	28 (49.1)

Videos that provided information about bruxism symptoms (p = 0.024), the risk factors during therapy (p = 0.001) and the results of treatment (p < 0.001), as well as the explanation of the muscle anatomy (p = 0.010) had substantially higher overall DISCERN scores. The average DISCERN score remained consistent regardless of whether the treatment phases were described, the prognosis was discussed, the presence of animations or diagrams was indicated, or the identity of the speaker (doctor or patient) was specified (p > 0.05) (Table 5).

There were no significant correlations between the mean total DISCERN score and the time elapsed since upload, the number of channel subscribers, the ratio of likes, or VPI. A weak positive correlation was observed between the DISCERN score and the view count (p = 0.044; r = 0.268). The DISCERN score was moderately positively correlated with the video duration (p = 0.002; r = 0.395), the number of comments (p = 0.013; r = 0.329), the number of likes (p = 0.010; r = 0.343), and the number of dislikes (p = 0.018; r = 0.350) (Table 4). The average *ICC* value was 0.823, indicating good agreement between the observers.

Discussion

Patients with bruxism are willing to undergo therapy due to the chronic symptoms associated with the disease, including fatigue, headaches, discomfort in the masticatory muscles and the temporomandibular joints (TMJs), and tooth hypersensitivity.[18] An increasing number of medical professionals are recommending the use of botulinum toxin. However, the term "toxin" continues to elicit apprehension among patients, despite the evidence supporting its efficacy in managing bruxism. According to a study by Zhang et al., the administration of botulinum toxin to the masseter muscles resulted in a notable reduction in occlusal force.[19] Furthermore, Al-Wayli observed a significant decrease in pain after adopting this approach.[20] It is there-

Table 3. Characteristics of the YouTube videos according to the source of upload

Characteristic	Source of upload			
	hospital/clinic	educational	news	patient
DISCERN total score	32.5 ±9.8[a]	30.3 ±6.5[a]	37.0 ±19.8[a]	33.0 ±5.5[a]
Question 1	4.0 ±1.3[a]	4.2 ±1.1[a]	4.0 ±1.4[a]	4.6 ±0.6[a]
Question 2	3.2 ±1.2[a]	3.2 ±0.9[a]	3.3 ±1.8[a]	3.3 ±0.8[a]
Question 3	3.1 ±1.2[a]	3.0 ±0.8[a]	3.0 ±1.4[a]	2.8 ±0.6[a]
Question 4	1.7 ±1.2[acd]	1.0 ±0.1[ab]	2.8 ±2.5[c]	1.1 ±0.3[bd]
Question 5	1.0 ±1.0[a]	1.0 ±0.0[a]	1.0 ±0.0[a]	1.0 ±0.0[a]
Question 6	2.5 ±0.8[a]	2.6 ±0.8[a]	2.8 ±0.4[a]	2.6 ±0.9[a]
Question 7	1.3 ±0.7[a]	1.0 ±0.1[a]	1.5 ±0.7[a]	1.1 ±0.4[a]
Question 8	1.7 ±0.9[ab]	1.5 ±0.8[ab]	2.5 ±2.1[a]	1.1 ±0.3[b]
Question 9	2.8 ±1.2[a]	2.6 ±1.0[a]	2.0 ±1.4[a]	2.3 ±0.9[a]
Question 10	2.3 ±0.9[a]	2.0 ±1.0[a]	2.3 ±1.1[a]	2.8 ±0.8[a]
Question 11	2.0 ±1.5[a]	1.9 ±1.4[a]	3.0 ±2.8[a]	2.0 ±1.1[a]
Question 12	1.1 ±0.3[a]	1.4 ±1.0[a]	1.8 ±1.1[a]	1.1 ±0.4[a]
Question 13	1.5 ±0.7[a]	1.7 ±0.7[a]	1.8 ±0.4[ab]	2.9 ±1.1[b]
Question 14	1.6 ±1.2[a]	1.4 ±1.1[a]	3.0 ±1.4[a]	1.6 ±0.8[a]
Question 15	2.2 ±1.0[ab]	1.8 ±0.9[a]	2.5 ±2.1[ab]	2.6 ±0.8[b]
Question 16	2.6 ±1.1[a]	2.3 ±0.9[a]	3.0 ±1.4[a]	2.3 ±0.7[a]
View count *n*	23,029 ±54,191[a]	22,092 ± 53,870[a]	1,040 ±1,208[a]	44,560 ± 63,846[a]
Video duration [s]	221 ±178[a]	221 ±139[a]	170 ±111[a]	917 ±1,578[a]
Time since upload [days]	973 ±643[a]	1,149 ±611[a]	1,633 ±52[a]	936 ±538[a]
Channel subscribers *n*	43,149 ±102,281[a]	84,602 ±247,099[a]	303,232 ± 428,178[a]	115,188 ±344,053[a]
Comments *n*	19 ±39[a]	39 ±73[a]	0 ±0[a]	136 ±230[a]
Likes *n*	272 ±786[a]	329 ±712[a]	4 ±5[a]	610 ±1,120[a]
Dislikes *n*	11 ±23[a]	9 ±17[a]	NA	47 ±92[a]
Ratio of likes	94.64 ±7.95[a]	91.49 ±13.53[a]	NA	95.63 ±4.11[a]
VPI	23.0 ±45.6[a]	25.8 ±41.0[a]	NA	49.1 ±56.6[a]

Data presented as *M* ±*SD*.
VPI – video power index; NA – data not available. The mean values in the same row and subtable not sharing the same superscript are significantly different at $p < 0.05$ in the two-sided test of equality for column means. The tests assume equal variances and are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction.

fore recommended that patients be provided with accurate information regarding the botulinum toxin treatment strategy.

To the best of our knowledge, this is the first research to use a validated instrument to evaluate the effectiveness of YouTube videos on the use of Botox for the treatment of bruxism. In our study, the majority of YouTube videos exhibited a low level of informational quality regarding Botox and bruxism. The videos were classified as very poor (26.3%), poor (61.4%), fair (10.5%), and good (1.8%).

Table 4. Descriptive statistics and correlations for the study variables

Variable	*M* ±*SD*	1	2	3	4	5	6	7	8	9
DISCERN total score	32.3 ±7.3	–	–	–	–	–	–	–	–	–
View count *n*	32,587 ±58,515	0.268*	–	–	–	–	–	–	–	–
Video duration [s]	561 ±1,156	0.395**	0.380**	–	–	–	–	–	–	–
Time since upload [days]	1,024 ±580	0.180	0.253	–0.223	–	–	–	–	–	–
Channel subscribers *n*	98.57 ±282.86	0.234	0.445**	0.312*	0.136	–	–	–	–	–
Comments *n*	82 ±175	0.329*	0.829**	0.640**	0.009	0.433**	–	–	–	–
Likes *n*	448 ±947	0.343*	0.924**	0.582**	0.066	0.549**	0.936**	–	–	–
Dislikes *n*	29 ±69	0.350*	0.888**	0.438**	0.367*	0.575**	0.796**	0.848**	–	–
Ratio of likes	94.43 ±8.06	0.085	–0.326*	–0.012	–0.364*	–0.222	–0.069	–0.153	–0.445**	–
VPI	38.6 ±51.8	0.220	0.873**	0.464**	–0.125	0.453**	0.816**	0.935**	0.745**	–0.153

M – mean; *SD* – standard deviation. * statistically significant ($p < 0.05$, Spearman's test); ** highly statistically significant ($p < 0.01$, Spearman's test).

Table 5. Relationships between video features and the total DISCERN score

Video features	DISCERN total score		*p*-value
	no	yes	
Were the symptoms of bruxism discussed?	30.4 ±7.0	34.6 ±7.1	0.024*
Were the risk factors during treatment explained?	30.0 ±6.2	37.3 ±7.2	0.001*
Were the results of treatment presented?	26.6 ±6.1	35.4 ±5.9	<0.001*
Were the steps of the procedure described?	32.6 ±8.1	32.0 ±6.5	0.462
Was the prognosis discussed?	32.1 ±7.1	36.5 ±10.0	0.442
Was any form of animation incorporated in the video?	31.7 ±6.4	32.6 ±7.7	0.986
Were there diagrams?	32.3 ±7.3	NA	–
Was the muscle anatomy explained?	30.9 ±6.6	37.8 ±7.1	0.010*
Was the speaker a doctor?	31.1 ±6.8	33.7 ±7.6	0.233
Was is a patient's experience?	31.4 ±7.6	32.9 ±7.1	0.546

* statistically significant; NA – data not available.

Factors affecting the DISCERN scores

The overall low DISCERN ratings indicate that the material regarding botulinum toxin therapy for bruxism on YouTube is of low quality and in need of improvement. The videos did not accurately depict the period during which the information utilized in the publications was created (mean (*M*): 1.0), nor did they describe the source of the information used in the video (*M*: 1.3) or suggest other sources that could be used to explore the topic (*M*: 1.2). Moreover, the videos presented the effects of not using bruxism treatment only to a small extent (*M*: 1.2). However, they expressed video aims, with a mean DISCERN score of 4.3, and provided substantial support for collaborative decision making (*M*: 2.3) (Table 1).

The lowest mean overall DISCERN scores were associated with educational videos. This might be attributable to the fact that some of the videos were produced for commercial gain rather than patient education. Additionally, a considerable portion of educational videos lacked information about the advantages and disadvantages of treatment, as well as guidance for collaborative

decision making (Table 3). Patients should be informed of the numerous risks and benefits associated with each treatment option so that they could engage in discourse with medical experts about specific treatment that would be relevant to their individual situation. The highest mean overall DISCERN scores were achieved by the videos uploaded by news channels, which also appear to have greater resources than other channels to produce high-quality videos with suitable information, animations and diagrams (Table 3).

As the provision of accurate and comprehensive information is a time-consuming process, longer videos demonstrated a moderately positive correlation with the DISCERN scores. A considerably higher overall DISCERN score was associated with videos that were more popular, as indicated by higher view counts and greater numbers of likes and dislikes. This may be attributed to the fact that users like to share videos with other viewers when the content is clear and comprehensive, which leads to an elevated view count. The time elapsed since upload, the number of channel subscribers, the ratio of likes, and VPI were not related to the DISCERN score (Table 4).

According to Table 5, the DISCERN scores for the videos that described the symptoms ($p = 0.024$), risk factors ($p = 0.001$) and outcomes ($p < 0.001$) of bruxism treatment were significantly higher. This information is necessary for making an informed decision regarding therapy. Patients are better informed about the potential consequences of onabotulinumtoxinA treatment if they are presented with the risk factors associated with the procedure. On the other hand, showing the results of treatment encourages patients to undergo this therapy.

Other studies have shown that higher-quality videos often last longer than lower-quality videos.[21,22] Contrary to studies that correlate the popularity of videos with a low quality,[23] our analysis indicated that more popular videos had richer information and obtained higher DISCERN scores. Our findings suggest that patients prefer to read comprehensive information regarding botulinum toxin treatment for bruxism rather than watch emotionally charged or more captivating video content. Our research did not support the claim made in other studies that adding animation or graphics to videos might improve their quality.[22,24,25] Nevertheless, diagrams and animations may prove useful to better understand the therapeutic process.

A study by Grippaudo et al. demonstrated the inadequate quality of information on Botox available online.[26] The Internet content, particularly on the websites of practitioners, did not provide sufficient information about the alternatives, statistical advantages and risk factors associated with Botox therapy. The authors speculate that the information provided about Botox may serve an advertising purpose, aiming to increase the number of Botox patients.[26] It is worth noting that a recent study conducted by Ornello et al. demonstrated the potential efficacy of botulinum toxin injections in reducing both the frequency and intensity of migraines.[27] Moreover, the excellent response status can be identified as early as after the first injection.[27] In a group of senior chronic migraine patients with a long history of migraines, botulinum toxin may offer considerable improvement over the first 3 treatment cycles, just as it would in younger individuals.[28] However, older patients are unable to obtain adequate information about botulinum toxin therapy from YouTube due to the potential restriction of their access to relevant and reliable sources on the issue. Additionally, older individuals may encounter difficulties in accurately evaluating the reliability of online sources of information, such as YouTube, which can render them more vulnerable to misinformation.

In a study published in 2000, entitled "How will the Internet change our health system?", it was reported that information on the Internet could not be limited by any policy aims, as it might prove to be a significant differentiator for the competing health portals and suppliers of health information.[29] Twenty-two years later, the issue regarding medical restrictions on the treatment information remains unchanged. According to Yagiz et al., YouTube is an inadequate source for students or specialists seeking high-quality information on botulinum toxin therapy for gummy smile.[30] Similarly, Patel et al. identified a paucity of reliable material on the use of botulinum toxin in cosmetic surgery on YouTube and other Internet websites.[31] The most knowledgeable medical experts in this field are dental professionals and cosmetic surgeons, who should modify the abundance of poor-quality online resources available to people. In order to help patients better understand the advantages and disadvantages of available treatment options and to facilitate informed decision making, it is important to evaluate the content of the various websites that provide medical information. Even if their primary subject is Botox, YouTube videos should provide a more accurate and comprehensive overview of the benefits and risks associated with accessible bruxism treatment options. Castillo-Abdul et al. observed that Spanish YouTube influencers who used Botox for cosmetic purposes exhibited positive attitudes toward the procedure-related content.[32] This highlights the commercial nature of the tutorial content and the interaction-seeking strategies employed, reflecting the lack of content focusing on the care required beyond the procedure in most cases.[32] Thus, it is recommended that experts provide unbiased material which covers every aspect of therapy, without including any promotional content, and which solely serves to support medical and scientific evidence instead of focusing on a patient's experience.

Limitations

Our study have some limitations. The analysis was constrained to videos published in English on a single video-sharing platform (YouTube). The results could have been

impacted by the collection of data on a single day, given the continual evolution of the Internet's informational landscape. Only the first 30 videos for each keyword at a specific time point (October 30, 2022) were included due to the fact that the top search results on YouTube are typically the most viewed and influential videos, and thus have the greatest impact on viewers. Furthermore, we used the incognito mode and erased all the browsing data from the Google web browser. However, a formal power analysis to determine the sample size was not conducted. In order to ensure the inclusion of videos that patients might use when searching for health information online, an effort was made to use common phrases rather than medical terminology in the search process. This approach may have led to the discovery of other, possibly less useful or deceptive videos. The search results were sorted using relevance-based ranking by default; however, it should be noted that search rankings may change over time and depend on the user's location. The fact that the 2 reviewers were DISCERN-trained medical dental students in their fifth and fourth years represents a strength of our study. DISCERN is a powerful tool that was developed not just for experts, but also for non-specialists to help them evaluate medical videos objectively.

Future directions

Future research could focus on evaluating the quality of information available on other online platforms, such as social media or patient support groups. Additionally, the impact of inaccurate or incomplete information on patient decision making and treatment outcomes could be investigated. Ultimately, it is essential to ensure that patients have access to accurate and comprehensive information to make informed decisions about their healthcare.

Conclusions

In conclusion, the findings of our study indicate that the quality of information about Botox therapy for bruxism available on YouTube is generally poor. The educational videos had the lowest overall DISCERN scores, with many of them seemingly created for commercial purposes rather than for the purpose of patient education. Conversely, the videos produced by news channels had the highest overall DISCERN scores, indicating that these channels have the requisite resources to produce high-quality videos with accurate information.

The findings of the present study have significant implications for healthcare providers and patients. Healthcare providers need to be aware that patients may be accessing inaccurate or incomplete information about Botox therapy for bruxism on YouTube, and should take steps to educate their patients about the risks and benefits of this treatment. Patients, on the other hand, should be cautious when accessing medical information on YouTube and should consult their healthcare providers before making any decisions regarding their health.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Beddis H, Pemberton M, Davies S. Sleep bruxism: An overview for clinicians. *Br Dent J*. 2018;225(6):497–501. doi:10.1038/sj.bdj.2018.757
2. Fernández-Núñez T, Amghar-Maach S, Gay-Escoda C. Efficacy of botulinum toxin in the treatment of bruxism: Systematic review. *Med Oral Patol Oral Cir Bucal*. 2019;24(4):e416–e424. doi:10.4317/medoral.22923
3. Pierce CJ, Chrisman K, Bennett ME, Close JM. Stress, anticipatory stress, and psychologic measures related to sleep bruxism. *J Orofac Pain*. 1995;9(1):51–56. PMID:7581205.
4. Rugh JD, Barghi N, Drago CJ. Experimental occlusal discrepancies and nocturnal bruxism. *J Prosthet Dent*. 1984;51(4):548–553. doi:10.1016/0022-3913(84)90312-3
5. Lobbezoo F, Naeije M. Bruxism is mainly regulated centrally, not peripherally. *J Oral Rehabil*. 2001;28(12):1085–1091. doi:10.1046/j.1365-2842.2001.00839.x
6. Klasser GD, Rei N, Lavigne GJ. Sleep bruxism etiology: The evolution of a changing paradigm. *J Can Dent Assoc*. 2015;81:f2. PMID:25633110.
7. Okeson JP. The effects of hard and soft occlusal splints on nocturnal bruxism. *J Am Dent Assoc*. 1987;114(6):788–791. doi:10.14219/jada.archive.1987.0165
8. Saletu A, Parapatics S, Saletu B, et al. On the pharmacotherapy of sleep bruxism: Placebo-controlled polysomnographic and psychometric studies with clonazepam. *Neuropsychobiology*. 2005;51(4):214–225. doi:10.1159/000085917
9. Long H, Liao Z, Wang Y, Liao L, Lai W. Efficacy of botulinum toxins on bruxism: An evidence-based review. *Int Dent J*. 2012;62(1):1–5. doi:10.1111/j.1875-595X.2011.00085.x
10. Aslam S. YouTube by the numbers: Stats, demographics and fun facts. https://www.omnicoreagency.com/youtube-statistics. Accessed June 15, 2023.
11. Erdem MN, Karaca S. Evaluating the accuracy and quality of the information in kyphosis videos shared on YouTube. *Spine (Phila Pa 1976)*. 2018;43(22):E1334–E1339. doi:10.1097/BRS.0000000000002691

12. Sommerhalder K, Abraham A, Zufferey MC, Barth J, Abel T. Internet information and medical consultations: Experiences from patients' and physicians' perspectives. *Patient Educ Couns*. 2009;77(2):266–271. doi:10.1016/j.pec.2009.03.028
13. The DISCERN Questionnaire. https://www.ndph.ox.ac.uk/research/research-groups/applied-health-research-unit-ahru/discern/the-discern-questionnaire. Accessed June 15, 2023.
14. Desai T, Shariff A, Dhingra V, Minhas D, Eure M, Kats M. Is content really king? An objective analysis of the public's response to medical videos on YouTube. *PloS One*. 2013;8(12):e82469. doi:10.1371/journal.pone.0082469
15. Charnock D, Shepperd S. Learning to DISCERN online: Applying an appraisal tool to health websites in a workshop setting. *Health Educ Res*. 2004;19(4):440–446. doi:10.1093/her/cyg046
16. Charnock D, Shepperd S, Needham G, Gann R. DISCERN: An instrument for judging the quality of written consumer health information on treatment choices. *J Epidemiol Community Health*. 1999;53(2):105–111. doi:10.1136/jech.53.2.105
17. Weil AG, Bojanowski MW, Jamart J, Gustin T, Lévêque M. Evaluation of the quality of information on the Internet available to patients undergoing cervical spine surgery. *World Neurosurg*. 2014;82(1–2):e31–e39. doi:10.1016/j.wneu.2012.11.003
18. Murali RV, Rangarajan P, Mounissamy A. Bruxism: Conceptual discussion and review. *J Pharm Bioallied Sci*. 2015;7(Suppl 1):S265–S270. doi:10.4103/0975-7406.155948
19. Zhang LD, Liu Q, Zou DR, Yu LF. Occlusal force characteristics of masseteric muscles after intramuscular injection of botulinum toxin A (BTX-A) for treatment of temporomandibular disorder. *Br J Oral Maxillofac Surg*. 2016;54(7):736–740. doi:10.1016/j.bjoms.2016.04.008
20. Al-Wayli H. Treatment of chronic pain associated with nocturnal bruxism with botulinum toxin. A prospective and randomized clinical study. *J Clin Exp Dent*. 2017;9(1):e112–e117. doi:10.4317/jced.53084
21. Ozsoy-Unubol T, Alanbay-Yagci E. YouTube as a source of information on fibromyalgia. *Int J Rheum Dis*. 2021;24(2):197–202. doi:10.1111/1756-185X.14043
22. Śledzińska P, Bebyn MG, Furtak J. Quality of YouTube videos on meningioma treatment using the DISCERN instrument. *World Neurosurg*. 2021;153:e179–e186. doi:10.1016/j.wneu.2021.06.072
23. Huang MM, Winoker JS, Allaf ME, Matlaga BR, Koo K. Evidence-based quality and accuracy of YouTube videos about nephrolithiasis. *BJU Int*. 2021;127(2):247–253. doi:10.1111/bju.15213
24. Szmuda T, Rosvall P, Hetzger TV, Ali S, Słoniewski P. YouTube as a source of patient information for hydrocephalus: A content-quality and optimization analysis. *World Neurosurg*. 2020;138:e469–e477. doi:10.1016/j.wneu.2020.02.149
25. Cassidy JT, Fitzgerald E, Cassidy ES, et al. YouTube provides poor information regarding anterior cruciate ligament injury and reconstruction. *Knee Surg Sports Traumatol Arthrosc*. 2018;26(3):840–845. doi:10.1007/s00167-017-4514-x
26. Grippaudo FR, Atzeni M, di Pompeo FS. Review of quality of patient information regarding Botox® cosmetic on the Internet. *J Plast Reconstr Aesthetic Surg*. 2016;69(3):e64–e66. doi:10.1016/j.bjps.2015.12.008
27. Ornello R, Baraldi C, Ahmed F, et al. Excellent response to onabotulinumtoxinA: Different definitions, different predictors. *Int J Environ Res Public Health*. 2022;19(17):10975. doi:10.3390/ijerph191710975
28. Altamura C, Ornello R, Ahmed F, et al. OnabotulinumtoxinA in elderly patients with chronic migraine: Insights from a real-life European multicenter study. *J Neurol*. 2023;270(2):986–994. doi:10.1007/s00415-022-11457-5
29. Goldsmith J. How will the Internet change our health system? *Health Aff (Millwood)*. 2000;19(1):148–156. doi:10.1377/hlthaff.19.1.148
30. Yagiz O, Yavuz GY, Keskinruzgar A, Acibadem E. Analyses of YouTube videos on Botox treatment of gummy smile. *J Craniofac Surg*. 2022;33(4):e433–e438. doi:10.1097/SCS.0000000000008375
31. Patel AA, Mulvihill L, Jin A, Patel A, Galiano RD. Websites or videos: Which offer better information for patients? A comparative analysis of the quality of YouTube videos and websites for cosmetic injectables. *Plast Reconstr Surg*. 2022;149(3):596–606. doi:10.1097/PRS.0000000000008827
32. Castillo-Abdul B, Jaramillo-Dent D, Romero-Rodriguez LM. 'How to Botox' on YouTube: Influence and beauty procedures in the era of user-generated content. *Int J Environ Res Public Health*. 2021;18(8):4359. doi:10.3390/ijerph18084359

Original paper

Searching for a relationship between the elemental composition of archaeological bones and the occurrence of caries

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Abstract

Background. Although the macroscopic assessment of dental caries and the assessment of bone elemental composition are quite different, efforts can be made to identify commonalities in the assessment of health and nutritional quality. Both indicators are correlated with dietary habits and are dependent on taphonomic processes occurring in the postmortem substrate. However, teeth exhibit structural resilience of their hard tissues to adverse environmental factors.

Objectives. The aim of the study was to establish a correlation between the elemental composition of bones and the presence of carious lesions.

Material and methods. The study material consisted of the following skeletal parts: 161 permanent teeth from 36 individuals and bridge fragments of 36 ribs. The presence of caries was assessed visually using a modified International Caries Detection & Assessment System (ICDAS II) scale. The rib samples were subjected to elemental analysis (zinc (Zn), iron (Fe), magnesium (Mg), calcium (Ca), phosphorus (P), strontium (Sr), barium (Ba)) using spectroscopic methods.

Results. The odontological and chemical analyses did not reveal any statistically significant relationships between the Ca/P diagenesis index and dental features. Postmortem tooth loss showed a weak correlation with the diagenesis index.

Conclusions. Discoloration, cracks and flaking of the dental crown surfaces may be associated with the intensity of Ca/P diagenesis. However, no significant correlation was found between these phenomena. Among other elements, only Zn levels exhibited a correlation with the caries index.

Keywords: diagenesis, bioarchaeology, bone elemental concentration

Background

One of the goals of dental studies of ancient populations is to accurately assess carious lesions on the surfaces of dental crowns of deciduous and permanent teeth. The loss of hard tooth tissue is significantly influenced by chemical and physical factors. The effective detection and quantification of carious lesions enables the estimation of the incidence and severity of caries within the studied population.[1]

The modern theories of the formation of carious lesions unanimously emphasize that caries is a dynamic process that varies in time and involves many microbiological factors as well as chemical and physical phenomena, resulting in the destruction of hard tooth tissues.[2] Among these, exogenous nutritional factors play an important role. Exogenous factors include processed foods with high viscosity and high levels of simple carbohydrates. The consumption of these products may result in an increased prevalence of advanced caries.[3]

Prior to the formation of medium and deep enamel defects as well as the destruction of dentin, characteristic discoloration is observed on the surfaces of dental crowns. This discoloration typically manifests as dull, chalky white spots associated with the loss of dihydroxyapatite. In the subsequent phase, black and brown spots can be observed within enamel micropores, which are caused by food-derived dyes.[4]

The biggest challenge in evaluating caries indicators, such as the severity of caries in ancient human populations, is accurately identifying the earliest carious lesions. This can be particularly challenging due to taphonomic changes, which can both mask the presence of early carious lesions or mimic them.[5] Another obstacle is postmortem tooth loss, which precludes the assessment of caries on absent teeth.

Incorrect or imprecise estimation of caries indicators in bioarchaeological studies affects the assessment of the masticatory organ condition and, indirectly, the hygienic and nutritional status of the examined population.[6] These studies implicitly provide indirect information on nutritional status, hygiene, eating habits, and socio-economic stratification in the population. Inaccurate assessment of the state of the masticatory organ indirectly results in an erroneous evaluation of the aforementioned aspects of the studied population.[1,7]

Chemical analysis of preserved hard tissues can be employed to obtain similar information about a given population. However, the relationship between diet and the elemental composition of bones obtained at archaeological sites is far from the simple "you are what you eat" model, and the method itself is prone to errors and overinterpretations. Nevertheless, with proper procedures, some metals can be successfully used in bioarchaeological research.[8] However, it is worth emphasizing that there is still a significant challenge (especially in the context of archaeological research) of determining the extent to which the original chemical composition of human bones has changed as a result of processes that occur after the death of an individual.[9,10] The knowledge about diagenetic changes (related to postmortem bone degradation) regarding the addition or removal of specific elements from human bones is incomplete.[10]

The most useful elements in the chemical analysis of bone excavation materials are calcium (Ca) and phosphorus (P). While not indicative of an individual's dietary habits, the calcium-to-phosphorus (Ca/P) ratio can be used to estimate the extent of diagenetic changes in decomposing bone, which are influenced by the chemical composition of the surrounding substrate. In the absence of substrate chemical influence, the Ca/P ratio in bone is expected to remain stable at approx. 2.1. However, due to diagenesis, it can increase significantly.[11–13]

The studies of other elements, such as barium (Ba), iron (Fe), magnesium (Mg), strontium (Sr), and zinc (Zn) remain more controversial. Theoretically, these elements could be used as potential biomarkers of diet assessment quality and its components, as well as indirectly to assess socio-environmental and economic differences that existed between the studied human groups.[14] However, in practice, the usefulness of elemental bone analysis is significantly impaired by postmortem diagenetic changes.[15] A bone deposited in soil is susceptible to element substitution, which results in composition changes over time.[16] For this reason, methods of diet reconstruction based on the elemental composition of extracted bone samples, quite commonly used in the 1970s and 1980s, have been the subject of increased criticism.[8,16,17] However, with careful analysis of the results and proper procedures, they can still be used successfully.[8,11,17]

Although the macroscopic assessment of dental caries and the assessment of elemental composition of bones are different markers, it is still possible to identify common points in the assessment of health and nutritional quality. Both indicators are correlated with diet and dependent on the taphonomic processes occurring in the postmortem substrate, despite the observed resilience of teeth's hard tissues to adverse environmental factors.[5] On the one hand, taphonomic processes occurring at the place of deposition of bone remains may have a secondary impact on the state of dentition (including the number of teeth preserved), the state of alveolar processes, enamel discoloration, etc., and thus the detection of caries. On the other hand, taphonomic processes can also impact the reliability of chemical analysis of elements.

The concentrations of Ba, Sr, Fe, Mg, and Zn in the rib samples were found to be outside the ranges of concentrations of these elements reported in bones of modern humans (without the influence of diagenesis), which were used as reference data. Therefore, we assumed that the diagenesis process may have influenced the concentration in the examined bones. Conversely, if the concentration

of a given element in the examined bone did not differ significantly from the so-called reference data, it was assumed that the diagenesis process had no impact on its concentration. Instead, its origin was attributed to the diet (i.e., the food and water consumed and drank by the individual to whom this bone belonged) and/or the biological condition of the individual's body. This interpretation is consistent with that used in other studies concerning the impact of diagenesis on the concentration of elements in human bones.[8] The reference data was obtained from the literature and is presented in Table 1.

However, this issue is highly complex.[27] Based on the aforementioned methodology, it is not possible to determine the standard limit of the concentration of a given element in human bone, above or below which (in the case of hypothetical loss of elements from bone) the processes of diagenesis occur. This is attributable to inter-individual variations in the concentration of these elements in the bones of living individuals. Therefore, only a significant deviation from the so-called reference data may be useful in determining whether the concentration of a specific element in bone is of diagenetic origin.[8,10]

Objectives

The question of whether there are significant links between these indicators remains open at present, given the indicated common dependencies. The answer to this question may be an attempt to assess the potential stochastic relationships between the state of dentition and the elemental composition of bones.

Material and methods

Population characteristics

The research material consisted of the following skeletal parts: 161 permanent teeth from 36 individuals and bridge fragments of 36 ribs from the collection of the Department of Anatomy, Wroclaw Medical University, Poland. Bone remains were obtained during the exploration of the archaeological site in the former church cemetery of St. Barbara in Wrocław, Poland (Fig. 1).

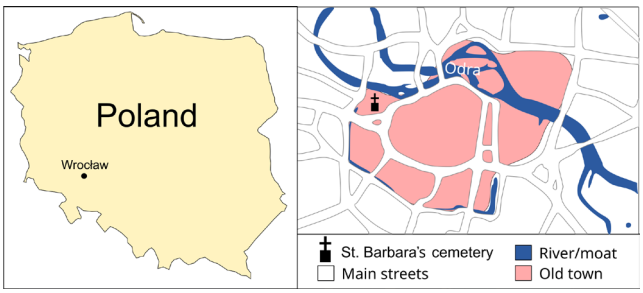

Fig. 1. Location of the cemetery from which the material was extracted

Table 1. Review of the concentrations of the elements analyzed in the bones of modern human populations

Element	Study	Rib bone samples (*n*)	Mean value (range) [µg/g]
Ba	Samudralwar and Robertson[19]	F&M (80)	2.54 (0.57–7.70)
	Zaichick et al.[18,20]	F&M (168)	0.94
	Brätter et al.[21]	F&M (12) cortical	36
		F&M (12) cancellous	19
	Yoshinaga et al.[22]	F (38)	2.86 (1.13–7.70)
		M (42)	2.24 (0.57–5.22)
Sr	Samudralwar and Robertson[19]	F&M (80)	29 (36–1,163)
	Takata et al.[23]	F&M (69)	400
	Yoshinaga et al.[22]	F (38)	334 (36–1,163)
		M (42)	252 (58–701)
	Yoshinaga et al.[24]	F&M (18)	176 (87–345)
	Brätter et al.[21]	F&M (12) cortical	62
		F&M (12) cancellous	58
Fe	Samudralwar and Robertson[19]	F&M (80)	140 (23.4–448)
	Yoshinaga et al.[24]	F&M (18)	31.2
	Crawford and Crawford[25]	F&M (13)	842 (224–917)
	Koch et al.[26]	F&M (45)	71
	Brätter et al.[21]	F&M (12) cortical	23
		F&M (12) cancellous	77
Mg	Samudralwar and Robertson[19]	F&M (80)	2,139 (1,118–2,876)
	Simpson et al.[27]	F&M (8)	620
	Crawford and Crawford[25]	F&M (18)	4,184 (2,886–5,321)
	Koch et al.[26]	F&M (45)	2,850
		F (28)	2,920
		M (17)	2,730
	Brätter et al.[21]	F&M (12) cortical	2,600
		F&M (12) cancellous	2,700
Zn	Samudralwar and Robertson[19]	F&M (80)	92.8 (53.6–128.0)
	Burak and Okólska[28]	F&M (2)	26 (18–34)
	Crawford and Crawford[25]	F&M (13)	220 (154–278)
	Brätter et al.[21]	F&M (12) cortical	180
		F&M (12) cancellous	144

Ba – barium; Sr – strontium; Fe – iron; Mg – magnesium; Zn – zinc; M – male; F – female.

The dating of the archaeological site at the church of St. Barbara was based on relative methodology, including historical sources, fragments of preserved epitaphs displayed in the church, stratigraphic research, and radioisotope methods.[28] The content of ^{14}C carbon isotope in the bone samples from 18 individuals across all stratigraphic

levels indicates that the cemetery was in use between 1695 and 1919 (±25 years). Based on the historical verification of the data, it can be inferred that the individuals whose bone remains were examined had been buried between the end of the late 17th and late 18th centuries.[11]

The cemetery was located in close proximity to the Gothic cemetery temple dedicated to Saint Barbara, a patron of hospitalization and poverty (Fig. 1). Historical sources, tombstone epitaphs and urban sociotopography from the 16th to 19th centuries indicate several distinct social groups interred in the cemetery, from which the archaeological material used in the research was derived. The groups include the poorer craftsmen, patients of numerous hospitals and shelters surrounding the cemetery, and a few wealthy townsmen from Protestant families.[28]

All skeletal remains used in the current study were excavated from the soil. As a result, they were subjected to diagenesis and a variety of taphonomic processes. The degree of diagenesis for each individual was estimated by calculating the Ca/P ratio.

Sex and age at death

Sex was determined by examining skull morphology and, when possible, the characteristics of the pelvic bone.[29,30] The age of individuals was estimated using both cranial and post-cranial skeletal features, including changes in the auricular surfaces of the ilia and pubic symphyses,[29,30] as well as the closure of cranial sutures and tooth crown wear.[30–32] If the post-cranial skeleton was not available, age estimation relied solely on the cranial skeleton.

Due to the poor state of preservation of the bone remains, it was not possible to determine the sex and biological age at the time of death in all cases (morphological gender was determined in 20 cases) (Table 2). The bone material and permanent dentition were subjected to further analysis in 28 adults and 8 juveniles (Table 2).

Table 2. Distribution of sex and age among the examined individuals

Variable		Individuals, *n*
Sex	male	16
	female	4
	unknown	16
Age	juveniles (<18 years)	8
	adults (≥18 years)	28

Evaluation of caries

The intensity of caries was visually assessed using a modified International Caries Detection & Assessment System (ICDAS II) scale, which is suitable for both daily clinical practice and epidemiological studies.[33] This scale has been modified for the study of archaeological materials.[6] The modified scoring system is presented in Table 3.

Table 3. Modified International Caries Detection & Assessment System (ICDAS II) scale criteria

Code	Criterion
1	No caries detected.
2	Distinct visual change in enamel.
3	Localized enamel breakdown (without clinical visual signs of dentinal involvement).
4	Underlying dark shadow from dentin.
5	Distinct cavity with visible dentin.
6	Extensive distinct cavity with visible dentin (>50% of the surface).

The objective of the individual assessment of caries intensity was to determine the stage of carious lesions with the highest degree among all lesions described in the upper and lower alveolar arches using the ICDAS II scale. In addition to evaluating the presence of caries on individual teeth, indicators were employed to estimate the intensity of caries in the examined individuals. The commonly used indicator, the caries index, was calculated by dividing the number of teeth with caries by the number of preserved teeth.[34]

Mineralization of research material

The samples were subjected to wet mineralization using a closed MARS 6 microwave system (CEM Corporation, Matthews, USA).

A homogeneous sample weighing between 0.1 g and 0.5 g was placed in preparation vessels, to which 5 cm^3 of concentrated nitric acid (V) (Chempur, Piekary Śląskie, Poland) was added. The samples were subjected to mineralization in the microwave sample preparation system. The minerals were quantitatively transferred to 10-cm^3 measuring vessels using redistilled water. Mineralization was conducted in accordance with the Polish standard PN-EN 13805:2003. Foodstuffs – Determination of trace elements – Pressure digestion.[35]

Determination of elements in the research material

The atomic absorption or emission spectrometry with an acetylene/air flame was employed to determine the Fe, Zn, Mg, and Ca contents in the materials. The analysis was conducted using a SpectraAA atomic absorption spectrometer (Spectralab Scientific Inc., Markham, Canada) and a Varian AA240FS flame attachment (Spectralab Scientific Inc.). The Sr and Ba levels were determined using inductively coupled plasma optical emission spectrometry (ICP-OES). The phosphorus content was quantified with a spectrophotometer (Thermo Spectronic Unicam UV-300; Thermo Fisher Scientific, Waltham, USA).

The accuracy of the method was validated using the National Institute of Standards and Technology (NIST) 1486 Bone Meal (Sigma-Aldrich, St. Louis, USA). The estimated measurement uncertainty was 5%.

The elements were determined in accordance with the following standards:

- Zn, Fe: PN-EN 12143:2000. Fruit and vegetable juices. Determination of soluble matter content by refractometric method[36];
- Mg, Ca: PN-EN ISO 6869:2000. Feedstuffs. Determination of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc by atomic absorption spectrometry.[37]

In order to assess the occurrence and intensity of diagenesis in examined rib bone samples, the mass ratio of Ca to P was calculated. A ratio above 2.16 is indicative of structural degradation of the bone (higher values indicate stronger bone degradation).[12,13]

Statistical analysis

The normality of the distribution for each variable was evaluated using the Shapiro–Wilk test. Pearson's analyses were performed using Statistica 13.1 software (TIBCO Software, Palo Alto, USA) to determine the correlation between dental condition traits, the concentration of the analyzed elements in the examined bones, and the diagenesis index. The assumptions of Pearson's correlation test were satisfied because at least 1 variable pair was normally distributed in each comparison.[38] The results were considered statistically significant for a p-value <0.05.

Results

Analysis of the prevalence of caries

The dental condition characteristics of the early modern population of Wrocław were based on an examination of carious changes in crowns and dental roots. This examination was conducted through a visual assessment of 161 permanent teeth obtained from 36 individuals. The total number of teeth in the alveolar arches was determined, including both carious and non-carious teeth, as well as those that had been lost antemortem. Information related to taphonomic processes included the number of teeth lost postmortem, as evidenced by the number of empty alveolar sockets. The characteristics of the severity of carious cavities were also presented, with a particular emphasis on the specific "predominance state", which represents the highest degree in the ICDAS II classification. Therefore, individuals were classified according to the most advanced form of dental caries, with the weaker forms of the disease on dental crowns being disregarded. In addition to the individual assessment of caries severity, caries intensity indexes were also calculated. The list of parameters for assessing the state of dentition in the form of descriptive statistics is presented in Table 4.

Table 4. Statistical data regarding the masticatory organs of the studied population

Variable	*Me*	Minimum	Maximum
Missing teeth, *n*	13	5	26
Teeth antemortem, *n*	0	0	24
Teeth postmortem, *n*	11	2	24
Teeth in the upper jaw, *n*	0	0	10
Teeth in the lower jaw, *n*	3	0	11
Teeth with caries, *n*	2	0	12
Teeth with caries in the crown, *n*	3	1	12
Teeth with caries in the root, *n*	0	0	2
Teeth with the highest caries grade, *n*	3	1	6

Me – median.

A considerable number of missing teeth were observed in the examined material. On average, approx. 13 teeth were outside the possibilities of dental assessment, with nearly 11 teeth lost postmortem. Undoubtedly, the decrease in the number of teeth available for examination may have influenced the further assessment of the distribution of caries. On average, 80% of the preserved teeth in the examined individuals were affected by carious lesions, with the maximum number of teeth affected by caries in a single case not exceeding 12. Among the carious lesions observed in 36 individuals, the most prevalent defect was classified as code 3 on the ICDAS II scale. An important finding about the nature of the disease in the study group is the relatively low average number of teeth lost antemortem, which may be linked to cariogenic processes (Table 4).

Diagenesis and the content of selected micronutrients

The range of values obtained for the diagenesis index (Ca/P ratio) for the examined human rib samples (n = 35; minimum Ca/P ratio = 2.099, maximum Ca/P ratio = 5.048) and the mean value of this trait (3.296) is indicative of the influence of diagenesis processes on these bones (with the exception of 1 sample, where the index value did not exceed 2.16) (Table 5). These results were expected due to 2 significant factors: the bones were derived from a cemetery dating to the 17th–18th centuries AD; and ribs are more susceptible to diagenesis processes compared to other bones, such as femur or skull bones.[39]

The mean concentration of Ba obtained in this study was 44.9 µg/g (range: 3.8–194.7 µg/g) (Table 5). Although this value is higher than the reference mean for this element in most specimens (approx. 65%) of the examined sample, the Ba concentration is within the widest reference range established for modern human ribs based on the available literature data (0.94–36.00 µg/g) (Table 1). Thus, the results indicate that diagenesis had a significant impact on the Ba concentration in approx. 35% of the examined bones.

Table 5. Summary statistics of the concentration of the analyzed elements in the rib bone sample

Element	Samples analyzed, *n*	*M* [μg/g]	*SD* [μg/g]	Range (min–max) [μg/g]
Ba	35	44.9	38.72	3.8–194.7
Sr	35	145.5	43.79	55.3–280.2
Fe	36	4,818.9	4,380.24	350.7–16,600.7
Mg	36	3,285.0	1,957.51	288.2–9,558.3
Zn	36	569.0	222.94	166.8–1,157.0
Ca	36	315,685.3	48,175.34	259,801.8–445,511.9
P	35	11,942.4	23,699.98	3,523.9–96,709.1
Ca/P ratio	35	3.3	0.62	2.1–5.0

Ca – calcium; P – phosphorus; *M* – mean; *SD* – standard deviation.

The mean Sr concentration in the examined rib samples (145.5 μg/g) (Table 5) was lower than the reference value (e.g., means: 252 μg/g or 334 μg/g) (Table 1). Furthermore, the obtained Sr concentrations (range: 55.3–280.2 μg/g) fall within the range of variability of this trait established for ribs of modern adult humans (36–1,163 μg/g) (Table 1). This suggests that diagenesis had no impact on the Sr concentration in the examined bones.

The mean Fe concentration was found to be 4,818.9 μg/g (range: 350.7–16,600.7 μg/g) (Table 5). This value is higher than the mean reference value (Table 1), and for most specimens, it is above the upper limit of the widest referential range (23.4–917.0 μg/g) (Table 1). Considering the prevailing view that elevated Fe levels in human bones from archaeological studies are likely caused by diagenesis,[8,40,41] a diagenetic origin is also suggested in this study.

The mean Mg concentration was 3,285 μg/g (range: 288.2–9,558.3 μg/g) (Table 5). According to the reference data, the widest range of variability for this trait (mean range: 620–4,184 μg/g) includes the Mg concentration values obtained for the majority of the specimens analyzed in this study. Therefore, a biogenic origin of Mg can be suggested for these specimens. A smaller subset of specimens included in the sample indicates higher Mg values (>4,184 μg/g). However, these values do not significantly deviate from the upper limit of the abovementioned reference range. In summary, the results indicate that the majority of the sample can be attributed to a biogenic origin of Mg.

The mean concentration of Zn (569 μg/g) significantly exceeds the reference averages established for the ribs of modern humans (e.g., 26 μg/g or 220 μg/g) (Table 1). Almost all Zn concentrations determined for the tested specimens (except for 1 individual) are higher than the upper limit of the widest reference range for this feature (18–278 μg/g) (Table 1). According to the method employed in this study, a large deviation from the upper limit of the reference range suggests a diagenetic origin of the Zn in the majority of the examined samples.

Correlation matrix: dental condition vs. elements and Ca/P ratio

The results of Pearson's analyses, conducted to determine the statistical relationship between element concentration and dental condition, indicated that only 2 correlations were statistically significant or close to the threshold of the confidence interval: first, a positive and weak correlation between the Ca/P ratio and the number of missing teeth; second, a negative and weak correlation between Zn concentration and the caries index (Table 6). No statistically significant correlation was found between any of the dental condition indicators and the Ca/P ratio.

Discussion

The objective of this study was to explore potential relationships between the elemental composition of bones and the state of dentition, as assessed through the analysis

Table 6. Results of the correlation analysis between the dental condition indicators and the content of selected micronutrients in the bone material

Variable	Zn		Fe		Mg		Ca		P		Sr		Ba		Ca/P ratio	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
Missing teeth	0.049	0.782	–0.119	0.501	–0.027	0.879	0.012	0.948	0.084	0.641	–0.225	0.208	–0.211	0.238	0.326	0.064
Teeth antemortem	–0.006	0.976	–0.255	0.146	0.185	0.296	–0.031	0.861	0.013	0.942	–0.143	0.427	–0.104	0.564	0.037	0.839
Teeth postmortem	0.059	0.742	0.151	0.393	–0.231	0.188	0.047	0.793	0.076	0.676	–0.084	0.644	–0.112	0.536	0.308	0.081
Teeth in the upper jaw	0.336	0.052	0.144	0.416	0.216	0.220	0.026	0.884	–0.131	0.467	–0.042	0.815	–0.156	0.386	0.019	0.917
Teeth in the lower jaw	0.109	0.540	0.039	0.826	–0.009	0.959	–0.088	0.622	–0.184	0.306	0.024	0.895	–0.027	0.882	–0.166	0.356
Teeth with caries	0.247	0.146	0.203	0.235	0.162	0.345	–0.196	0.251	–0.238	0.169	0.087	0.618	0.035	0.844	–0.205	0.238
Teeth with caries in the crown	0.263	0.133	0.179	0.311	0.190	0.282	–0.083	0.641	–0.189	0.293	0.095	0.601	0.018	0.920	–0.164	0.361
Teeth with caries in the root	0.173	0.327	0.220	0.212	–0.257	0.143	–0.064	0.720	–0.105	0.560	–0.052	0.774	–0.125	0.488	0.093	0.605
Teeth with the highest caries grade	–0.099	0.576	0.072	0.684	–0.146	0.411	–0.087	0.627	0.007	0.969	–0.077	0.671	–0.229	0.200	0.074	0.684
Caries index	–0.336*	0.045*	0.177	0.303	–0.004	0.981	–0.204	0.234	0.254	0.142	0.028	0.871	0.285	0.097	–0.048	0.782

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

of carious lesions. The impact of secondary diagenesis processes was also taken into account.

We aimed to detect a relationship between the concentration of specific elements (Ba, Sr, Mg, Fe, and Zn) in bones, the index of the diagenesis intensity (Ca/P ratio) of these bones, and the broader dental condition traits of the examined individuals, while taking into account the potential impact of diagenesis on the concentration of the abovementioned elements. With regard to the diagenesis index, the study aimed to assess the relationship between the intensity of diagenesis in human bones and 2 types of features characterizing the condition of the teeth: the number of teeth (the number of teeth lost postmortem may be positively related to the intensity of diagenesis influencing the bones); and the intensity of caries development (a positive correlation between the index of caries intensity and the intensity of diagenesis suggests the influence of diagenesis on the occurrence of changes that mimic caries, particularly in early caries).

Macroscopic observations of carious cavities indicate that the disease course was more intense among the studied population from early modern Wrocław. However, the reduction in the number of observations resulting from postmortem tooth loss could have affected the assessment of caries intensity. The individual nature of carious defects, according to modern standards, indicates moderate caries localized to specific areas. On average, carious lesions were recorded at code 3 on the ICDAS II scale, indicating limited local penetration into dental crowns without affecting the dentin.

The results of the odontological and chemical analyses did not reveal statistically significant relationships between the Ca/P diagenesis index and any of the macroscopic variables (Table 6). For the number of teeth lost postmortem (r = 0.308, p = 0.081), the result is on the verge of statistical significance, suggesting a possible but weak dependence.

Changes observed on the surface of dental crowns and exposed parts of dental roots, such as macroscopically visible discoloration, flaky surfaces, cracks, and chips, were hypothesized to be most related to the Ca/P diagenesis index. This association was considered plausible given that secondary diagenesis processes often correlate indirectly with the poor macroscopic preservation of remains.[42] This, in turn, could result in false positive assessments of lesions conducted macroscopically, which may appear similar to cariogenic defects but are actually specific pseudopathologies. However, the analysis demonstrated no correlation between the caries index and the Ca/P ratio (r = –0.048, p = 0.782). This suggests that dental assessment of the teeth condition should be considered an independent phenomenon in relation to the process of secondary diagenesis, at least in the context of the tested archaeological material. Numerous studies indicate that dentition is minimally affected by postmortem environmental factors and remains largely unaltered.[43]

Bone tissue undergoes antemortem modifications due to metabolic activity (e.g., as a consequence of disease, inflammatory processes and hyperplasia) and mechanical injuries. In contrast, its secondary transformations occur postmortem due to taphonomic factors, such as substrate pH, water flow and the chemical composition of the burial site. The passage of time significantly affects the preservation of bone material.[44] Thus, the mineral composition of the deposited remains may vary depending on the type and intensity of diagenetic factors affecting the deposited bones, such as those in burial pits. Changes in the chemical composition of bone tissue may be the basis for further transformations and damages of a macroscopic nature, both in terms of its volume and structure.[45]

In the case of searching for potential correlations between the concentration of the analyzed element in the bones and traits of the dental condition, the results suggest a biogenic origin for Sr and Mg, and partially for Ba (significant diagenesis is suggested in approx. 35% of the sampled bones). The majority of the examined bones exhibited predominantly diagenetic origins for Fe and Zn.

The prevailing view is that the concentration of Ba and Sr in human bones is related to diet.[8,46–48] The majority of studies suggest that diagenesis has no effect on the levels of these elements in bones. However, contrary results have been reported, indicating that diagenetic influences may be present.[49–52] Thus, the comparison of the results obtained with the so-called reference data was helpful in determining whether, in the case of analyzed bone samples, the level of these elements is related to the influence of diagenesis. Notably, the relationship between the geographical area inhabited by human populations (especially archaeological) and the specific concentration of Ba and Sr in bones is a consequence of the composition of available water and the type of vegetation occurring in a given area. It is commonly believed that modifying one's diet to include a greater proportion of plant-based foods will result in the elevated levels of Sr and Ba in bones.[53,54] However, the relationship between the Sr and Ba content in food and Sr and Ba levels in the bones of an individual consuming that food remains unclear.[55] In light of our interpretation that the influence of diagenesis on the Ba concentration concerned about 35% of bones in the sample, the presence of Ba of diagenetic origin could potentially influence the results of the statistical analysis, as evidenced by the absence of a significant correlation between the level of this element and dental condition traits.

Studies on the impact of a diet without Sr in rats and guinea pigs indicated reduced calcification of bones and teeth and an increase in the occurrence of dental caries.[56] The results of these studies may, therefore, suggest a relationship between the concentration of this element in human bones and dental condition. Thus, it is probable that a low concentration of Sr in bones may be associated with a higher risk of the occurrence of caries. The statistical analysis of the sample revealed no statistically significant relationships between the Sr level and the analyzed dental

condition features, including those describing the occurrence of caries. The relatively narrow range of Sr concentrations obtained in our sample, when compared to the available data (Table 1), suggests that the diets of the examined individuals were likely similar, as was the composition of the water they consumed. The limited variability in Sr concentration may have reduced the likelihood of detecting a relationship between this element and the occurrence of dental caries. Therefore, further research on this matter is necessary, including a sample of individuals with more diverse diets. Considering the high metabolic turnover rate of rib bones,[39] future studies should also include other types of bones, such as the femur.

Previous studies on the impact of diagenesis on Mg concentration in human bones have yielded disparate results, with some indicating the influence of diagenesis on Mg levels and others suggesting no such influence.[52,57,58] Diagenesis processes may cause a decrease or an increase in Mg concentration in bones.[59] Magnesium is important for overall body functioning, including bone mineralization and its development.[60–62] Approximately 60% of Mg present in the human body is stored in bones.[63] Its deficiency affects bone density,[62] and animal studies have demonstrated that a diet with inadequate Mg contributes to the development of osteoporosis.[62] Elevated levels of Mg in bones may influence bone metabolism and cause defective mineralization.[64] In this study, the statistical analysis results showed no relationship between the dental condition traits and Mg concentration in bones. Further studies with larger bone samples (free from the effects of diagenesis) are required to determine whether a positive relationship exists between Mg concentration and good dental condition.

If our interpretation that Fe and Zn in the examined bones predominantly originate from diagenesis is correct, the possibility of detecting potential relationships between the concentration of these elements of biogenic origin and the traits of the dental condition is strongly obscured by the phenomenon of diagenesis. Thus, the significant correlation found in this study between Zn concentration and 2 traits of the dental condition is difficult to explain. The negative and weak correlation between Zn concentration and the caries intensity index ($r = -0.336$, $p = 0.045$) may be attributed to the influence of diagenesis on Zn concentration.

This result is close to the threshold of statistical significance, which suggests that caution should be exercised against dismissing it as a false positive result. Nevertheless, in the absence of a correlation between caries indicators and other chemical elements, several premises indicate that this correlation may not be merely coincidental.

Regarding Zn compounds, the variations in Zn ion concentration in bone material attributable to the type of sediment and soil acidity result from the substitution of Ca in hydroxyapatite crystals. This implies that the proportion of Zn ions in postmortem bones may be greater than in new bone tissue. The degree of tissue Zn saturation is more intense in wet and acidic deposits, similar to the concentration changes observed for other metals.[44] However, these changes are not significant and should not deviate from the typical range observed in new bone tissue. In addition, the elevated concentration of Zn ions in bone tissue may be the result of an individual's diet consumed during their lifetime.[39]

Zinc compounds participate in the prevention of caries at multiple levels, through direct and indirect interactions with tooth tissue. In both cases, the endogenous distribution of Zn ions is dependent on the presence of suitable nutrients.

The indirect effect focuses on the presence of Zn compounds in saliva, the concentration of which varies depending on the salivary gland responsible for their production. The parotid glands are the source of the lowest concentration of Zn compounds, while the sublingual and submandibular salivary glands are the primary sources of these compounds. Zinc plays an important role in the protective function of saliva. It is hypothesized that Zn compounds present in saliva are a constant source of enamel remineralization. During this process, the lost Ca ions in the hydroxyapatite are replaced with Zn ions.[65] As with fluorine compounds, which play a similar role in layer remodeling, a decline in Zn concentrations in enamel is observed during the second and third decades of life. Zinc ions in saliva compete with Ca ions for a place in the enamel. The loss of Ca ions is a direct consequence of the development of caries.

It is not possible to exclude with certainty the potential importance of biogenic Zn contained in bones in shaping the negative correlation between Zn concentration and the caries intensity index. The corresponding differences in bone Zn levels may also be attributed to dietary differences, accompanied by an increase in Zn level due to diagenesis. Such variations may have influenced the results. Maintaining an adequately high Zn level in a living organism (within the natural range needed to maintain internal homeostasis) is associated with overall biological health.[27] The second significant correlation obtained in this study is the positive correlation between the Zn concentration and the number of examined teeth (i.e., individuals with higher Zn concentration tended to have more preserved teeth). A greater number of preserved teeth indicates a lower rate of postmortem loss, as well as a lower rate of antemortem loss. This suggests that individuals with a greater number of preserved teeth may exhibit healthier biological conditions. Unless influenced by diagenesis, this correlation could suggest the importance of Zn as a marker of overall bodily health.

Conclusions

The evaluation of bone mineral composition as a method for assessing the quality of life in historical populations has been a subject of discussion for many years. Linking the elemental content in bone tissue provides a potentially valuable tool for the reconstruction of lifestyle factors related to socioeconomic aspects such as diet, masticatory organ health and general health awareness.

However, proper evaluation in chemical studies of hard tissues of the human body is associated with certain limitations, which must be considered when drawing conclusions based on the elemental composition of dental or bone material. The current research on early modern urban populations highlights 2 main limitations. First, the degree of diagenesis has a significant impact on the obtained results. Second, the chemical composition of bones exhibits variability and instability during ontogenesis, which limits the applicability of results to a narrow period, spanning a few weeks to a few months before death.

Therefore, the assessment of the presence and severity of long-term diseases such as caries provides insights into the mineral composition of bone tissue and may potentially correlate it with persistent nutritional deficiencies. Despite the influence of diagenetic factors on the preservation of the studied elements in bone minerals, elevated concentrations of Zn ions in bone material may be attributable to specific dietary habits influenced by socioeconomic factors. Although the results only approached statistical significance, in light of the limitations associated with diagenesis, it is important to mention the potential association between elevated Zn levels and reduced caries intensity among former inhabitants of Wrocław.

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.

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References

1. Temple DH. Caries: The Ancient Scourge. In: Irish JD, Scott GR, eds. *A Companion to Dental Anthropology*. 1st ed. Hoboken, NJ: John Wiley & Sons, Inc.; 2015:433–449. doi:10.1002/9781118845486.ch26
2. Pitts NB, Zero DT, Marsh PD, et al. Dental caries. *Nat Rev Dis Primers*. 2017;3:17030. doi:10.1038/nrdp.2017.30
3. van Meijeren-van Lunteren AW, Voortman T, Wolvius EB, Kragt L. Adherence to dietary guidelines and dental caries among children: A longitudinal cohort study. *Eur J Public Health*. 2023;33(4):653–658. doi:10.1093/eurpub/ckad097
4. Bilge NH, Yeşiltepe S, Törenek Ağırman K, Çağlayan F, Bilge OM. Investigation of prevalence of dental anomalies by using digital panoramic radiographs. *Folia Morphol (Warsz)*. 2018;77(2):323–328. doi:10.5603/FM.a2017.0087
5. Schmidt CW, Quataert R, Zalzala F, D'Anastasio R. Taphonomy of Teeth. In: Schotsmans EMJ, Márquez-Grant N, Forbes SL, eds. *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment*. 1st ed. Hoboken, NJ: John Wiley & Sons, Inc.; 2017:92–100. doi:10.1002/9781118953358.ch7
6. Dąbrowski P, Grzelak J, Kulus M, Staniowski T. Diagnodent and VistaCam may be unsuitable for the evaluation of dental caries in archeological teeth. *Am J Phys Anthropol*. 2019;168(4):797–808. doi:10.1002/ajpa.23785
7. Cox M, Mays S, eds. *Human Osteology in Archaeology and Forensic Science*. Cambridge, UK: Cambridge University Press; 2006:228–236.
8. Rasmussen KL, Milner G, Skytte L, Lynnerup N, Thomsen JL, Boldsen JL. Mapping diagenesis in archaeological human bones. *Herit Sci*. 2019;7(1):41. doi:10.1186/s40494-019-0285-7
9. Price TD, Blitz J, Burton J, Ezzo JA. Diagenesis in prehistoric bone: Problems and solutions. *J Archaeol Sci*. 1992;19(5):513–529. doi:10.1016/0305-4403(92)90026-Y
10. Rasmussen KL, Milner GR, Delbey T, et al. Trace element distribution in human cortical bone microstructure: The potential for unravelling diet and social status in archaeological bones. *Herit Sci*. 2020;8(1):111. doi:10.1186/s40494-020-00457-1
11. Dąbrowski P, Kulus MJ, Grzelak J, Olchowy C, Staniowski T, Paulsen F. Nutritional reconstruction in an early modern population: Searching for a relationship between dental microwear and bone element composition. *Ann Anat*. 2022;240:151884. doi:10.1016/j.aanat.2021.151884
12. Demirci S, Kayaturk N. Chemical analysis of fossil bones. *PACT (Rixensart)*. 1995;(45):111–118. https://open.metu.edu.tr/handle/11511/7199. Accessed June 26, 2024.
13. Hedges REM. Bone diagenesis: An overview of processes. *Archaeometry*. 2002;44(3):319–328. doi:10.1111/1475-4754.00064
14. Lambert JB, Weydert-Homeyer JM. The fundamental relationship between ancient diet and the inorganic constituents of bone as derived from feeding experiments. *Archaeometry*. 1993;35(2):279–294. doi:10.1111/j.1475-4754.1993.tb01043.x
15. Szostek K, Stepańczak B, Szczepanek A, et al. Diagenetic signals from ancient human remains – Bioarchaeological applications. *Mineralogia*. 2011;42(2–3):93–112. doi:10.2478/v10002-011-0009-4
16. Burton JH, Price TD. The Use and Abuse of Trace Elements for Paleodietary Research. In: Ambrose SH, Katzenberg MA, eds. *Advances in Archaeological and Museum Science. Volume 5. Biogeochemical Approaches to Paleodietary Analysis*. Dordrecht, the Netherlands: Kluwer Academic Publishers; 2002:159–171. doi:10.1007/0-306-47194-9_8
17. Wathen CA, Isaksson S, Lidén K. On the road again – A review of pretreatment methods for the decontamination of skeletal materials for strontium isotopic and concentration analysis. *Archaeol Anthropol Sci*. 2022;14(3):45. doi:10.1007/s12520-022-01517-2
18. Zaichick V, Zaichick S, Karandashev V, Nosenko S. The effect of age and gender on Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Sr, V, and Zn contents in rib bone of healthy humans. *Biol Trace Elem Res*. 2009;129(1–3):107–115. doi:10.1007/s12011-008-8302-9
19. Samudralwar DL, Robertson JD. Determination of major and trace elements in bones by simultaneous PIXE/PIGE analysis. *J Radioanal Nucl Chem*. 1993;169(1):259–267. doi:10.1007/BF02046801
20. Zaichick S, Zaichick V, Karandashev V, Nosenko S. Accumulation of rare earth elements in human bone within the lifespan. *Metallomics*. 2011;3(2):186–194. doi:10.1039/c0mt00069h
21. Brätter P, Gawlik D, Lausch J, Rösick U. On the distribution of trace elements in human skeletons. *J Radioanal Chem*. 1977;37(1):393–403. doi:10.1007/BF02520545
22. Yoshinaga J, Suzuki T, Morita M. Sex- and age-related variation in elemental concentrations of contemporary Japanese ribs. *Sci Total Environ*. 1989;79(3):209–221. doi:10.1016/0048-9697(89)90337-9
23. Takata MK, Saiki M, Sumita NM, Saldiva PHN, Pasqualucci CA. Activation analysis methods and applications. *J Radioanal Nucl Chem*. 2005;264(1):5–8. doi:10.1007/s10967-005-0666-0
24. Yoshinaga J, Suzuki T, Morita M, Hayakawa M. Trace elements in ribs of elderly people and elemental variation in the presence of chronic diseases. *Sci Total Environ*. 1995;162(2–3):239–252. doi:10.1016/0048-9697(95)04470-I

25. Crawford MD, Crawford T. Lead content of bones in a soft and a hard water area. *Lancet*. 1969;1(7597):699–701. doi:10.1016/s0140-6736(69)92649-x
26. Koch HJ, Smith ER, McNeely J. Analysis of trace elements in human tissues. II. The lymphomatous diseases. *Cancer*. 1957;10(1):151–160. doi:10.1002/1097-0142(195701/02)10:1<151::aid-cncr2820100122>3.0.co;2-p
27. Simpson R, Cooper DML, Swanston T, Coulthard I, Varney TL. Historical overview and new directions in bioarchaeological trace element analysis: A review. *Archaeol Anthropol Sci*. 2021;13(1):24. doi:10.1007/s12520-020-01262-4
28. Burak M, Okólska H. *Cmentarze dawnego Wrocławia*. Wrocław, Poland: Muzeum Architektury we Wrocławiu; 2007:100–128.
29. White TD, Black MT, Folkens PA. *Human Osteology*. 3rd ed. Cambridge, MA: Elsevier Academic Press; 2012:51–99.
30. Buikstra JE, Ubelaker DH, eds. *Standards for Data Collection from Human Skeletal Remains*. Arkansas Archeological Survey; 1994:17–38.
31. Lovejoy CO. Dental wear in the Libben population: Its functional pattern and role in the determination of adult skeletal age at death. *Am J Phys Anthropol*. 1985;68(1):47–56. doi:10.1002/ajpa.1330680105
32. Meindl RS, Lovejoy CO. Ectocranial suture closure: A revised method for the determination of skeletal age at death based on the lateral-anterior sutures. *Am J Phys Anthropol*. 1985;68(1):57–66. doi:10.1002/ajpa.1330680106
33. Braga MM, Oliveira LB, Bonini GAVC, Bönecker M, Mendes FM. Feasibility of the International Caries Detection and Assessment System (ICDAS-II) in epidemiological surveys and comparability with standard World Health Organization Criteria. *Caries Res*. 2009;43(4):245–249. doi:10.1159/000217855
34. Erdal YS, Duyar İ. A new correction procedure for calibrating dental caries frequency. *Am J Biol Anthropol*. 1999;108(2):237–240. doi:10.1002/(SICI)1096-8644(199902)108:2<237::AID-AJPA8>3.0.CO;2-Z
35. gov.pl. Zakres działalności laboratorium badania żywności. https://www.gov.pl/attachment/75016d78-d3db-4192-a077-f09ad186f030. Accessed July 19, 2024.
36. Institute of Agricultural and Food Biotechnology – State Research Institute. Propozycje dotyczące metod oznaczania jakości, metod próbobrania, niezbędnego wyposażenia wykorzystywanego do oceny jakości, znakowania, opakowań i warunków przechowywania najbardziej perspektywicznych produktów rolno-spożywczych oferowanych na Platformie Żywnościowej. https://platformazywnosciowa.com.pl/wp-content/uploads/2019/12/RAPORT-NR-4-IBPRS.pdf. Accessed July 19, 2024.
37. ISO 6869:2000. Animal feeding stuffs – Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc – Method using atomic absorption spectrometry. https://www.iso.org/standard/33707.html. Accessed July 19, 2024.
38. Sedgwick P. Pearson's correlation coefficient. *BMJ*. 2012;345:e4483. doi:10.1136/bmj.e4483
39. López-Costas O, Lantes-Suárez Ó, Martínez Cortizas A. Chemical compositional changes in archaeological human bones due to diagenesis: Type of bone vs soil environment. *J Archaeol Sci*. 2016;67:43–51. doi:10.1016/j.jas.2016.02.001
40. Buikstra JE. Multiple Elements: Multiple Expectations. In: Price TD, ed. *The Chemistry of Prehistoric Human Bone*. Cambridge, UK: Cambridge University Press; 1989:155–210.
41. Williams AMM, Siegele R. Iron deposition in modern and archaeological teeth. *Nucl Instrum Methods Phys Res*. 2014;335:19–23. doi:10.1016/j.nimb.2014.06.003
42. Kendall C, Eriksen AMH, Kontopoulos I, Collins MJ, Turner-Walker G. Diagenesis of archaeological bone and tooth. *Palaeogeogr Palaeoclimatol Palaeoecol*. 2018;491:21–37. doi:10.1016/j.palaeo.2017.11.041
43. Humphrey LT. Chemical and Isotopic Analyses of Dental Tissues. In: Irish JD, Scott GR, eds. *A Companion to Dental Anthropology*. 1st ed. Hoboken, NJ: Wiley-Blackwell; 2015:499–513. doi:10.1002/9781118845486.ch30
44. Krajcarz MT. Alteration of the metal content in animal bones after 2.5-year experimental exposure to sediments. *Archaeol Anthropol Sci*. 2019;11(1):361–372. doi:10.1007/s12520-017-0533-2
45. Sorg MH. Differentiating trauma from taphonomic alterations. *Forensic Sci Int*. 2019;302:109893. doi:10.1016/j.forsciint.2019.109893
46. Rasmussen KL, Skytte L, Pilekær C, et al. The distribution of mercury and other trace elements in the bones of two human individuals from medieval Denmark – The chemical life history hypothesis. *Herit Sci*. 2013;1(1):10. doi:10.1186/2050-7445-1-10
47. Schutkowski H, Herrmann B, Wiedemann F, Bocherens H, Grupe G. Diet, status and decomposition at Weingarten: Trace element and isotope analyses on early mediaeval skeletal material. *J Archaeol Sci*. 1999;26(6):675–685. doi:10.1006/jasc.1998.0384
48. Lösch S, Moghaddam N, Grossschmidt K, Risser DU, Kanz F. Stable isotope and trace element studies on gladiators and contemporary Romans from Ephesus (Turkey, 2nd and 3rd Ct. AD) – Implications for differences in diet. *PLoS One*. 2014;9(10):e110489. doi:10.1371/journal.pone.0110489
49. Trueman CN, Tuross N. Trace elements in recent and fossil bone apatite. *Rev Mineral Geochem*. 2002;48(1):489–521. doi:10.2138/rmg.2002.48.13
50. Fabig A, Herrmann B. Trace elements in buried human bones: Intra-population variability of Sr/Ca and Ba/Ca ratios – Diet or diagenesis? *Naturwissenschaften*. 2002;89(3):115–119. doi:10.1007/s00114-001-0294-7
51. Hancock RGV, Grynpas MD, Alpert B. Are archaeological bones similar to modern bones? An INAA assessment. *J Radioanal Nucl Chem*. 1987;110(1):283–291. doi:10.1007/BF02055031
52. Lambert JB, Vlasak S, Simpson, Szpunar CB, Buikstra JE. Bone diagenesis and dietary analysis. *J Hum Evol*. 1985;14(5):477–482. doi:10.1016/S0047-2484(85)80026-9
53. González-Weller D, Rubio C, Gutiérrez ÁJ, et al. Dietary intake of barium, bismuth, chromium, lithium, and strontium in a Spanish population (Canary Islands, Spain). *Food Chem Toxicol*. 2013;62:856–868. PMID:24416776.
54. Burton JH, Price TD, Cahue L, Wright LE. The use of barium and strontium abundances in human skeletal tissues to determine their geographic origins. *Int J Osteoarchaeol*. 2003;13(1–2):88–95. doi:10.1002/oa.661
55. Burton JH, Wright LE. Nonlinearity in the relationship between bone Sr/Ca and diet: Paleodietary implications. *Am J Phys Anthropol*. 1995;96(3):273–282. doi:10.1002/ajpa.1330960305
56. Underwood EJ. *Trace Elements in Human and Animal Nutrition*. 4th ed. Cambridge, MA: Elsevier Academic Press; 2012:183.
57. Lambert JB, Xue L, Buikstra JE. Physical removal of contaminative inorganic material from buried human bone. *J Archaeol Sci*. 1989;16(4):427–436. doi:10.1016/0305-4403(89)90017-4
58. Byrne KB, Parris DC. Reconstruction of the diet of the Middle Woodland Amerindian population at Abbott Farm by bone trace-element analysis. *Am J Biol Anthropol*. 1987;74(3):373–384. doi:10.1002/ajpa.1330740309
59. Parker RB, Toots H. Trace Elements in Bones as Paleobiological Indicators. In: Behrensmeyer AK, Hill AP, eds. *Fossils in the Making: Vertebrate Taphonomy and Paleoecology*. Chicago, IL: University of Chicago Press; 1988:197.
60. Maguire ME, Cowan JA. Magnesium chemistry and biochemistry. *Biometals*. 2002;15(3):203–210. doi:10.1023/A:1016058229972
61. Leidi M, Dellera F, Mariotti M, Maier JAM. High magnesium inhibits human osteoblast differentiation in vitro. *Magnes Res*. 2011;24(1):1–6. doi:10.1684/mrh.2011.0271
62. Castiglioni S, Cazzaniga A, Albisetti W, Maier JAM. Magnesium and osteoporosis: Current state of knowledge and future research directions. *Nutrients*. 2013;5(8):3022–3033. doi:10.3390/nu5083022
63. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium: An update on physiological, clinical and analytical aspects. *Clin Chim Acta*. 2000;294(1–2):1–26. doi:10.1016/s0009-8981(99)00258-2
64. Navarro-González JF, Mora-Fernández C, García-Pérez J. Clinical implications of disordered magnesium homeostasis in chronic renal failure and dialysis. *Semin Dial*. 2009;22(1):37–44. doi:10.1111/j.1525-139X.2008.00530.x
65. Sejdini M, Begzati A, Salihu S, Krasniqi S, Berisha N, Aliu N. The role and impact of salivary Zn levels on dental caries. *Int J Dent*. 2018;2018:8137915. doi:10.1155/2018/8137915

Original paper

Polymorphisms within genes encoding Ikaros family proteins IKZF1 and IKZF3 in multiple myeloma patients treated with thalidomide

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Abstract

Background. Multiple myeloma (MM) is a hematological malignancy characterized by the presence of abnormal plasma cells. It is associated with anemia, bone lesions and renal dysfunction. Immunomodulatory drugs (IMiDs) are commonly used in MM treatment. Recent studies indicate that their therapeutic effect is caused by binding to cereblon (CRBN), which in turn causes the degradation of 2 important immune cell regulatory factors, IKZF1 and IKZF3. These are necessary for the anti-myeloma effect of IMiDs. Their expression level has been shown to affect MM survival and response to treatment. Potentially important single-nucleotide polymorphisms (SNPs) in the genes coding for IKZF1 and IKZF3 have been identified, but they have not been analyzed in MM patients before.

Objectives. The study was designed to establish the relationship between 4 SNPs in the genes coding for IKZF1 (rs61731359, rs4132601 and rs10272724) and IKZF3 (rs907091), and MM survival, response to treatment and other parameters.

Material and methods. The study involved 222 MM patients, as well as 100 control individuals. The *IKZF1* and *IKZF3* genotypes were determined by the LightSNiP assay. Genotyping was performed in the real-time polymerase chain reaction (PCR) LightCycler 480 device.

Results. No difference was observed between the patients and the controls for any of the SNPs, but the *IKZF1* and *IKZF3* variants were associated with various clinical parameters. Allele *IKZF1* rs4132601 *G* was more common in the patients with worse response to first-line therapy ($p = 0.040$), particularly in the patients treated with thalidomide ($p = 0.017$). The patients tended to have worse overall survival. *IKZF3* rs907091 *CC* was detected more commonly in the patients in stage I than in those in stages II and III, according to the International Staging System (ISS) criteria ($p = 0.015$). This genotype was also associated with a higher albumin level ($p = 0.033$), and was less common in the patients with the albumin level below 3.5 g/dL ($p = 0.030$).

Conclusions. Our results suggest that *IKZF1* rs4132601 and *IKZF3* rs907091 may affect response to treatment and progression in patients with MM.

Keywords: single nucleotide polymorphism, multiple myeloma, Ikaros transcription factor

Introduction

Multiple myeloma (MM) is an incurable hematological malignancy, which is characterized by the monoclonal growth of abnormal cells derived from transformed plasma cells. Multiple myeloma is a progressive disease that evolves from monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) into symptomatic MM.[1] It is estimated that 35,730 new MM cases will be diagnosed in the United States in 2023 and 12,590 patients will die due to the disease.[2] In the Polish population, MM accounts for 18% of all hematological malignancies, and over 2,000 new cases are reported annually.[3] The disease is diagnosed mostly in elderly people, and the median age at diagnosis is 69 years.[2] Multiple myeloma is associated with poor prognosis; however, new drugs have greatly improved 5-year overall survival and patients' quality of life (QoL).[4,5]

Multiple myeloma typically leads to and is associated with anemia, lytic bone lesions, hypercalcemia, and renal dysfunction.[6] It is believed that both environmental and genetic factors are responsible for the development of MM.[1] Drugs currently available for MM treatment include proteasome inhibitors, monoclonal antibodies, 3-phosphoinositoside kinase inhibitors, and immunomodulatory drugs (IMiDs). Immunomodulatory drugs are a group that comprises thalidomide and its derivatives – lenalidomide and pomalidomide. They exert their therapeutic effect by binding to cereblon (CRBN), a component of the E3 ubiquitin ligase complex.[7,8] By binding to CRBN, IMiDs change the substrate specificity of the E3 ubiquitin ligase complex, and cause it to ubiquitinate and target for proteasomal degradation two B cell transcription factors – Ikaros family zinc finger protein 1 (IKZF1, Ikaros) and Ikaros family zinc finger protein 3 (IKZF3, Aiolos). Ikaros family proteins are known to be the major regulators of immune cell development, particularly with regard to $CD4^+$ T helper cells, as well as B cells during their early and late development.[9,10] IKZF1 acts as a tumor suppressor in normal cells, but in MM cells, it becomes a transcriptional activator of oncogenes and promotes cancer cell survival.[11] The knockdown and overexpression of IKZF1 and IKZF3 reduces the sensitivity of MM cells to IMiDs.[12–14] Both IKZF1 and IKZF3 are needed for IMiDs to work, and the inactivation of either of them leads to MM cell cycle arrest.[15] The degradation of IKZF1 and IKZF3 may lead to MM cell apoptosis through the downregulation of interferon regulatory factor 4 (IRF4) and c-MYC. It has also been shown that IKZF1 and IKZF3 repress interferon-stimulated genes (ISGs), including *CD38*, the expression of which is increased upon IMiD treatment.

Earlier studies established that the IKZF1 expression levels affected response to therapy and survival in patients treated with IMiDs.[16–18] However, as of yet, no studies have investigated whether genetic variability within the genes coding for IKZF1 and IKZF3 may affect survival or response to treatment in MM patients. Our previous studies showed that the single-nucleotide polymorphisms (SNPs) located in the genes associated with IMiD metabolism might correlate with response to treatment and survival in MM patients.[19–21] In the present study, we aimed to analyze selected SNPs in the *IKZF1* and *IKZF3* genes in the context of MM. Three SNPs were in *IKZF1* (rs61731359, a missense Asn>Asp substitution; rs4132601, a 3' untranslated region (3'UTR) variant and a potential miRNA binding site; and rs10272724, located near *IKZF1* and affecting survival in acute lymphoblastic leukemia) and one in *IKZF3* (rs907091, a 3'UTR variant and a potential miRNA binding site).[22–25] The 4 SNPs were analyzed in relation to MM susceptibility, response to treatment, survival, and association with diagnostic and prognostic markers, such as albumin, beta-2-microglobulin (β2-M), C-reactive protein (CRP), or calcium (Ca).

Material and methods

Patients and controls

The study included 222 patients diagnosed with MM, 111 women and 111 men, aged 37–88 years (median age: 64 years). The only inclusion criterion for patients to enter the study was a diagnosis of MM. The exclusion criterion was a diagnosis of any other malignancy.

According to the International Staging System (ISS) criteria, 54 patients were in stage I, 67 in stage II and 81 in stage III.[26] Detailed information on patients is presented in Table 1. In addition, 100 healthy blood donors (50 women and 50 men) served as a control group. All patients and control individuals were from the Lower Silesia region in western Poland, and were treated between 2015 and 2020. The study was performed in accordance with the Declaration of Helsinki, and received the approval of the Ethics Committee at Wroclaw Medical University, Poland (no. KB-297/2016).

Sample size

The sample size analysis was conducted based on one of the primary objectives of the study, assuming a lower occurrence of the *IKZF1* rs4132601 *G* allele in patients with better response (complete or very good partial remission) to first-line therapy (effect size: 0.30). The minimum required sample size to detect this difference, assuming $\alpha = 5\%$, a power of 90% and a confidence level of 95%, was 184 patients in total. Additionally, a 10% risk of incomplete data was considered. The final minimum required sample size was 203 participants. The sample size analysis was performed using the G*Power software.[27]

Table 1. Patients' characteristics

Data		Number of patients
Age [years]	≤60	70
	>60	137
Sex	M	111
	F	111
ISS	I	54
	II	67
	III	81
Hypercalcemia	yes	38
	no	154
Anemia	yes	69
	no	126
β2-M [μg/mL]	≤3	74
	>3	126
CRP [mg/L]	≤5	75
	>5	64
Albumin [mg/dL]	≤3.5	71
	>3.5	86

ISS – International Staging System; β2-M – beta-2-microglobulin; CRP – C-reactive protein; M – male; F – female.

Immunomodulating therapy

Regarding first-line therapy, 127 patients were treated with the thalidomide-containing regimens (thalidomide 100 mg/day), including 89 patients treated with cyclophosphamide, thalidomide and dexamethasone (CTD) up to 6 cycles, and the subsequent continuous thalidomide monotherapy. Of the rest of the patients, 76 were not treated with thalidomide, and 19 patients lacked data on thalidomide treatment. Response to first-line therapy was as follows: complete remission (CR) in 37 patients; very good partial remission (VGPR) in 29 patients; partial remission (PR) in 77 patients; minor remission (MR) in 11 patients; stable disease (SD) in 19 patients; and progressive disease (PD) in 12 patients. For the patients treated with the thalidomide-containing regimens, the response was as follows: CR in 24 patients; VGPR in 18 patients; PR in 49 patients; MR in 8 patients; SD in 15 patients; and PD in 8 patients. Response criteria are well described in a previous study.[3]

Genotyping

DNA was extracted from the samples of peripheral blood taken on ethylenediaminetetraacetic acid (EDTA), using Maxwell® 16 Blood DNA Purification Kit (Promega Corp., Madison, USA) or QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany), following the manufacturers' recommendations. The *IKZF1* and *IKZF3* genotypes were determined by the LightSNiP assay (TIB Molbiol, Berlin, Germany), which uses specific probes allowing to distinguish genotypes based on melting curves (melting curve genotyping). Genotyping was performed in the real-time polymerase chain reaction (PCR) LightCycler 480 device (Roche Diagnostics, Rotkreuz, Switzerland) on a 96-well plate. A negative control with no DNA was included. Conditions for PCR were as follows: 95°C for 10 min; 45 cycles of 95°C for 10 s, 60°C for 10 s and 72°C for 15 s. This was then followed by one cycle of 95°C for 30 s and 40°C for 2 min, and gradual melting from 75°C to 40°C. The genotypes were distinguished based on the melting temperatures of the samples and the shape of their melting curves.

Statistical analysis

The null hypothesis that there is no difference in the presence of the *IKZF1*/*IKZF3* alleles between patients and controls was tested with Fisher's exact test or the χ^2 test, using the website http://vassarstats.net. The genotypes were tested for deviation from the Hardy–Weinberg equilibrium using the χ^2 test. Survival was assessed with the Wilcoxon test and the Kaplan–Meier survival curves. Other associations were tested either with Fisher's exact test or the Mann–Whitney *U* test. All of these tests, except for Fisher's exact test and the χ^2 test, were performed with the Real Statistics Resource Pack for Microsoft Excel (https://real-statistics.com/free-download/real-statistics-resource-pack). A p-value <0.05 was considered statistically significant.

Results

Distribution of the *IKZF1* and *IKZF3* alleles and genotypes among patients with multiple myeloma and control individuals

All patients and control individuals (control group) were genotyped for rs4132601, rs10272724 and rs907091, while 203 patients and all control individuals were genotyped for rs61731359. Details about the genotype and allele frequencies are presented in Table 2. Genotype frequencies for all 4 SNPs were in accordance with the Hardy–Weinberg equilibrium in both patients and controls. We detected no statistically significant differences between the patients and the controls in any of the 4 studied SNPs, in any of the 3 investigated models (dominant, recessive and additive) (Table 3). Regarding rs61731359*A*>*G*, only allele *A* was detected, as all the subjects carried genotype *AA*. Two *IKZF1* SNPs, rs4132601 and rs10272724, were in very high linkage disequilibrium (r^2 = 0.96, D' = 0.98), prompting us to select only one of them for further analysis. Due to the reasons listed above, rs61731359 and rs10272724 were not included in further analysis.

Table 2. *IKZF1* and *IKZF3* genotypes and alleles

Genotypes and alleles		MM patients	Controls $N = 100$
IKZF1 rs61731359 *A>G* $N = 203$ (MM patients)	*AA*	203 (100.0%)	100 (100.0%)
	AG	0 (0.0%)	0 (0.0%)
	GG	0 (0.0%)	0 (0.0%)
	A	1.000	1.000
	G	0.000	0.000
IKZF1 rs4132601 *T>G* $N = 222$ (MM patients)	*TT*	132 (59.5%)	61 (61.0%)
	TG	77 (34.7%)	34 (34.0%)
	GG	13 (5.9%)	5 (5.0%)
	T	0.768	0.780
	G	0.232	0.220
IKZF1 rs10272724 *T>C* $N = 222$ (MM patients)	*TT*	133 (59.9%)	60 (60.0%)
	TC	76 (32.2%)	35 (35.0%)
	CC	13 (5.9%)	5 (5.0%)
	C	0.770	0.775
	T	0.230	0.225
IKZF3 rs907091 *C>T* $N = 222$ (MM patients)	*CC*	42 (18.9%)	17 (17.0%)
	CT	116 (52.3%)	53 (53.0%)
	TT	64 (28.8%)	30 (30.0%)
	C	0.451	0.439
	T	0.549	0.561

MM – multiple myeloma.

Association of the *IKZF1* and *IKZF3* polymorphisms with response to immunomodulating therapy

Allele *IKZF1* rs4132601 *G* was less common among the patients with better response (CR-VGPR) to first-line therapy (19/66 vs. 54/119; $p = 0.040$). This association could also be observed in the subgroup of patients treated with thalidomide (9/42 vs. 35/80; $p = 0.017$) (Fig. 1). Additionally, we observed a trend for worse response to treatment in the patients with allele *IKZF3* rs907091 *C*, although this was not statistically significant ($p = 0.086$) (Fig. 2).

Table 3. Case–control comparison between the multiple myeloma (MM) patients and the controls in 3 genetic models for *IKZF1* and *IKZF3* single-nucleotide polymorphisms (SNPs)

Model		*p*-value
Dominant	rs61731359 *A>G*	1.000
	rs4132601 *T>G*	0.807
	rs10272724 *T>C*	1.000
	rs907091 *C>T*	0.757
Recessive	rs61731359 *A>G*	1.000
	rs4132601 *T>G*	0.802
	rs10272724 *T>C*	0.802
	rs907091 *C>T*	1.000
Additive	rs61731359 *A>G*	1.000
	rs4132601 *T>G*	0.937
	rs10272724 *T>C*	0.951
	rs907091 *C>T*	0.932

Association of the *IKZF1* and *IKZF3* polymorphisms with survival and disease progression

Overall and disease-free survival analyses were conducted for the *IKZF1* and *IKZF3* SNPs. While no statistically significant associations were observed for any of the analyzed SNPs, we noticed that the patients with allele *IKZF1* rs4132601 *G* tended to have shorter overall survival than the patients without it ($p = 0.104$) (Fig. 3).

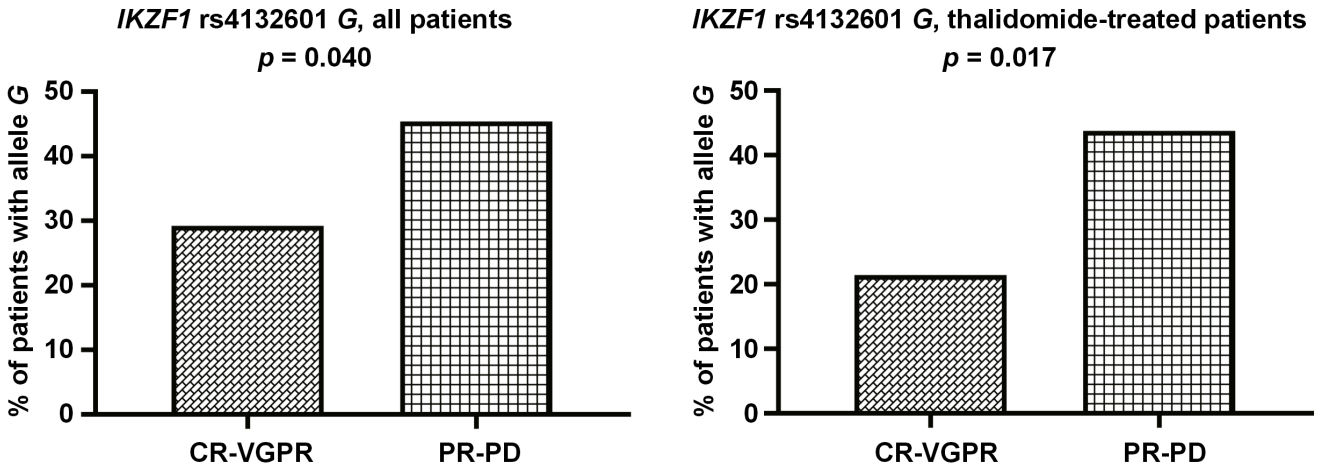

Fig. 1. Distribution of the *IKZF1* rs4132601 *G* allele in the patients after first-line therapy showing complete or very good partial remission (CR-VGPR), and in the patients showing only partial remission, minor remission, no remission, or progression of the disease (PR-PD)

The left panel illustrates the association for all patients, regardless of the type of treatment, while the right panel includes only the patients undergoing treatment that included thalidomide. In both groups, allele *G* was less commonly detected.

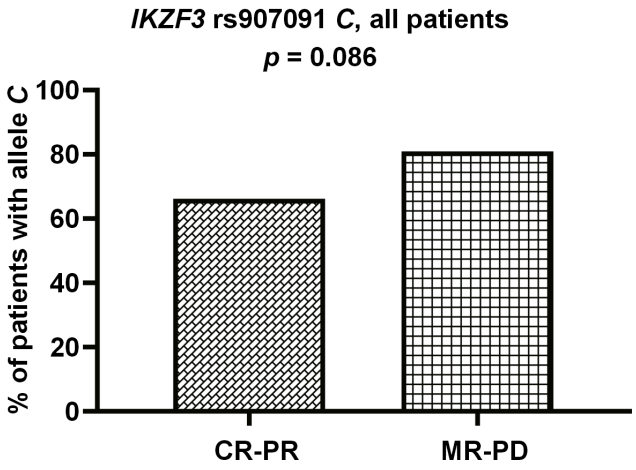

Fig. 2. Distribution of the *IKZF3* rs907091 *C* allele in the patients after first-line therapy showing complete, very good or partial remission (CR-PR), and in the patients showing only minor remission, no remission or progression of the disease (MR-PD)

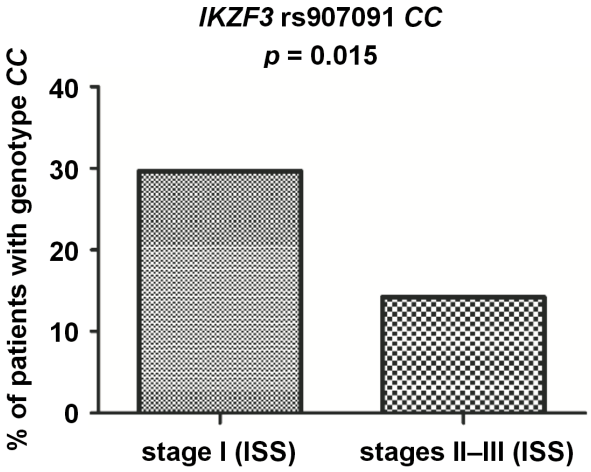

Fig. 4. Distribution of the *IKZF3* rs907091 *CC* genotype in the patients characterized by being in either stage I, or stage II–III of the disease, according to the International Staging System (ISS) criteria

The *CC* genotype was more prevalent among the patients in the initial stage.

Regarding *IKZF3*, we found genotype rs907091 *CC* to be more common in the patients in stage I than in those in stages II and III, according to the ISS criteria (16/54 vs. 21/148; $p = 0.015$) (Fig. 4).

Associations of the *IKZF1* and *IKZF3* polymorphisms with other prognostic factors

We observed that the median albumin blood concentration was slightly higher in the patients with genotype *IKZF3* rs907091 *CC* than in the patients without this genotype (3.80 g/dL vs. 3.55 g/dL; $p = 0.033$). Furthermore, we noticed that *CC* homozygosity was less common in the patients with the albumin levels below 3.5 g/dL than in those with the albumin levels above 3.5 g/dL (9/71 vs. 24/86; $p = 0.030$) (Fig. 5), with the albumin concentration below 3.5 g/dL being associated with worse prognosis.[28] Additionally, the patients with genotype *IKZF1* rs4132601 *GG* tended to have lower albumin concentration than the patients without this genotype (3.35 g/dL vs. 3.65 g/dL; $p = 0.057$).

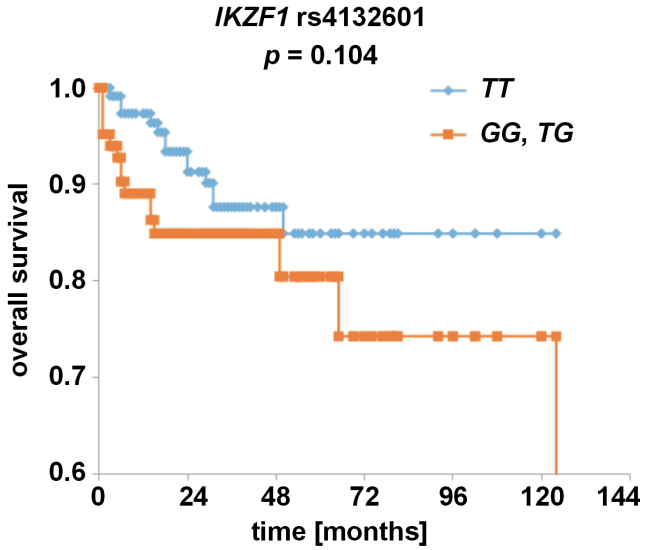

Fig. 3. Kaplan–Meier curves for *IKZF1* rs4132601, visualizing overall survival

The red line shows overall survival for the patients with allele *G* (genotypes *GG* and *TG*), while the blue line represents the patients lacking allele *G* (genotype *TT*).

Regarding other clinical parameter – hemoglobin – allele *IKZF3* rs907091 *C* was more commonly detected in the patients with abnormal hemoglobin blood levels (<10 g/dL) than in the patients with normal levels (55/69 vs. 81/126; $p = 0.034$) (Fig. 6). We also observed that the patients with allele *IKZF1* rs4132601 *G* tended to have higher Ca blood levels (9.53 mg/dL vs. 9.25 mg/dL; $p = 0.082$) and slightly higher age on diagnosis (65.0 years vs. 63.5 years; $p = 0.053$) than the patients without this allele, although this was not statistically significant. No associations regarding the levels of β2-M, creatinine and CRP, or the presence of light chains were detected.

Discussion

Immunomodulatory drugs are a major group of drugs used to treat MM. However, their mechanism of action has not been fully elucidated. It has been established that the molecular target of IMiDs is CRBN. While a ubiquitin ligase-independent pathway for IMiD action has been described, the anti-myeloma effect of IMiDs is most often ascribed to the degradation of IKZF1 and IKZF3 by the E3 ubiquitin ligase complex upon IMiD binding to CRBN.[12,13,25] In the present study, we investigated the *IKZF1* and *IKZF3* genetic variants and analyzed their influence on survival, response to treatment and diagnostic

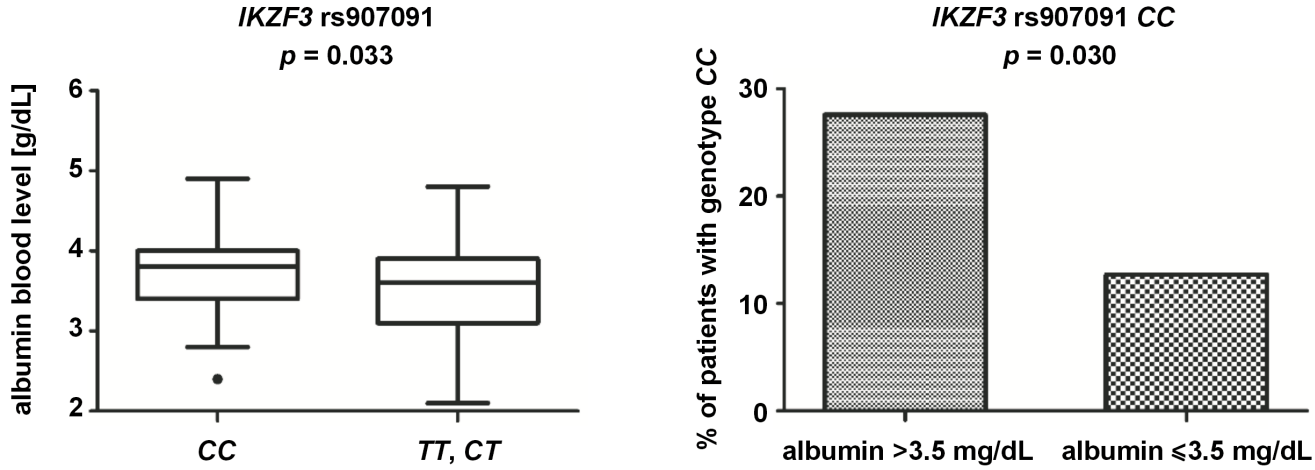

Fig. 5. Albumin blood levels in the patients carrying the *IKZF3* rs907091 *CC* genotype

The left panel shows differences in the albumin blood concentration between the patients with genotype *CC* and those without it (genotypes *TT* and *CT*). The *CC* genotype was associated with higher albumin levels. The right panel shows the distribution of the *CC* genotype in the patients with the albumin levels above and below 3.5 mg/dL, with the levels below the 3.5 mg/dL cut-off point being associated with worse prognosis. The patients with albumin lower than 3.5 mg/dL less commonly presented with the *CC* genotype.

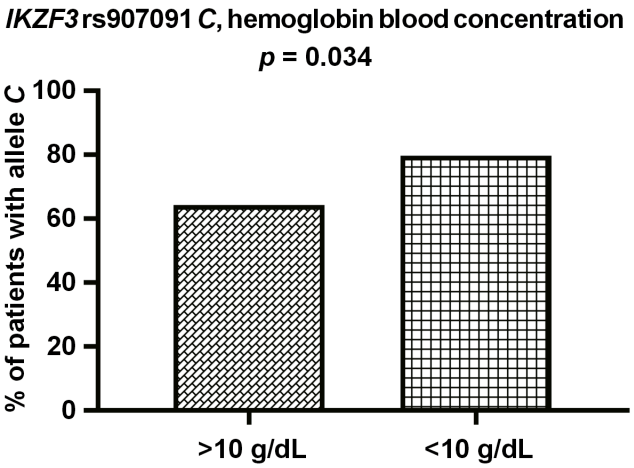

Fig. 6. Hemoglobin blood levels in the patients carrying the *IKZF3* rs907091 *C* allele

The graphs show differences in the distribution of allele *C* in the patients with higher and lower hemoglobin blood concentration. Allele *C* was more common in the patients with lower hemoglobin blood concentration.

markers in MM patients. Previous studies found differences in survival and response to treatment between patients with different IKZF1 expression levels,[16–18] which may be caused by the interference of the genetic background. To the best of our knowledge, there has been no study analyzing the influence of SNPs in the genes coding for IKZF1 or IKZF3 on MM. Our study showed that allele rs4132601 *G* was associated with worse response to treatment, especially in patients treated with thalidomide, and might be more common in patients with shorter overall survival, although the latter association was not statistically significant. Furthermore, the *G* allele tended to be more common in patients with higher Ca blood levels, while the *GG* genotype tended to be more frequent in patients with lower albumin blood levels, which are associated with worse prognosis.[25] However, it was not associated with susceptibility to MM.

IKZF1 rs4132601 was first identified as a risk variant in childhood acute lymphoblastic leukemia (ALL) in a study by Papaemmanuil et al,[29] and has mostly been studied in the context of this disease. The researchers found the *G* allele to be associated with a higher risk for ALL and a lower *IKZF1* mRNA expression level in a dose-dependent fashion.[29] This association with the ALL risk was confirmed in various European, North African, Middle Eastern and Southeast Asian populations, although it was not detected in studies on ALL patients from Taiwan, Latvia and Iran.[24,30–39] The rs4132601 SNP was also analyzed in Polish diffuse large B cell lymphoma (DLBCL) patients.[40] While the SNP was not associated with DLBCL susceptibility, allele *G* was found to correlate with longer progression-free and overall survival,[40] contrary to our results in MM. This may be attributed to differences between the 2 diseases and the fact that DLBCL is not treated with IMiDs; therefore, the role of IKZF1 in this disease may be different from its role in MM. It is worth noting that our study showed that rs4132601 is in strong linkage disequilibrium with another *IKZF1* SNP, rs10272724. It is thus possible that the effects ascribed to rs4132601 may, in fact, be due to a genetic variation in rs10272724.

Another SNP covered in our present study was rs907091, located in the gene coding for IKZF3, also known as Aiolos. Both IKZF1 and IKZF3 are needed for IMiDs to work, and the inactivation of either of them can lead to MM cell cycle arrest.[15] We found rs907091 *CC* to be more common in MM patients with albumin levels above 3.5 g/dL (a marker of better prognosis) and to correlate with higher albumin levels in general. The rs907091 *CC* was also more commonly detected in patients in stage I of MM than in those in more advanced stages II and III, according to the ISS criteria. These findings may suggest

that rs907091 *CC* is associated with better prognosis and slower progression of MM. On the other hand, we also observed that allele rs907091 *C* was more often detected in patients with abnormally low (<10 g/dL) hemoglobin levels. *IKZF3* rs907091 was first described in a haplotype-based analysis that found it to be associated with gene expression changes.[41] *IKZF3* rs907091 is located in a predicted miR-326 recognition element (binding site) and miR-326 is expected to bind to rs907091 *T* (a variant absent in rs907091 *CC* homozygotes). Accordingly, patients with rs907091 *T* are reported to have lower *IKZF3* expression.[42] The rs907091 *T* was also been described to be associated with a higher risk for systemic lupus erythematosus (SLE) and multiple sclerosis (MS), with the *CC* genotype playing a protective role.[42–45] The protective effect of the *CC* genotype in these studies seems to correspond well with our results with regard to MM, although it should be noted that SLE and MS differ significantly from MM. No studies on *IKZF3* rs907091 have been conducted in the context of MM or other hematologic malignancies.

Our study also included rs61731359, a functionally relevant *IKZF1* SNP causing a missense Asn>Asp amino acid change.[22] To the best of our knowledge, this variant has not been analyzed before in any group of patients. It did not have a polymorphic character in our group of MM patients and control individuals, as only one allele (*A*) was detected. This absence of allele *G* may have been due to the relatively small number of patients and controls investigated. Further studies on rs61731359 in MM and other diseases may be needed to elucidate its role.

Limitations

The relatively small number of patients and controls is a major limitation of our study. Another limitation that could have influenced our results is the non-homogeneous therapy used for MM treatment. Further studies on larger cohorts of patients are needed to confirm our results.

Conclusions

In conclusion, our present study shows that rs4132601 in the gene coding for IKZF1 and rs907091 in the gene coding for IKZF3 may be associated with response to treatment, survival, and the diagnostic and prognostic parameters in MM patients, such as the stage of the disease and albumin blood concentration.

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Wroclaw Medical University, Poland (approval No. KB-297/2016).

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Fonseca R, Blood E, Rue M, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood*. 2003;101(11):4569–4575. doi:10.1182/blood-2002-10-3017
2. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: A systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388(10053):1545–1602. doi:10.1016/S0140-6736(16)31678-6
3. Dmoszyńska A, Usnarska-Zubkiewicz L, Walewski J, et al. Recommendations of Polish Myeloma Group concerning diagnosis and therapy of multiple myeloma and other plasmacytic dyscrasias for 2017 [in Polish]. *Acta Haematol Pol*. 2017;48(2):55–103. doi:10.1016/j.achaem.2017.05.003
4. Kobayashi H, Abe Y, Miura D, et al. Clinical features and predictive factors of early mortality in patients with MM outside of clinical trials. *Blood*. 2018;132(Suppl 1):5593. doi:10.1182/blood-2018-99-113750
5. Kyle RA, Therneau TM, Rajkumar SV, et al. A long- term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med*. 2002;346(8):564–569. doi:10.1056/NEJMoa01133202
6. Chen M, Zhao Y, Xu C, Wang X, Zhang X, Mao B. Immunomodulatory drugs and the risk of serious infection in multiple myeloma: Systematic review and meta-analysis of randomized and observational studies. *Ann Hematol*. 2018;97(6):925–944. doi:10.1007/s00277-018-3284-y
7. Ito T, Ando H, Suzuki T, et al. Identification of a primary target of thalidomide teratogenicity. *Science*. 2010;327(5971):1345–1350. doi:10.1126/science.1177319
8. Chang XB, Stewart AK. What is the functional role of the thalidomide binding protein cereblon? *Int J Biochem Mol Biol*. 2011;2(3):287–294. PMID:22003441. PMCID:PMC3193296.
9. Powell MD, Read KA, Sreekumar BK, Oestreich KJ. Ikaros zinc finger transcription factors: Regulators of cytokine signaling pathways and $CD4^+$ T helper cell differentiation. *Front Immunology*. 2019;10:1299. doi:10.3389/fimmu.2019.01299
10. Sellars M, Kastner P, Chan S. Ikaros in B cell development and function. *World J Biol Chem*. 2011;2(6):132–139. doi:10.4331/wjbc.v2.i6.132
11. Osada N, Kikuchi J, Iha H, et al. c-FOS is an integral component of the IKZF1 transactivator complex and mediates lenalidomide resistance in multiple myeloma. *Clin Transl Med*. 2023;13(8):e1364. doi:10.1002/ctm2.1364
12. Lu G, Middleton RE, Sun H, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science*. 2014;343(6168):305–309. doi:10.1126/science.1244917

13. Krönke J, Udeshi ND, Narla A, et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science*. 2014;343(6168):301–305. doi:10.1126/science.1244851
14. Abe Y, Ishida T. Immunomodulatory drugs in the treatment of multiple myeloma. *Jpn J Clin Oncol*. 2019;49(8):695–702. doi:10.1093/jjco/hyz083
15. Fedele PL, Willis SN, Liao Y, et al. IMiDs prime myeloma cells for daratumumab-mediated cytotoxicity through loss of Ikaros and Aiolos. *Blood*. 2018;132(20):2166–2178. doi:10.1182/blood-2018-05-850727
16. Krönke J, Kuchenbauer F, Kull M, et al. IKZF1 expression is a prognostic marker in newly diagnosed standard-risk multiple myeloma treated with lenalidomide and intensive chemotherapy: A study of the German Myeloma Study Group (DSMM). *Leukemia*. 2017;31(6):1363–1367. doi:10.1038/leu.2016.384
17. Bolomsky A, Hübl W, Spada S, et al. IKAROS expression in distinct bone marrow cell populations as a candidate biomarker for outcome with lenalidomide-dexamethasone therapy in multiple myeloma. *Am J Hematol*. 2017;92(3):269–278. doi:10.1002/ajh.24634
18. Tachita T, Kinoshita S, Ri M, et al. Expression, mutation, and methylation of cereblon-pathway genes at pre- and post-lenalidomide treatment in multiple myeloma. *Cancer Sci*. 2020;111(4):1333–1343. doi:10.1111/cas.14352
19. Butrym A, Rybka J, Łacina P, et al. Polymorphisms within beta-catenin encoding gene affect multiple myeloma development and treatment. *Leuk Res*. 2015;39(12):1462–1466. doi:10.1016/j.leukres.2015.10.007
20. Butrym A, Łacina P, Rybka J, et al. Cereblon and IRF4 variants affect risk and response to treatment in multiple myeloma. *Arch Immunol Ther Exp (Warsz)*. 2016;64(Suppl 1):151–156. doi:10.1007/s00005-016-0442-6
21. Łacina P, Butrym A, Mazur G, Bogunia-Kubik, K. *BSG* and *MCT1* genetic variants influence survival in multiple myeloma patients. *Genes (Basel)*. 2018;9(5):226. doi:10.3390/genes9050226
22. Yang L, Luo Y, Wei J. Integrative genomic analyses on Ikaros and its expression related to solid cancer prognosis. *Oncol Rep*. 2010;24(2):571–577. doi:10.3892/or_00000894
23. Xu Z, Taylor JA. SNPinfo: Integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic Acids Res*. 2009;37(Web Server issue):W600–W605. doi:10.1093/nar/gkp290
24. Bahari G, Hashemi M, Naderi M, Taheri M. *IKZF1* gene polymorphisms increased the risk of childhood acute lymphoblastic leukemia in an Iranian population. *Tumour Biol*. 2016;37(7):9579–9586. doi:10.1007/s13277-016-4853-0
25. Eichner R, Heider M, Fernández-Sáiz V, et al. Immunomodulatory drugs disrupt the cereblon–CD147–MCT1 axis to exert antitumor activity and teratogenicity. *Nat Med*. 2016;22(7):735–743. doi:10.1038/nm.4128
26. Greipp PR, San Miguel J, Durie BG et al. International staging system for multiple myeloma. *J Clin Oncol*. 2005;23(15):3412–3420. doi:10.1200/JCO.2005.04.242
27. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods*. 2009;41(4):1149–1160. doi:10.3758/BRM.41.4.1149
28. Kim JE, Yoo C, Lee DH, Kim SW, Lee JS, Suh C. Serum albumin level is a significant prognostic factor reflecting disease severity in symptomatic multiple myeloma. *Ann Hematol*. 2010;89(4):391–397. doi:10.1007/s00277-009-0841-4
29. Papaemmanuil E, Hosking FJ, Vijayakrishnan J, et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. *Nat Genet*. 2009;41(9):1006–1010. doi:10.1038/ng.430
30. Lautner-Csorba O, Gézsi A, Semsei AF, et al. Candidate gene association study in pediatric acute lymphoblastic leukemia evaluated by Bayesian network based Bayesian multilevel analysis of relevance. *BMC Med Genomics*. 2012;5:42. doi:10.1186/1755-8794-5-42
31. Tayel SI, El-Hefnway SM, Abo El-Fotoh WM, El-Zayat RS. The genetic variants of *IKZF1* gene linked with the growing risk of childhood acute lymphoblastic leukaemia. *Curr Mol Med*. 2019;19(1):32–39. doi :10.2174/1566524019666190219123900
32. Al-Absi B, Razif MF, Noor SM, et al. Contributions of *IKZF1*, *DDC*, *CDKN2A*, *CEBPE*, and *LMO1* gene polymorphisms to acute lymphoblastic leukemia in a Yemeni population. *Genet Test Mol Biomarkers*. 2017;21(10):592–599. doi:10.1089/gtmb.2017.0084
33. Mullighan CG, Su X, Zhang J, et al.; Children's Oncology Group. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med*. 2009;360(5):470–480. doi:10.1056/NEJMoa0808253
34. Vijayakrishnan J, Sherborne AL, Sawangpanich R, Hongeng S, Houlston RS, Pakakasama S. Variation at 7p12.2 and 10q21.2 influences childhood acute lymphoblastic leukemia risk in the Thai population and may contribute to racial differences in leukemia incidence. *Leuk Lymphoma*. 2010;51(10):1870–1874. doi:10.3109/10428194.2010.511356
35. Mosaad YM, El-Ziny MA, Darwish A, et al. *IKZF1* rs4132601 and rs11978267 gene polymorphisms and acute lymphoblastic leukemia: Relation to disease susceptibility and outcome. *J Pediatr Hematol Oncol*. 2020;42(7):420–428. doi:10.1097/MPH.0000000000001874
36. Al-Zayan NR, Ashour MJ, Abuwarda HN, Sharif FA. *ARID5B*, *IKZF1*, *GATA3*, *CEBPE*, and *CDKN2A* germline polymorphisms and predisposition to childhood acute lymphoblastic leukemia. *Pediatr Hematol Oncol*. 2024;14(2):103–113. doi:10.1080/08880018.2023.2234946
37. Lin CY, Li MJ, Chang JG, et al. High-resolution melting analyses for genetic variants in *ARID5B* and *IKZF1* with childhood acute lymphoblastic leukemia susceptibility loci in Taiwan. *Blood Cells Mol Dis*. 2014;52(2–3):140–145. doi:10.1016/j.bcmd.2013.10.003
38. Kreile M, Piekuse L, Rots D, Dobele Z, Kovalova Z, Lace B. Analysis of possible genetic risk factors contributing to development of childhood acute lymphoblastic leukaemia in the Latvian population. *Arch Med Sci*. 2016;12(3):479–485. doi:10.5114/aoms.2016.59920
39. Bardsiri MS, Zehtab S, Karami N, Farsinejad A, Ehsan M, Fatemi A. Association of *IKZF1* and *CDKN2A* gene polymorphisms with childhood acute lymphoblastic leukemia: A high-resolution melting analysis. *BMC Med Genomics*. 2022;15(1):171. doi:10.1186/s12920-022-01325-6
40. Bielska M, Borowiec M, Jesionek-Kupnicka D, et al. Polymorphism in *IKZF1* gene affects clinical outcome in diffuse large B-cell lymphoma. *Int J Hematol*. 2017;106(6):794–800. doi:10.1007/s12185-017-2315-0
41. Verlaan DJ, Berlivet S, Hunninghake GM, et al. Allele-specific chromatin remodeling in the *ZPBP2/GSDMB/ORMDL3* locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet*. 2009;85(3):377–393. doi:10.1016/j.ajhg.2009.08.007
42. Richardson K, Lai CQ, Parnell LD, Lee YC, Ordovas JM. A genome-wide survey for SNPs altering microRNA seed sites identifies functional candidates in GWAS. *BMC Genomics*. 2011;12:504. doi:10.1186/1471-2164-12-504
43. Cai X, Qiao Y, Diao C, et al. Association between polymorphisms of the *IKZF3* gene and systemic lupus erythematosus in a Chinese Han population. *PloS One*. 2014;9(10):e108661. doi:10.1371/journal.pone.0108661
44. Farh KKH, Marson A, Zhu J, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature*. 2015;518(7539):337–343. doi:10.1038/nature13835
45. Keshari PK, Harbo HF, Myhr KM, Aarseth JH, Bos SD, Berge T. Allelic imbalance of multiple sclerosis susceptibility genes *IKZF3* and *IQGAP1* in human peripheral blood. *BMC Genet*. 2016;17:59. doi:10.1186/s12863-016-0367-4

Original paper

Artificial intelligence versus semi-automatic segmentation of the inferior alveolar canal on cone-beam computed tomography scans: A pilot study

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Abstract

Background. The inferior alveolar canal (IAC) is a fundamental mandibular structure. It is important to conduct a precise pre-surgical evaluation of the IAC to prevent complications. Recently, the use of artificial intelligence (AI) has demonstrated potential as a valuable tool for dentists, particularly in the field of oral and maxillofacial radiology.

Objectives. The aim of the study was to compare the segmentation time and accuracy of AI-based IAC segmentation with semi-automatic segmentation performed by a specialist.

Material and methods. Thirty individual IACs from 15 anonymized cone-beam computed tomography (CBCT) scans of patients with at least 1 lower third molar were collected from the database of Poznan University of Medical Sciences, Poland. The IACs were segmented by a trainee in the field of oral and maxillofacial radiology using a semi-automatic method and automatically by an AI-based platform (Diagnocat). The resulting segmentations were overlapped with the use of Geomagic Studio, reverse engineering software, and then subjected to a statistical analysis.

Results. The AI-based segmentation closely matched the semi-automatic method, with an average deviation of 0.275 ±0.475 mm between the overlapped segmentations. The mean segmentation time for the AI-based method (175.00 s) was similar to that of the semi-automatic method (175.67 s).

Conclusions. The results of the study indicate that AI-based tools may offer a reliable approach for the segmentation of the IAC in the context of dental pre-surgical planning. However, further comprehensive studies are required to compare the methods and consider their limitations more comprehensively.

Keywords: accuracy, diagnosis, artificial intelligence, segmentation, inferior alveolar canal

Introduction

The inferior alveolar canal (IAC), also known as the mandibular canal, is a bony structure within the internal border of the mandible that carries the inferior alveolar nerve (IAN), artery and vein.[1,2] The IAN is responsible for providing sensory innervation to the lower lip, mandibular teeth, chin, lower gingiva, and buccal mucosa, as well as motor innervation to the jaw muscles, enabling movement.[3,4] Damage to the IAC can result in numbness and paresthesia in the affected areas.[5] Therefore, it is essential to conduct an accurate pre-surgical evaluation of the IAC based on the specific procedure being performed. Such procedures include implant placement, extraction of third molars, root canal treatment, and orthognathic surgery, among others.[6–9] This evaluation is typically conducted through radiographic image analysis, such as two-dimensional (2D) panoramic X-rays or three-dimensional (3D) X-rays, to prevent complications.

Cone-beam computed tomography (CBCT) is a commonly used imaging technique in dentistry that generates images using a fan-shaped beam of X-rays.[10] Cone-beam computed tomography produces high-resolution images that can be reconstructed into a 3D image, rendering it a valuable tool for evaluating facial anatomy, including the IAC and its variations.[11–13] Ozturk et al. identified 3 distinct configurations for the IAC within the mandible (Fig. 1).[14]

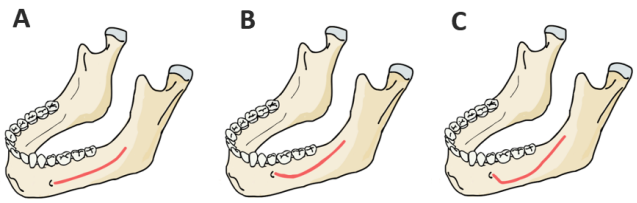

Fig. 1. Mandibular canal path alterations

A. Straight configuration: the terminal part of the inferior alveolar canal (IAC) is nearly at the level of the mental foramen; B. Catenary-like configuration: the canal is almost at the level of the mental foramen and forms a U shape to reach the mental foramen; C. Progressively descending from posterior to anterior: the IAC moves downward gradually until it reaches the molar region, where it ascends to reach the mental foramen.

The term "artificial intelligence" (AI) is used to describe the ability of computer systems and other machines to simulate human cognitive functions, including decision-making, problem-solving and visual perception.[15,16] In recent years, AI has been increasingly adopted in the field of dentistry, reflecting a transition toward the 4th industrial revolution, also known as Industry 4.0.[17] This term refers to the integration of modern technologies, such as AI, robotics and the Internet of things (IoT), into various sectors.[17,18]

Machine learning (ML) algorithms are a type of AI that enables computer systems to enhance their performance on a specific task through experience.[19] Deep learning (DL) is a form of ML that is particularly useful for tasks that require processing large amounts of data and extracting complex patterns and features.[19] The accuracy of AI, including DL algorithms, in performing automatic segmentation of the IAC has been evaluated in previous studies.[20] However, to the best of our knowledge, none of these studies have compared the segmentation time of AI-based segmentation to semi-automatic segmentation. Furthermore, the methodologies used in these studies differed from those employed in our research. Our retrospective pilot study aims to address this gap by comparing the segmentation time and accuracy of AI-based IAC segmentation with semi-automatic segmentation performed by a specialist.

Material and methods

Image dataset

Fifteen anonymized CBCT images, performed for the purposes of implant planning and third molar extractions, were obtained from the database of Poznan University of Medical Sciences, Poland. The scans were selected in accordance with the established inclusion criteria (Table 1). Each of the scans included both the right and left canals, allowing for a total of 30 IACs to be retrospectively analyzed. The CBCT images were registered during the years 2020 and 2021 using dental imaging system (CRANEX® 3D; Soredex, Milwaukee, USA) and stored in the DICOM (Digital Imaging and Communications in Medicine) file format. The CBCT images were taken using the following settings: an X-ray tube voltage of 90 kV; an X-ray tube current of 10 mA; a voxel size of 0.25 mm; and a field of view (FOV) ranging from 600 mm × 800 mm to 1,600 mm × 1,300 mm.

Semi-automatic segmentation

The IAC tracing, integrated into the Romexis® software, v. 6.2 (Planmeca, Helsinki, Finland), was employed to perform the semi-automatic segmentation (ground

Table 1. Inclusion and exclusion criteria for the study

Inclusion criteria	Exclusion criteria
– patient's age ≥18 years – sufficient FOV to visualize the entire lower jaw – presence of at least 1 lower third molar erupted or impacted	– patient's age <18 years – insufficient FOV to visualize the entire lower jaw – artifacts – third molars not present

FOV – field of view.

truth method) of the IAC on cross-sectional views of the 15 images collected. The tool requires the user to designate points along the canal, with the software automatically computing the canal's pathway through progressive extension from these designated points. The task was performed by a trainee in oral and maxillofacial radiology (JI). During the segmentation process, the diameter of the cylinder representing the nerve replica was set at 1.50 mm. Subsequently, the IACs on both sides were saved as a single STL (Standard Triangle Language) file (Fig. 2). The time required to complete the segmentation was recorded from the commencement of the process to its conclusion.

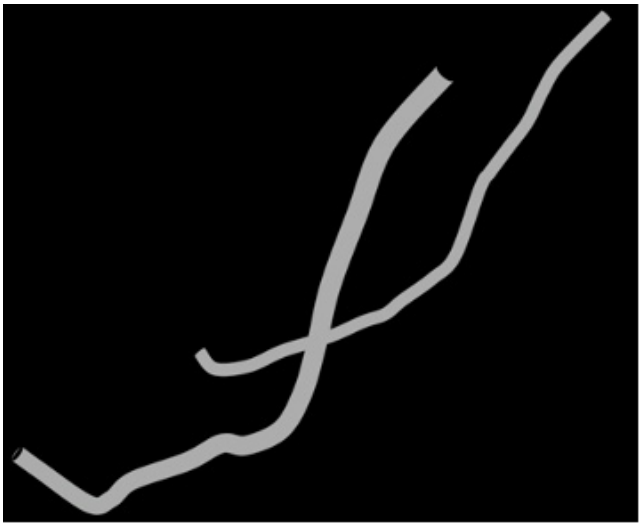

Fig. 2. Results of the semi-automatic segmentation of the IAC performed by an investigator using Romexis® software

Automatic segmentation

Artificial intelligence was used to perform the automatic tracing of the IAC in the same anonymized images that had been previously segmented semi-automatically. The images were uploaded to Diagnocat (DGNCT LLC, Miami, USA), an online AI-based platform designed for the storage and processing of dental images based on a U-Net-like architecture algorithm. The AI algorithm automatically generated the IAC tracing and saved it as an STL file (Fig. 3). The time required to complete the process was recorded, with an average Internet speed of 290 Mbps.

Evaluation of the 3D models obtained from 2 segmentation methods

Following the completion of the segmentation process using both methods, the STL files were exported to Geomagic Studio (3D Systems, Morrisville, USA). The software was used to overlap the segmented IAC produced by both methods onto the same image (3D registration), facilitating a 3D visualization for the purpose

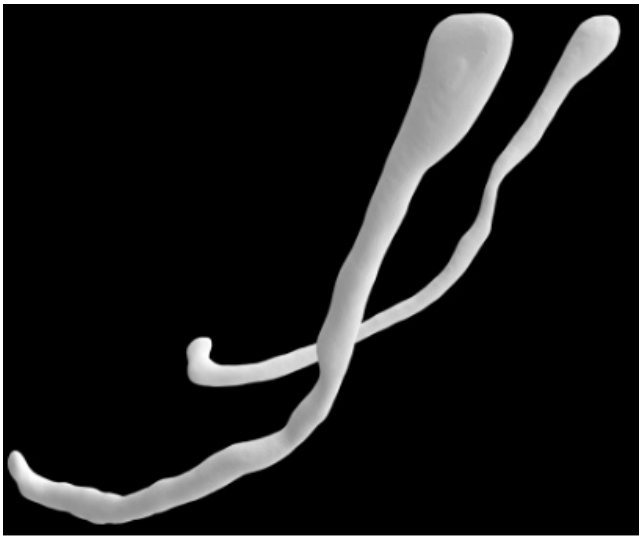

Fig. 3. Results of the automatic segmentation of the IAC performed using the Diagnocat platform

of evaluating the accuracy of the AI model in comparison to the semi-automatic method (Fig. 4).

As a first step, a pre-registration was conducted using the 3-point method to ensure accurate orientation of the models in relation to one another within the 3D space. This step was performed by an experienced Geomagic Studio user (MR). In the second step, the automatic registration procedure was initiated (Fig. 4A).

The software uses 100 iterations to calculate and minimize the mean square error of the global distance between the surfaces of the overlapping structures without requiring input from an operator. To compare the surface results of the 3D models, the 3D Compare command in Geomagic Control software (Geomagic Studio; 3D Systems) was used, which generated numerical results, including volumetric deviation and average distance, as well as a color map with 15 segments, each representing a different level of volumetric deviation. The average distance was then compared for the purpose of visualizing and assessing the deviation in the individual IAC areas of the 3D models (Fig. 4B).

Statistical analysis

The statistical analysis was conducted using the IBM SPSS Statistics for Windows software, v. 29.0 (IBM Corp., Armonk, USA). The numerical results of the 3D evaluation were subjected to a descriptive statistical analysis and summarized using mean and standard deviation ($M \pm SD$). The normality of the numerical variables was evaluated using the Shapiro–Wilk test, with a significance level of $p < 0.05$. Subsequently, an inferential statistical analysis was performed using the non-parametric Mann–Whitney U test for numerical variables with a non-normal distribution. All inferential tests were conducted in accordance with the assumptions of a 95% confidence interval (CI) and a p-value of less than 0.05.

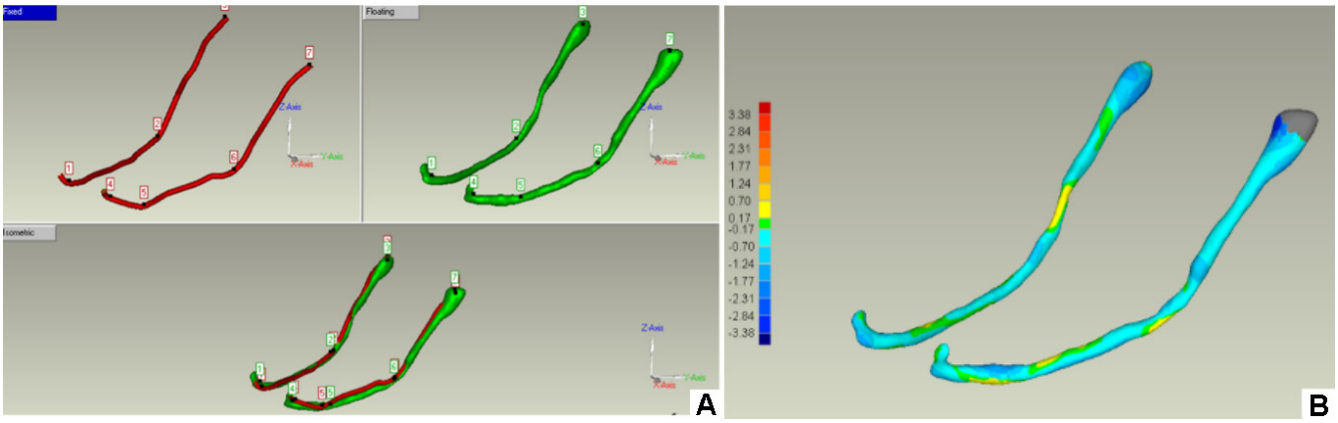

Fig. 4. Evaluation of the accuracy of an artificial intelligence (AI) model in comparison to a semi-automatic model

A. Overlapping the segmented IACs obtained by both methods using Geomagic Studio software: red color (semi-automatic segmentation); green color (automatic segmentation); B. 3D comparison deviation chromatogram after the overlapping of the segmented IACs: blue color (minus direction of deviation); red color (plus direction of deviation); and green color (average value).

Results

The results of the overlap analysis between the semi-automatic and automatic segmentation methods, in terms of average distance, are shown in Table 2. These values were calculated for each side (left and right) of the included patients. The mean value of the average distance across all patients was found to be 0.275 ±0.475 mm.

The segmentation time was recorded for both methods and subjected to the Mann–Whitney *U* test for comparison. The recorded times for the semi-automatic and automatic segmentation methods were 175.67 ±49.08 s and 175.00 ±68.08 s, respectively. The Mann–Whitney *U* test demonstrated that the difference in time between the 2 methods was not statistically significant ($p = 0.389$).

Table 2. Results of the overlap analysis

Patient number	Side	Distance [mm]
1	left	0.329 ±0.485
	right	0.234 ±0.422
2	left	0.304 ±0.292
	right	0.337 ±0.323
3	left	0.301 ±0.409
	right	0.197 ±0.462
4	left	0.186 ±0.795
	right	0.120 ±0.797
5	left	0.308 ±0.337
	right	0.167 ±0.359
6	left	0.277 ±0.385
	right	0.309 ±0.213
7	left	0.321 ±0.323
	right	0.253 ±0.403
8	left	0.419 ±0.449
	right	0.399 ±0.605
9	left	0.145 ±0.900
	right	0.168 ±0.733
10	left	0.327 ±0.365
	right	0.300 ±0.337
11	left	0.269 ±0.285
	right	0.207 ±0.342
12	left	0.226 ±0.705
	right	0.322 ±0.477
13	left	0.397 ±0.406
	right	0.387 ±0.322
14	left	0.161 ±0.961
	right	0.154 ±0.791
15	left	0.427 ±0.329
	right	0.328 ±0.266
Total		0.275 ±0.475

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

Discussion

Imaging techniques such as CBCT have significantly improved the ability to detect and segment the IAC.[21] Accurate detection of the IAC is vital in pre-surgical planning for dental procedures to prevent injury, particularly in view of the diverse variations that may be encountered.[22,23] However, semi-automatic detection of the IAC in CBCT images can be time-consuming and prone to human error.

Recently, there has been a growing trend in using and developing automated algorithms for the detection of the IAC in CBCT images.[20] These algorithms employ ML techniques, such as DL, to analyze the images and accurately locate the IAC. Artificial intelligence has the potential to markedly enhance the efficiency and precision of pre-surgical planning for dental procedures, reducing the risk of nerve injury. Additionally, AI has the potential to significantly increase the speed and efficiency of tasks such as image analysis, as well as assist in diagnosis.[24,25]

The automation of repetitive and time-consuming tasks by AI enables medical professionals to prioritize direct patient care, ultimately improving patient outcomes.[24,25] This research compares the AI-based IAC segmentation performed by Diagnocat (DGNCT LLC) with the semi-automatic segmentation performed by a specialist in terms of segmentation time and accuracy.

The average distance, representing the level of deviation between the points on the surface of the overlapped structures, was used in order to evaluate the accuracy of IAC segmentation performed by both methods. The average distance is a recommended metric for assessing non-regular complex shapes and quantitatively evaluating overlapping structures.[26–28] The results obtained from comparing the average distance of the overlapped semi-automatic segmentation and Diagnocat segmentation of the 30 IACs present the value of 0.275 ±0.475 mm. In contrast to a study conducted by Jaskari et al., our research yielded superior outcomes, with an average symmetric surface distance of 0.45 mm for both canals (with a *SD* of 0.12 mm for the left canal and 0.11 mm for the right canal).[29] These results suggest that the segmentation generated by Diagnocat is highly accurate, approaching the precision of the semi-automatic segmentation performed by an operator. A lower average distance indicates greater similarity in shape and volume between the 2 structures, and a higher accuracy of the automatic segmentation.

A variety of methodologies exist for medical image segmentation. Some involve user interaction for support, a process known as energy minimization. Others utilize DL, which enhances accuracy by comparing its predictions with real data.[30] However, the second method encounters challenges with including user feedback and potential corrections in the process.[30] Upon investigating the reasons behind the average distance differences observed in certain cases, we observed that Diagnocat tends to segment the IAC along the radiolucent path, following a straight pattern. However, in practice, some discrepancies were noted (Fig. 5).

The results of the segmentation time analysis indicate that the semi-automatic and automatic segmentation methods require a similar amount of time. The mean time for the semi-automatic method was 175.67 ±49.08 s, while the mean time for the automatic method was 175.00 ±68.08 s. The Mann–Whitney *U* test revealed no statistically significant difference in time between the 2 techniques. A standardized assessment technique was used in our study to ensure a comprehensive and uniform comparison. For the semi-automatic method, the time was recorded from the initiation of segmentation by the operator until its completion. For the AI-based evaluation, the time was recorded after the activation of the segmentation command until the generation of the segmented output. This is in contrast to a study conducted by Lahoud et al., in which the overall time was recorded from the initial DICOM file upload to the software/AI-based model to the visualization of results.[31] Their findings demonstrated that AI exhibited a significantly higher processing speed than the manual segmentation, with a factor of 107.[31]

Limitations

This study has several limitations that should be considered when interpreting the results. The 3D models of the IAC obtained from the semi-automatic and automatic segmentation methods differ in shape. In the semi-automatic procedure, the shape of the IAC is that of a tube with a fixed diameter. In contrast, the shape of the IAC in the automatic procedure varies along the length of the canal.

Another limitation is that Diagnocat segmentation results in a wide segmentation of the IAC at the mandibular entrance. In contrast, the semi-automatic method yields a narrow segmentation at the same location. This discrepancy in segmentation width may lead to errors when the results of the 2 methods are overlapped and compared (Fig. 6).

Additionally, in the case of the AI-based automatic segmentation, the algorithm did not correctly detect the IAC

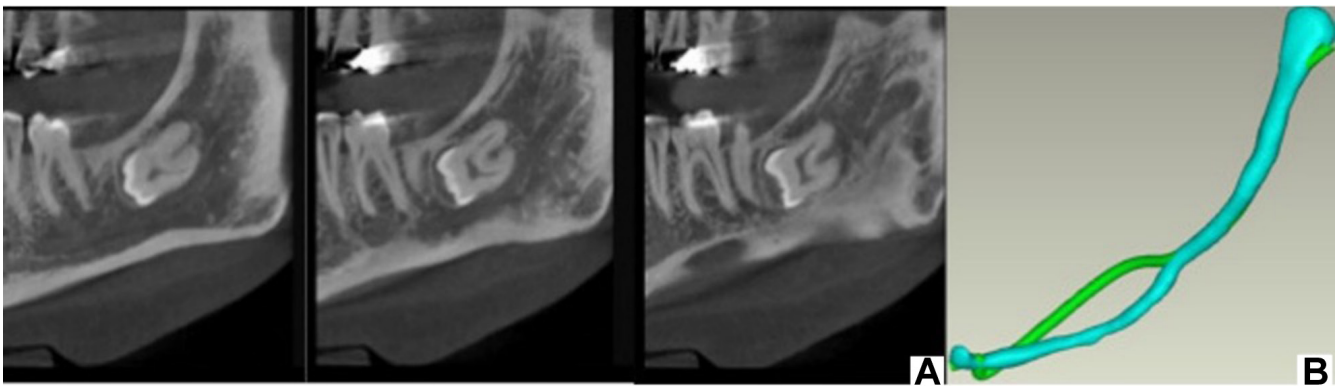

Fig. 5. Discrepancies between the semi-automatic segmentation and Diagnocat segmentation

A. Corrected sagittal views of the left IAC of patient 4; B. Overlapped IAC segmentations (left side of patient 4) in Geomagic Studio software: green region (semi-automatic segmentation); blue region (AI-based segmentation).

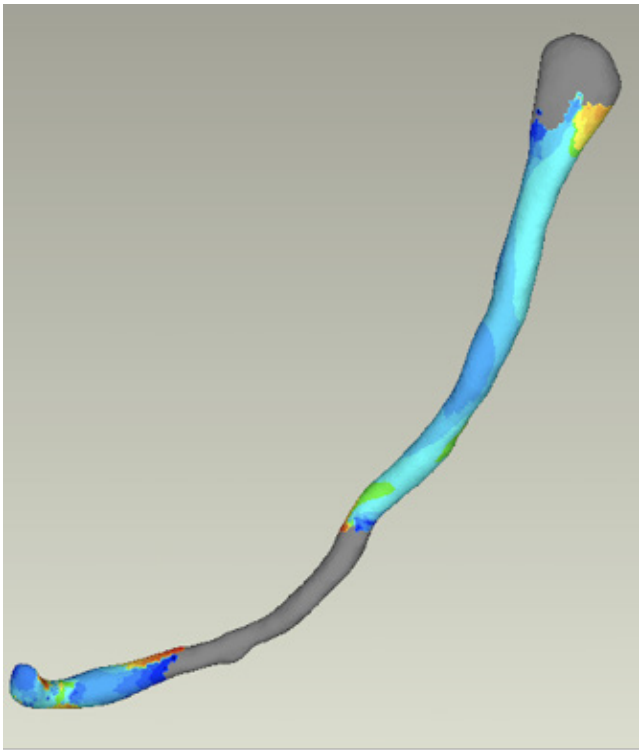

Fig. 6. Results of the overlapping procedure with the area of error indicated in gray

in some areas of the CBCT image. This resulted in an underestimation or even complete absence of the IAC diameter, which in turn led to the exclusion of portions of the 3D model (Fig. 7).

The current findings must be viewed with caution, as the sample size was relatively small. Further research with a larger number of participants is required to confirm these results.

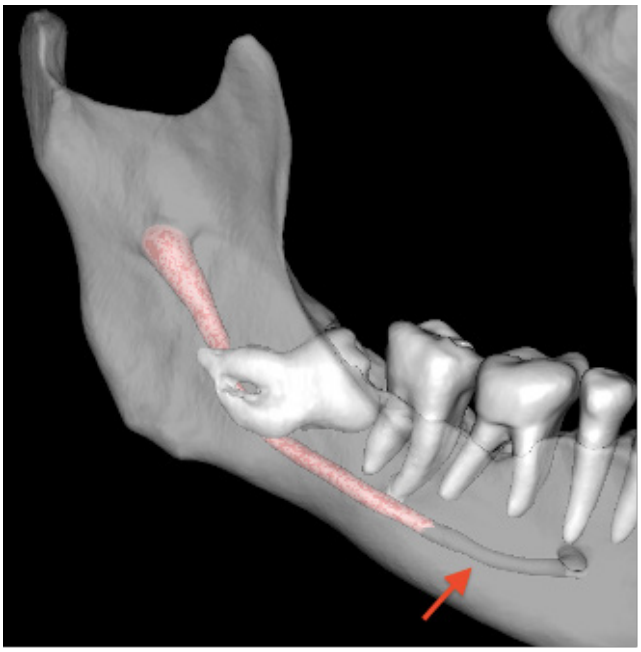

Fig. 7. Area not identified by Diagnocat segmentation of the IAC (red arrow)

Conclusions

The results of our study demonstrated a low mean distance and a non-significant discrepancy in time between the AI-based and semi-automatic segmentation methods. The findings indicate that the AI-based segmentation has the potential to serve as a reliable assisting tool in pre-surgical planning for dental procedures. Further studies are required to make more direct comparisons and take into account the limitations mentioned in our study.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and exempted from ethical approval on the grounds of its observational nature. The Ethics Committee of Poznan University of Medical Sciences granted a waiver of patient consent for the use of anonymized radiographs in this retrospective study.

Data availability

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

Consent for publication

Not applicable.

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References

1. Iwanaga J, Matsushita Y, Decater T, Ibaragi S, Tubbs RS. Mandibular canal vs. inferior alveolar canal: Evidence-based terminology analysis. *Clin Anat*. 2021;34(2):209–217. doi:10.1002/ca.23648
2. Yaghmaei M, Mashhadiabbas F, Shahabi S, Zafarbakhsh A, Yaghmaei S, Khojasteh A. Histologic evaluation of inferior alveolar lymphatics: An anatomic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;112(5):564–567. doi:10.1016/j.tripleo.2010.11.013
3. Waldman SD. Inferior Alveolar Nerve Block. In: *Atlas of Interventional Pain Management*. 4th ed. Elsevier eBooks; 2015:75–78. doi:10.1016/b978-0-323-24428-2.00021-8
4. Shankland WE. The trigeminal nerve. Part IV: The mandibular division. *Cranio*. 2001;19(3):153–161. doi:10.1080/08869634.2001.11746164S
5. Li Y, Ling Z, Zhang H, et al. Association of the inferior alveolar nerve position and nerve injury: A systematic review and meta-analysis. *Healthcare (Basel)*. 2022;10(9):1782. doi:10.3390/healthcare10091782
6. Juodzbalys G, Wang HL, Sabalys G. Injury of the inferior alveolar nerve during implant placement: A literature review. *J Oral Maxillofac Res*. 2011;2(1):e1. doi:10.5037/jomr.2011.2101
7. 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

8. Castro R, Guivarc'h M, Foletti JM, Catherine JH, Chossegros C, Guyot L. Endodontic-related inferior alveolar nerve injuries: A review and a therapeutic flow chart. *J Stomatol Oral Maxillofac Surg*. 2018;119(5):412–418. doi:10.1016/j.jormas.2018.04.012
9. Phillips C, Essick G. Inferior alveolar nerve injury following orthognathic surgery: A review of assessment issues. *J Oral Rehabil*. 2011;38(7):547–554. doi:10.1111/j.1365-2842.2010.02176.x
10. Venkatesh E, Elluru SV. Cone beam computed tomography: Basics and applications in dentistry. *J Istanb Univ Fac Dent*. 2017;51(3 Suppl 1):S102–S121. doi:10.17096/jiufd.00289
11. Kaasalainen T, Ekholm M, Siiskonen T, Kortesniemi M. Dental cone beam CT: An updated review. *Phys Med*. 2021;88:193–217. doi:10.1016/j.ejmp.2021.07.007
12. Jung YH, Cho BH. Radiographic evaluation of the course and visibility of the mandibular canal. *Imaging Sci Dent*. 2014;44(4):273–278. doi:10.5624/isd.2014.44.4.273
13. Piskórz M, Bukiel A, Kania K, Kałkowska D, Różyło-Kalinowska I. Retromolar canal: Frequency in a Polish population based on CBCT and clinical implications – a preliminary study. *Dent Med Probl*. 2023;60(2):273–278. doi:10.17219/dmp/147005
14. Ozturk A, Potluri A, Vieira AR. Position and course of the mandibular canal in skulls. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2012;113(4):453–458. doi:10.1016/j.tripleo.2011.03.038
15. Collins C, Dennehy D, Conboy K, Mikalef P. Artificial intelligence in information systems research: A systematic literature review and research agenda. *Int J Inf Manag*. 2021;60:102383. doi:10.1016/j.ijinfomgt.2021.102383
16. Xu Y, Liu X, Cao X, et al. Artificial intelligence: A powerful paradigm for scientific research. *Innovation (Camb)*. 2021;2(4):100179. doi:10.1016/j.xinn.2021.100179
17. Xu M, David JM, Kim SH. The fourth industrial revolution: Opportunities and challenges. *Int J Financ Res*. 2018;9(2):90–95. doi:10.5430/ijfr.v9n2p90
18. Maqbool R, Saiba MR, Ashfaq S. Emerging industry 4.0 and Internet of things (IoT) technologies in the Ghanaian construction industry: Sustainability, implementation challenges, and benefits. *Environ Sci Pollut Res Int*. 2023;30(13):37076–37091. doi:10.1007/s11356-022-24764-1
19. Janiesch C, Zschech P, Heinrich K. Machine learning and deep learning. *Electron Mark*. 2021;31:685–695. doi:10.1007/s12525-021-00475-2
20. Issa J, Olszewski R, Dyszkiewicz-Konwińska M. The effectiveness of semi-automated and fully automatic segmentation for inferior alveolar canal localization on CBCT scans: A systematic review. *Int J Environ Res Public Health*. 2022;19(1):560. doi:10.3390/ijerph19010560
21. Del Lhano NC, Ribeiro RA, Martins CC, Picorelli Assis NMS, Devito KL. Panoramic versus CBCT used to reduce inferior alveolar nerve paresthesia after third molar extractions: A systematic review and meta-analysis. *Dentomaxillofac Radiol*. 2020;49(4):20190265. doi:10.1259/dmfr.20190265
22. Agbaje JO, de Casteele EV, Salem AS, Anumendem D, Lambrichts I, Politis C. Tracking of the inferior alveolar nerve: Its implication in surgical planning. *Clin Oral Investig*. 2017;21(7):2213–2220. doi:10.1007/s00784-016-2014-x
23. Reda R, Zanza A, Bhandi S, De Biase A, Testarelli L, Miccoli G. Surgical–anatomical evaluation of mandibular premolars by CBCT among the Italian population. *Dent Med Probl*. 2022;59(2):209–216. doi:10.17219/dmp/143546
24. van Leeuwen KG, de Rooij M, Schalekamp S, van Ginneken B, Rutten MJCM. How does artificial intelligence in radiology improve efficiency and health outcomes? *Pediatr Radiol*. 2022;52(11):2087–2093. doi:10.1007/s00247-021-05114-8
25. Ahuja AS. The impact of artificial intelligence in medicine on the future role of the physician. *PeerJ*. 2019;7:e7702. doi:10.7717/peerj.7702
26. Fenster A, Chiu B. Evaluation of segmentation algorithms for medical imaging. *Conf Proc IEEE Eng Med Biol Soc*. 2005;2005:7186–7189. doi:10.1109/IEMBS.2005.1616166
27. Taha AA, Hanbury A. Metrics for evaluating 3D medical image segmentation: Analysis, selection, and tool. *BMC Med Imaging*. 2015;15:29. doi:10.1186/s12880-015-0068-x
28. Brock KK, Mutic S, McNutt TR, Li H, Kessler ML. Use of image registration and fusion algorithms and techniques in radiotherapy: Report of the AAPM Radiation Therapy Committee Task Group No. 132. *Med Phys*. 2017;44(7):e43–e76. doi:10.1002/mp.12256
29. Jaskari J, Sahlsten J, Järnstedt J, et al. Deep learning method for mandibular canal segmentation in dental cone beam computed tomography volumes. *Sci Rep*. 2020;10(1):5842. doi:10.1038/s41598-020-62321-3
30. Trimpl MJ, Primakov S, Lambin P, Stride EPJ, Vallis KA, Gooding MJ. Beyond automatic medical image segmentation – the spectrum between fully manual and fully automatic delineation. *Phys Med Biol*. 2022;67(12). doi:10.1088/1361-6560/ac6d9c
31. Lahoud P, Diels S, Niclaes L, et al. Development and validation of a novel artificial intelligence driven tool for accurate mandibular canal segmentation on CBCT. *J Dent*. 2022;116:103891. doi:10.1016/j.jdent.2021.103891

Original paper

Evaluating the accuracy of one-piece and three-piece 3D-printed indirect bonding transfer trays: An in vitro study

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

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Abstract

Background. Recent developments in computer-aided design/computer-aided manufacturing (CAD/CAM) and 3D printing have enabled the fabrication of digital indirect bonding (IDB) transfer trays. These modern products require thorough investigation.

Objectives. The aim of the study was to determine the accuracy of one-piece and three-piece IDB transfer trays in vitro.

Material and methods. An initial dental scan (IDS) of a randomly selected patient with digitally positioned brackets served as the master scan (MS) for designing 16 IDB transfer trays of each type. They were 3D printed and used for bonding 448 brackets to the models. Subsequently, the models were scanned with a TRIOS® 3 Intraoral Scanner (3Shape A/S, Copenhagen, Denmark), producing actual scans (ASs). The accuracy of bracket positioning was measured digitally on both MSs and ASs. The measurements were compared to the Objective Grading System for dental casts provided by the American Board of Orthodontics (ABO).

Results. The 2 types of IDB transfer trays showed comparable accuracy. All linear errors were within the clinically acceptable range, whereas the angular measurements demonstrated significant variability, resulting in clinically unacceptable transfer errors that ranged from 3.3% to 90.3%.

Conclusions. The study results cannot be unconditionally extrapolated to other types of IDB transfer trays due to the diversity of their properties and features. The study evaluated the in vitro accuracy of IDB transfer trays. The revealed number of errors may be even higher in vivo due to limitations in visibility, salivary flow, interference from the tongue, and difficulties in achieving a proper fit of the IDB transfer tray to the teeth.

Keywords: orthodontics, 3D printing, computer-aided design, orthodontic brackets, IBT resin

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Introduction

Bracket positioning represents a significant challenge in orthodontic treatment with fixed appliances, the objective of which is to achieve the best results with minimal archwire bending or bracket repositioning. In this regard, the indirect bonding (IDB) technique offers considerable promise. This method, which employs transfer trays, was first proposed in 1972 by Silverman et al.[1] Since that time, various modes of IDB have been developed, and numerous studies have been conducted to examine the precision of bracket positioning with IDB transfer trays.[2–17]

Current IDB transfer trays are manufactured from a variety of materials, each with distinct properties, that are important in orthodontic treatment.[3,4,6] Transfer trays made of thermoplastic materials are known for their ease of use and adaptability to the dental arch. However, their flexibility and susceptibility to distortion raise concerns about the precision of bracket placement.[4,6] Conversely, silicone-based transfer trays offer improved rigidity and accuracy, although their increased stiffness can sometimes complicate their application.[3,4,6,11,13] Substantial advancements in computer-aided design/computer-aided manufacturing (CAD/CAM) have enabled the digital design and fabrication of IDB transfer trays using resin for 3D printing. The digitization of IDB transfer tray fabrication, particularly through resin 3D printing, represents a significant advancement in orthodontic treatment modalities. These modern, digitally-designed products offer previously unattainable customization. However, rigorous scrutiny is required to validate their reliability and efficacy. An important aspect of this advancement is the introduction of IDB materials in 3D printing. These materials, which are characterized by softness and susceptibility to damage, differ from traditional 3D-printing materials[18–20] and cannot endure extended storage or undergo polishing like other materials used in dental applications.

The objective of our study was to evaluate the precision of bracket bonding using IDB transfer trays created with 3D-printed biocompatible resin. The focus of the study is on assessing the accuracy of these trays in both one-piece and three-piece designs, while also highlighting the advancements in material science and digital technology in orthodontics. This study addresses not only the technical aspects of IDB tray fabrication but also the clinical implications of these advancements, evaluating their impact on treatment outcomes and efficacy.

Material and methods

IDB transfer tray management

A randomly selected patient's occlusion was scanned using a TRIOS® 3 Intraoral Scanner (3Shape A/S, Copenhagen, Denmark) with an accuracy of 6.9 μm, resulting in the initial dental scan (IDS). The patient presented with Angle Class I malocclusion on the right side and Angle Class II malocclusion on the left side, in addition to dental crowding, a normal overbite and an increased overjet. Subsequently, virtual pre-torqued brackets (Victory Series™ LP Roth 022 APC Flash-Free; 3M ESPE, St. Paul, USA) were placed on the virtual teeth of the IDS using Ortho Analyzer 2019 software (3Shape A/S), resulting in the master scan (MS). Based on the MS, transfer trays for the IDB were designed to rest solely on the teeth, thus avoiding contact with the gingiva and fully covering the brackets, with the exception of the undercuts of the gingival bracket wings. The thickness of each IDB transfer tray reached 2.3 mm around the brackets and at least 4 mm in the occlusal part.

At this stage, the virtual IDB transfer trays were divided into 2 groups based on the transfer mode: Group I – one-piece IDB transfer trays (n = 16); and Group II – three-piece IDB transfer trays (n = 16). In Group II, the IDB transfer trays were designed similarly to those in Group I, but were divided in CAD software into 3 segments: an anterior segment extending from the right canine to the left canine; and 2 lateral segments extending from the first premolars to the second molars. The IDB transfer trays were subsequently 3D printed on the SprintRay Pro 95 3D printer (SprintRay Inc., Los Angeles, USA) using a transparent biocompatible resin specially designed for IDB transfer trays (NextDent Ortho IBT; Nextdent, Soesterberg, the Netherlands), with an accuracy of 50 μm (Fig. 1).

The IDS was 3D printed using Model Gray resin (SprintRay Inc.) with an accuracy of 50 μm, serving as the initial model (IM), and replicated 32 times. The obtained models were coated with Transbond XT LC Adhesive Kit (3M ESPE). The material was applied to the models using compressed air and cured on each tooth with a Valo™ X polymerization lamp (Ultradent Products, Inc., South Jordan, USA) in normal mode for 20 s. Metal brackets with pre-applied adhesive were used in the study (Victory Series™ LP Roth 022 APC Flash-Free; 3M ESPE). The brackets were manually placed in the IDB transfer trays from both

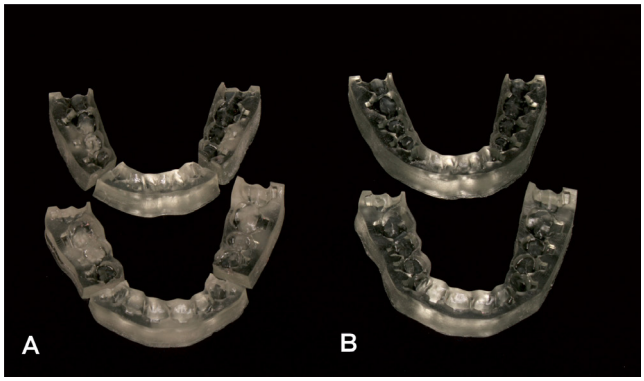

Fig. 1. 3D-printed indirect bonding (IDB) transfer trays
A. Three-piece tray; B. One-piece tray.

study groups and bonded to the models. The adhesive was polymerized with the Valo™ X polymerization lamp in Xtra Power mode, which was repeated twice for 3 s. All brackets were bonded by the same clinician.

The models with bonded brackets were scanned using the TRIOS 3 Intraoral Scanner, thereby facilitating the acquisition of the actual scans (ASs). In order to eliminate reflections on the brackets during scanning, the models with bonded brackets were coated with a thin layer of Helling 3D Scan Spray (Helling GmbH, Heidgraben, Germany) with an average particle size of 2.8 µm.

Measurements

Using GOM Inspect V8 SR1 software (Carl Zeiss GOM Metrology GmbH, Braunschweig, Germany), the ASs with brackets bonded using 3D-printed IDB transfer trays were superimposed on the MSs of the patient's dentition with brackets generated by the Ortho Analyzer software. Only the lingual and occlusal surfaces of the dentition were selected to ensure that the superimposition did not include the brackets. To prevent bias in the imposition, the dental arches were divided into 3 sections: from the right second molar to the right first premolar; from the right canine to the left canine; and from the left first premolar to the left second molar. The superimposition was conducted for each section separately in order to enable the identification of the optimal fit at the local level (Fig. 2). To determine the differences between the bracket positions in the MSs and the ASs, the "3-2-1" technique was applied using the local X, Y and Z coordinate system, with the reference points illustrated in Fig. 3. The 2 coordinate systems were compared using GOM Inspect software to calculate the differences between bracket positions in the MSs and the ASs and provide the linear or angular errors. The linear errors in the mesio-distal, linguo-vestibular and occluso-gingival directions were measured as the distances between the bracket positions in the MS and the AS in relation to the X-, Y- and Z-axes, respectively. Any toric, oblique, or rotational errors were measured as the inclination of the bracket positions from the MS to the AS in relation to the X-, Y- and Z-axes and noted as torque, tip and rotation, respectively. For linear measurements, a positive value indicated mesial, vestibular, or occlusal bracket displacement. For angular measurements, a positive value indicated palatal/lingual crown torque, mesial tipping, or disto-vestibular rotation of the bracket. To assess the repeatability of the "3-2-1" technique, measurements of the same model were repeated 3 times by the same experienced operator, 7 days apart.

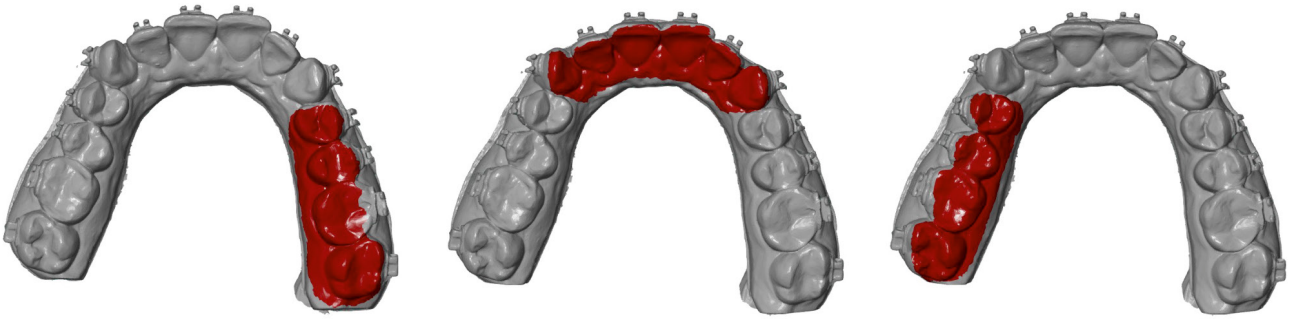

Fig. 2. Superimposition of the model
Areas selected for the local "best fit" are marked in red.

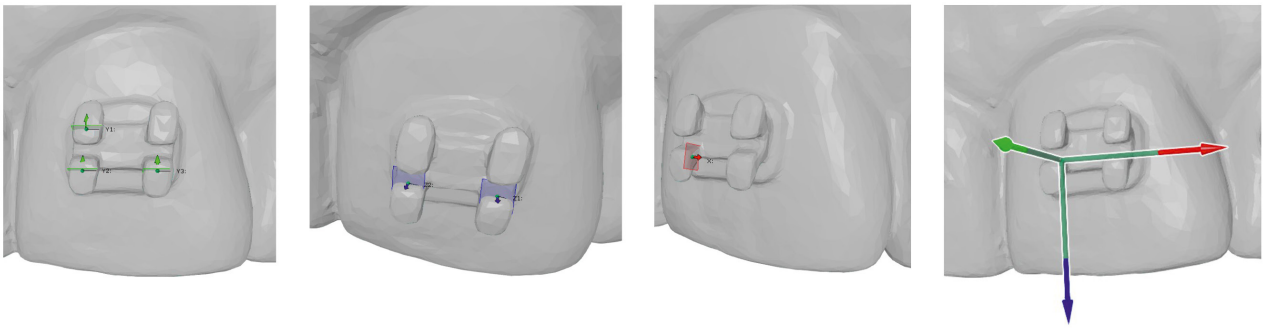

Fig. 3. Reference points determining the local coordinate system using the "3-2-1" technique
The color red is used to indicate reference points on the X-axis, the color green is used to indicate reference points on the Y-axis, and the color blue is used to indicate reference points on the Z-axis.

In accordance with the American Board of Orthodontics (ABO) Objective Grading System,[21] linear errors ≤0.5 mm in the proper alignment are considered clinically acceptable. This criterion was adhered to in our study, thereby enabling a reliable evaluation of the results. Additionally, given that a marginal ridge discrepancy of 0.5 mm in an average-sized molar would result in a crown tip deviation of 2°, angular errors ≤2° were also defined as clinically acceptable.

Statistical analysis

The repeatability of the measurements was assessed using Lin's concordance correlation coefficient. Student's *t*-test was performed to evaluate the bracket placement errors, and Fisher's exact test was used to assess the prevalence of clinically acceptable transfer errors. All statistical analyses were conducted using Statistica® v. 13.3 software (TIBCO Software Inc., Palo Alto, USA).

Results

Out of a total of 448 bonded brackets, 14 were ineligible for measurements due to various reasons, including debonding during the IDB procedure or incomplete capture by the scanner. The transfer accuracy of 434 brackets was examined: 219 from Group I; and 215 from Group II, allowing for a total of 2,604 bracket positioning measurements.

Repeatability of measurements

Lin's concordance correlation coefficient demonstrated high repeatability of the measurements, with coefficients in the order of 0.9 (α = 5%) at 0.980, 0.994, 0.998, 0.982, 0.986, and 0.997 for mesio-distal, linguo-vestibular, occluso-gingival, toric, oblique, and rotational displacements, respectively.

Errors of placement

Overall, more significant bracket displacements resulting in substantial changes in positions between the MS and the AS were observed in the mandible and in Group II (Table 1,2).

Maxilla: significant results

The molar brackets in Group I demonstrated linear errors in 3 directions, resulting in more mesial, vestibular and gingival positions in the ASs compared to the MSs. The molar brackets in Group II showed significant vestibular displacement. The brackets placed on the incisors and canines in both groups were positioned more vestibularly. The brackets placed on the incisors, canines and premolars in both groups displayed additional palatal crown torque, although this torque change did not exceed 2° for the canines in Group II. The premolar brackets in Group I exhibited oblique displacement, resulting in additional distal tipping (Table 1).

Mandible: significant results

In Group I, all brackets on the incisors, canines and premolars were displaced mesially. The incisor and molar brackets in both groups, as well as the premolar brackets in Group II, exhibited vestibular displacement. The premolar brackets in Group I, as well as the molar brackets in both groups, were displaced gingivally, whereas the canine brackets from Group II were shifted toward the occlusal plane. The incisor, premolar and molar brackets in both groups, as well as the canine brackets in

Table 1. Descriptive statistics of the errors in the maxilla

Displacement	Incisors				Canines				Premolars				Molars			
	Group I		Group II		Group I		Group II		Group I		Group II		Group I		Group II	
	M ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value
M-D [mm]	−0.00 ±0.07	0.699	−0.02 ±0.08	0.182	−0.00 ±0.10	0.869	−0.06 ±0.13	0.110	0.02 ±0.07	0.097	0.02 ±0.08	0.144	0.06 ±0.09	0.001*	0.01 ±0.25	0.876
L-V [mm]	0.06 ±0.04	<0.001*	0.06 ±0.06	<0.001*	0.09 ±0.09	0.001*	0.17 ±0.08	<0.001*	0.00 ±0.07	0.981	0.04 ±0.06	0.001*	0.08 ±0.06	<0.001*	0.08 ±0.13	0.001*
O-G [mm]	−0.02 ±0.13	0.503	−0.15 ±0.24	0.001*	0.01 ±0.17	0.898	−0.08 ±0.33	0.341	0.00 ±0.11	0.987	−0.03 ±0.08	0.024*	−0.13 ±0.16	<0.001*	0.06 ±0.53	0.536
Torque [mm]	2.1 ±2.3	<0.001*	2.1 ±2.6	<0.001*	3.8 ±2.8	<0.001*	1.6 ±2.1	0.009*	4.8 ±3.2	<0.001*	4.6 ±1.8	<0.001*	−0.6 ±2.5	0.164	0.0 ±3.4	0.963
Tip [mm]	0.5 ±1.5	0.087	0.4 ±2.5	0.382	−1.5 ±4.7	0.213	−2.5 ±8.1	0.234	−1.1 ±1.5	<0.001*	−0.6 ±2.0	0.109	0.1 ±2.4	0.797	−0.1 ±5.9	0.946
Rotation [mm]	0.1 ±1.2	0.570	0.1 ±1.1	0.792	−1.1 ±4.6	0.348	0.1 ±1.7	0.814	0.1 ±1.2	0.570	0.1 ±1.1	0.792	−1.1 ±4.6	0.348	0.1 ±1.7	0.814

M-D – mesio-distal; L-V – linguo-vestibular; O-G – occluso-gingival; *M* – mean; *SD* – standard deviation; * statistically significant ($p < 0.05$, Student's *t*-test).

Table 2. Descriptive statistics of the errors in the mandible

Displacement	Incisors				Canines				Premolars				Molars			
	Group I		Group II		Group I		Group II		Group I		Group II		Group I		Group II	
	M ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value	*M* ±*SD*	*p*-value
M-D [mm]	0.13 ±0.14	<0.001*	0.06 ±0.13	0.076	0.03 ±0.09	0.043*	0.02 ±0.15	0.460	0.03 ±0.09	0.043*	0.02 ±0.15	0.460	0.00 ±0.11	0.824	0.00 ±0.09	0.983
L-V [mm]	0.09 ±0.11	<0.001*	0.10 ±0.12	<0.001*	0.04 ±0.10	0.120	0.03 ±0.30	0.305	0.00 ±0.07	0.981	0.04 ±0.06	0.001*	0.08 ±0.06	<0.001*	0.08 ±0.13	0.001*
O-G [mm]	−0.01 ±0.20	0.840	0.02 ±0.29	0.686	−0.05 ±0.26	0.486	0.28 ±0.33	0.004*	−0.17 ±0.18	<0.001*	−0.03 ±0.27	0.506	−0.11 ±0.31	0.049*	−0.34 ±0.28	<0.001*
Torque [mm]	1.9 ±3.3	0.003*	2.4 ±3.6	0.001*	1.1 ±2.6	0.111	1.3 ±2.3	0.036*	3.4 ±2.6	<0.001*	2.5 ±3.3	<0.001*	2.2 ±4.6	0.011*	4.6 ±3.1	<0.001*
Tip [mm]	1.7 ±2.2	<0.001*	2.6 ±2.2	<0.001*	0.3 ±4.1	0.797	−0.3 ±3.2	0.669	−0.2 ±1.8	0.490	1.2 ±3.2	0.046*	0.2 ±3.5	0.764	0.6 ±4.6	0.475
Rotation [mm]	−1.0 ±1.5	0.001*	−0.2 ±1.2	0.411	−0.1 ±5.5	0.921	−1.7 ±2.0	0.003*	0.3 ±2.9	0.556	0.1 ±1.6	0.733	−0.2 ±1.6	0.407	0.0 ±1.4	0.936

* statistically significant ($p < 0.05$, Student's *t*-test).

Group II, exhibited lingual crown torque. Mesial tipping of the incisor brackets in both groups and the premolar brackets in Group II was also observed. However, mesial rotation of the canine brackets occurred only in Group II (Table 2).

Prevalence of clinically acceptable transfer errors

With regard to linear errors, no measurements exceeded the ABO criteria in either group. However, for angular measurements, toric, oblique and rotational errors exceeding 2° were observed in both groups, with toric errors being the most affected (Fig. 4,5).

Comparison of inter-group accuracy

Regarding linear measurements, both groups demonstrated an equal accuracy of the IDB transfer trays in all dimensions, with no errors exceeding 0.5 mm (Fig. 4,5).

With regard to angular measurements, the toric displacement resulting in palatal/lingual crown torque was comparable in both groups. The only significant inter-group differences were observed in molar bracket positions, with a greater number of errors in oblique and rotational angulations in Group II in the maxilla and in oblique angulation in Group II in the mandible. The mandibular incisor brackets exhibited a more pronounced rotation in Group I (Fig. 4,5).

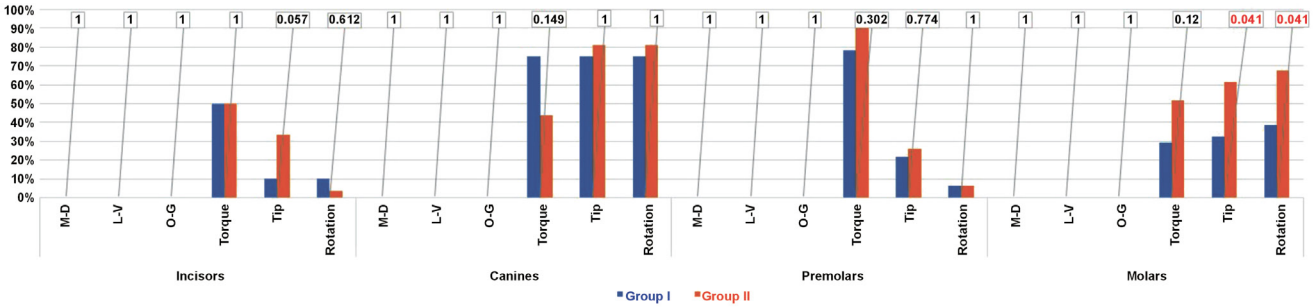

Fig. 4. Percentage distribution of measurements exceeding the acceptable deviations during bracket positioning in both groups in the maxilla
M-D – mesio-distal; L-V – linguo-vestibular; O-G – occluso-gingival. *P*-values are presented in the frames.

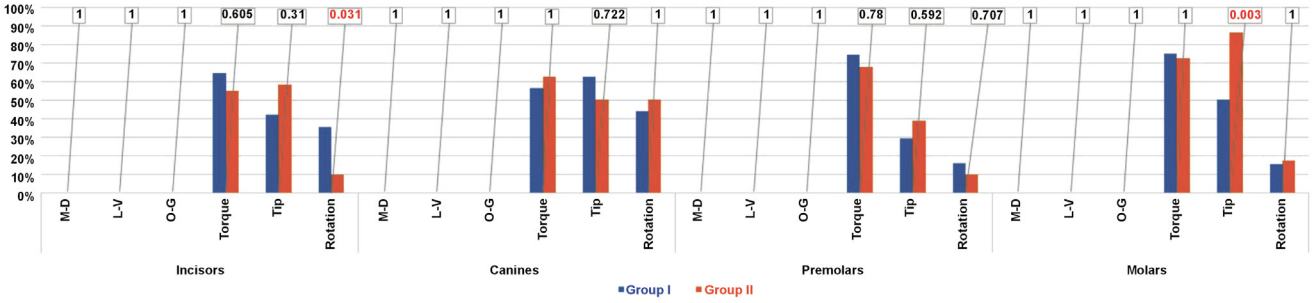

Fig. 5. Percentage distribution of measurements exceeding the acceptable deviations during bracket positioning in both groups in the mandible

Discussion

The size of our study sample was determined based on the literature.[3,6] Out of 448 bonded brackets, we assessed 434 due to a failure rate reaching 1.7% in Group I and 2.7% in Group II, which is comparable with values found in published papers.[6,11,16]

An in vitro study by Pottier et al. evaluated the precision of hard 3D-printed IDB transfer trays compared to soft silicone transfer trays and demonstrated the reliability of the "3-2-1" measurement technique, which justifies its application in our study.[11]

Currently, 6 technologies are used in 3D printing: stereolithography (SLA); digital light processing (DLP); fused filament fabrication (FFF); selective laser sintering (SLS); liquid crystal display (LCD); and PolyJet. Hazeveld et al. suggested the use of DLP or PolyJet prints, as they proved to be more precise than SLA.[22] However, due to the rapid evolution of 3D printing and the differences in printer designs, there is no scientific evidence to suggest that 1 method is superior in terms of providing the most accurate models. At the time of designing our study, the SprintRay Pro 95 3D printer was one of the most advanced DLP printers. To minimize bias associated with the printing process, both the models and the IDB transfer trays were printed with the highest possible accuracy of the material used, which was 50 μm.

To minimize the influence of adhesive thickness on bracket position, brackets with pre-applied adhesive were used in our study. The amount of adhesive was kept to a minimum and standardized.

A study by Zhang et al. compared 6 currently available intraoral scanners and demonstrated that the TRIOS 3 Intraoral Scanner had the highest precision (4.5 ±0.9 μm) and accuracy (6.9 ±0.9 μm), justifying its selection for our study.[23] Lab scanners were excluded due to their inability to precisely capture undercuts. Nevertheless, spraying the brackets with a thin layer of scan spray was necessary to avoid reflections of the stripe light by the metal surface. The average particle size of the spray used in the present study was 2.8 μm. Although spraying the brackets by an experienced dentist significantly improved the homogeneity of the layer thickness, a resultant unquantifiable systematic error cannot be entirely excluded.[24] Studies have shown that coating thickness may vary from 13.3 μm to 49.1 μm.[24]

The mechanical properties of the materials used for fabricating IDB transfer trays play a crucial role in ensuring the precision of bracket placement. Although these materials are easy to use and support the adaptability of IDB transfer trays, they are simultaneously prone to fragility, softness and flexibility. As highlighted by Paradowska-Stolarz et al., the mechanical properties of these materials can lead to deformation under force, resulting in deviations from the intended bracket position.[18] However, studies by Schwärzler et al. demonstrate that using higher hardness materials for the fabrication of IDB transfer trays can adversely impact the effectiveness of the bonding procedure.[14,15]

The 3D inspection and mesh processing software (GOM Inspect) presents data with an accuracy of up to 1 μm. In order to implement the "3-2-1" technique, 6 points were required to be marked in predefined positions. We could not rule out the possibility of inaccuracies occurring while marking the points. Therefore, we calculated Lin's concordance correlation coefficients, which demonstrated significantly consistent repeatability of the measurements.

To ensure the accuracy of the measurements, an automatic scan overlay was used in the initial stage. It was obtained via MS and AS superimposition along the greatest number of matching areas. This method revealed discrepancies in the posterior parts of the dental arches, which demonstrated the limitations of the intraoral scanner. Despite its high resolution, the intraoral scan does not accurately represent the shape of the entire dental arch, as evidenced by Anh et al.[25] Such discrepancies could also result from the model printing process itself, as demonstrated in a study by Kim et al.[5] Therefore, to avoid the influence of these discrepancies on the results of our study, we sectioned the scans into 3 parts to obtain the local "best fit".

Both types of IDB transfer trays enabled precise bracket positioning in the linear dimensions, consistent with the results of several in vitro studies.[3,5,6,9,12] Although significant vestibular and gingival displacement of the brackets was observed, none exceeded the ABO criteria. However, to enhance the precision in the occluso-gingival dimension, it is recommended to apply gentle pressure in the occlusal direction on the bracket after fitting the IDB transfer tray. To ensure better accuracy in the linguo-vestibular dimension, the vestibular part of the tray should be thicker. This increases the stiffness and makes the tray less prone to deformation.

With regard to angular errors, bracket positioning was found to be less accurate and characterized by great variability, resulting in clinically unacceptable transfer errors that ranged from 3.3% to 90.3%. These results align with those reported in the literature.[3,6,9,13,16,26] Niu et al. suggested that the low accuracy of angular bracket positioning may be attributed to the design of the IDB transfer tray, specifically the filling of all undercuts in the MS, which may result in incomplete adhesion of the IDB transfer tray to the bracket surface.[9] This limitation may impair the ability to control angular bracket positioning and may be considered a drawback of our study. Nevertheless, von Glasenapp et al. compared the accuracy of IDB transfer trays with jigs that fill the inner spaces of brackets, similar to those used in our study. The authors did not note the superiority of any of the evaluated IDB transfer trays in terms of angular control, indicating that filling the undercuts may not be the optimal method for preventing angular errors.[12] Thicker or more rigid IDB transfer trays

could enhance the angular control; however, increased thickness or rigidity may interfere with transfer tray removal and result in bond failures.

The use of CAD/CAM allows for the design and fabrication of IDB transfer trays in countless ways. However, many modalities may be affected by factors that influence the properties of trays. These factors may occur at any stage of tray manufacturing, from design through production, and they have implications for printing technology, material selection and post-processing methods. Bracket systems, which may vary in design and dimensions, may also influence the accuracy and precision of IDB transfer trays in bracket positioning.

Conclusions

In summary, it should be emphasized that our study results cannot be unconditionally extrapolated to other types of IDB transfer trays due to their diverse properties and features. Furthermore, it is important to note that our study evaluated the in vitro accuracy of the IDB transfer trays. It is possible that the error rate may be even higher in vivo due to limitations in visibility, salivary flow, interference from the tongue, and difficulties in achieving a proper fit of the IDB transfer tray to the teeth.

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.

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References

1. Silverman E, Cohen M, Gianelly AA, Dietz VS. A universal direct bonding system for both metal and plastic brackets. *Am J Orthod*. 1972;62(3):236–244. doi:10.1016/s0002-9416(72)90264-3
2. Duarte MEA, Gribel BF, Spitz A, Artese F, Miguel JAM. Reproducibility of digital indirect bonding technique using three-dimensional (3D) models and 3D-printed transfer trays. *Angle Orthod*. 2020;90(1):92–99. doi:10.2319/030919-176.1
3. Grünheid T, Lee MS, Larson BE. Transfer accuracy of vinyl polysiloxane trays for indirect bonding. *Angle Orthod*. 2016;86(3):468–474. doi:10.2319/042415-279.1
4. Castilla AE, Crowe JJ, Moses JR, Wang M, Ferracane JL, Covell DA Jr. Measurement and comparison of bracket transfer accuracy of five indirect bonding techniques. *Angle Orthod*. 2014;84(4):607–614. doi:10.2319/070113-484.1
5. Kim J, Chun YS, Kim M. Accuracy of bracket positions with a CAD/CAM indirect bonding system in posterior teeth with different cusp heights. *Am J Orthod Dentofacial Orthop*. 2018;153(2):298–307. doi:10.1016/j.ajodo.2017.06.017
6. Schmid J, Brenner D, Recheis W, Hofer-Picout P, Brenner M, Crismani AG. Transfer accuracy of two indirect bonding techniques – An in vitro study with 3D scanned models. *Eur J Orthod*. 2018;40(5):549–555. doi:10.1093/ejo/cjy006
7. Koo BC, Chung CH, Vanarsdall RL. Comparison of the accuracy of bracket placement between direct and indirect bonding techniques. *Am J Orthod Dentofacial Orthop*. 1999;116(3):346–351. doi:10.1016/s0889-5406(99)70248-9
8. Aguirre MJ, King GJ, Waldron JM. Assessment of bracket placement and bond strength when comparing direct bonding to indirect bonding techniques. *Am J Orthod*. 1982;82(4):269–276. doi:10.1016/0002-9416(82)90461-4
9. Niu Y, Zeng Y, Zhang Z, Xu W, Xiao L. Comparison of the transfer accuracy of two digital indirect bonding trays for labial bracket bonding. *Angle Orthod*. 2021;91(1):67–73. doi:10.2319/013120-70.1
10. Bachour PC, Klabunde R, Grünheid T. Transfer accuracy of 3D-printed trays for indirect bonding of orthodontic brackets. *Angle Orthod*. 2022;92(3):372–379. doi:10.2319/073021-596.1
11. Pottier T, Brient A, Turpin YL, et al. Accuracy evaluation of bracket repositioning by indirect bonding: Hard acrylic CAD/CAM versus soft one-layer silicone trays, an in vitro study. *Clin Oral Investig*. 2020;24(11):3889–3897. doi:10.1007/s00784-020-03256-x
12. von Glasenapp J, Hofmann E, Süpple J, Jost-Brinkmann PG, Koch PJ. Comparison of two 3D-printed indirect bonding (IDB) tray design versions and their influence on the transfer accuracy. *J Clin Med*. 2022;11(5):1295. doi:10.3390/jcm11051295
13. Gündoğ H, Arman Özçırpıcı A, Pamukçu H. Transfer accuracy of three indirect bonding trays: An in vitro study with 3D scanned models. *Turk J Orthod*. 2023;36(1):1–9. doi:10.4274/TurkJOrthod.2022.2022.34
14. Schwärzler A, Nemec M, Lettner S, Rank C, Schedle A, Jonke E. 3D printed indirect bonding trays: Transfer accuracy of hard versus soft resin material in a prospective, randomized, single-blinded clinical study. *Dent Mater*. 2023;39(11):1058–1065. doi:10.1016/j.dental.2023.09.011
15. Schwärzler A, Lettner S, Nemec M, Rank C, Schedle A, Jonke E. CAD/CAM indirect bonding trays using hard versus soft resin material: A single-blinded in vitro study. *Dent Mater*. 2023;39(9):831–838. doi:10.1016/j.dental.2023.07.003
16. Hoffmann L, Sabbagh H, Wichelhaus A, Kessler A. Bracket transfer accuracy with two different three-dimensional printed transfer trays vs silicone transfer trays. *Angle Orthod*. 2022;92(3):364–371. doi:10.2319/040821-283.1
17. Panayi NC, Tsolakis AI, Athanasiou AE. Digital assessment of direct and virtual indirect bonding of orthodontic brackets: A clinical prospective cross-sectional comparative investigation. *Int Orthod*. 2020;18(4):714–721. doi:10.1016/j.ortho.2020.09.007
18. Paradowska-Stolarz AM, Wieckiewicz M, Mikulewicz M, et al. Comparison of the tensile modulus of three 3D-printable materials used in dentistry. *Dent Med Probl*. 2023;60(3):505–511. doi:10.17219/dmp/166070
19. Paradowska-Stolarz A, Malysa A, Mikulewicz M. Comparison of the compression and tensile modulus of two chosen resins used in dentistry for 3D printing. *Materials (Basel)*. 2022;15(24):8956. doi:10.3390/ma15248956
20. Paradowska-Stolarz A, Wieckiewicz M, Kozakiewicz M, Jurczyszyn K. Mechanical properties, fractal dimension, and texture analysis of selected 3D-printed resins used in dentistry that underwent the compression test. *Polymers (Basel)*. 2023;15(7):1772. doi:10.3390/polym15071772
21. Casko JS, Vaden JL, Kokich VG, et al. Objective grading system for dental casts and panoramic radiographs. American Board of Orthodontics. *Am J Orthod Dentofacial Orthop*. 1998;114(5):589–599. doi:10.1016/s0889-5406(98)70179-9

22. Hazeveld A, Huddleston Slater JJR, Ren Y. Accuracy and reproducibility of dental replica models reconstructed by different rapid prototyping techniques. *Am J Orthod Dentofacial Orthop*. 2014;145(1):108–115. doi:10.1016/j.ajodo.2013.05.011
23. Zhang YJ, Shi JY, Qian SJ, Qiao SC, Lai HC. Accuracy of full-arch digital implant impressions taken using intraoral scanners and related variables: A systematic review. *Int J Oral Implantol (Berl)*. 2021;14(2):157–179. PMID:34006079.
24. Dehurtevent M, Robberecht L, Béhin P. Influence of dentist experience with scan spray systems used in direct CAD/CAM impressions. *J Prosthet Dent*. 2015;113(1):17–21. doi:10.1016/j.prosdent.2014.07.006
25. 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
26. Sabbagh H, Khazaei Y, Baumert U, Hoffmann L, Wichelhaus A, Janjic Rankovic M. Bracket transfer accuracy with the indirect bonding technique – A systematic review and meta-analysis. *J Clin Med*. 2022;11(9):2568. doi:10.3390/jcm11092568

Original paper

Electrodeposited hydroxyapatite coating on titanium after ultrashort-pulsed laser processing for a novel surface of endosseous implants

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Conflict of interest
None declared

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Abstract

Background. Ceramic endosseous implant coatings have gained esteem due to their favorable osteoinductive and osteoconductive properties. However, such a layer may be prone to failure under in vivo conditions, which necessitates its modification.

Objectives. The aim of the present study was to modify an electrodeposited hydroxyapatite (HA) coating on titanium (Ti) with ultrashort-pulsed lasers for the incorporation of the ceramic into the sample surface and the texturing of the metal surface. The obtained surface was planned for application on the endosseous implant surface to enhance osseointegration. To our knowledge, such laser modification of a HA coating has not been performed previously.

Material and methods. Four different HA coatings were created (A–D). Each coating was conditioned with 4 different laser irradiations (1-4 to 4-4), carried out using different power, velocity and frequency settings. The surface features of the laser-irradiated coatings were analyzed.

Results. The laser modifications of the HA coatings resulted in 2 kinds of surfaces. Laser-induced periodic surface structure (LIPSS) texturing could be observed on quadrants 1-4 to 3-4, with parallel grooves and HA crystals melted and sintered into spherical structures. The 4-4 laser surface conditioning did not altered the needle-like morphology of the HA coating. The LIPSS–fusion modification decreased the water contact angle of the samples.

Conclusions. The ultrashort-pulsed laser modification of the HA coating for regimes 1-4 to 3-4 resulted in the LIPSS texturing of the Ti surface with HA sinterization. Further biological analyses are necessary to evaluate the cell and tissue response to such laser-modified HA coating on Ti.

Keywords: titanium, hydroxyapatite, laser processing, periodic surface structure texturing

Introduction

Osseointegration, defined as a direct structural and functional connection between ordered living bone and the surface of a load-carrying implant, is a key factor of clinical success in dental implant rehabilitation.[1,2] The goal of modern implantology is rapid and strong osseointegration. There are many factors affecting osseointegration, such as implant factors, host factors, the surgical technique, the healing time, and the loading conditions.[3] Among implant factors, implant composition, biocompatibility, macroscopic and microscopic surface topography and treatment, osteogenic biological coatings, surface energy, and wettability can influence the osseointegration process.[3] Continuous research is focused on developing an implant with features favoring osseointegration. There are numerous procedures to modify the implant surface. Surface topography can be modified by blasting or etching.[4,5] A biological coating can be obtained by adding compounds affecting osteoblasts, such as hydroxyapatite (HA).[6,7] Wettability can be improved by the immersion of the modified titanium (Ti) implant in an isotonic solution at low pH or by photo-functionalization.[2,8]

One of the more recent techniques of surface modification is laser treatment. Precise, organized, nano- or micrometer surface structures,[9,10] can be manufactured using this technique, with a reduced risk of surface contamination.[11] Among different kinds of laser surface treatment, ultrashort-pulsed lasers have recently been investigated as a potential structuring tool for biomedical implants.[12,13] The technique focuses energy in time and space, resulting in laser-induced periodic surface structure (LIPSS) texturing, with minimal thermal damage. The LIPSS are self-organized formations observed on the surfaces treated with polarized laser radiation.[10]

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is a mineral from the family of apatites.[14,15] It is a natural component of bones and tooth tissue. Hydroxyapatite in the form of crystals provides the structural stability and hardness of the bone. It is also involved in the bone regeneration process, since it exhibits osteoinductive and osteoconductive properties. Synthetic HA, on the other hand, is currently used for bone repair, as well as for bone regeneration. In implantology, HA coatings on the implant surface have been used for many years as bioactive coatings.[16,17] Such a ceramic coating improves osseointegration and provides long-term in vivo functionality.[16,17] Those features are related to the great capacity of HA for adsorbing proteins, improving osteoblast proliferation, enhancing bone formation, reducing bone loss, and augmenting osteogenic activity through the direct release of calcium (Ca) and phosphate ions.[7,17,18] Hydroxyapatite used as a coating for endosseous implants has been reported to be an effective material for the bone–implant interface because of its similarity to the mineral phase of natural bone tissue.[7] Hydroxyapatite coatings can be applied on the implant surface with different techniques, such as plasma spraying, biomimetic deposition, the sol–gel technique, or electrophoretic deposition.[7,14,17] Some techniques, i.e., plasma spraying, utilize high temperatures, generating a large amount of amorphous HA and providing a microcracked thick coating.[7,14] Such a layer is prone to failure under in vivo conditions because of its high bio-dissolution rate, disintegration with the formation of debris particles, and the risk of delamination caused by low bond strength with Ti.[14,17] Conversely, the electrodeposition of HA is conducted at relatively low temperatures, eliminating the risk of the synthesis of amorphous HA.[19] The dissolution and bond strength degradation of the electrodeposited coatings are reported to be much lower than those of the plasma-sprayed coatings.[19,20] Also, a coating thickness of less than 1 μm increases delamination resistance.[19,21] The electrodeposition process can also result in good conformability to the shape of the component and in coating homogeneity.[19] However, the elevated temperature during electrodeposition results in decreased coating adhesion to the substrate as compared to the processes conducted at room temperature.[19,22] To incorporate HA into the Ti surface and reduce the risk of delamination, laser conditioning is applied. The obtained LIPSS are also meant to enhance cell differentiation, improving the osseointegration of such modified endosseous implants.[23,24]

In this article, we present a thin HA coating on Ti, obtained by electrophoretic deposition processed with laser irradiation, using different laser parameters, for the incorporation of the ceramic into the sample surface and the texturing of the metal surface. The influence of laser irradiation on the ceramic coating was analyzed, and the modification with the most promising osteoconductive properties was determined. To our knowledge, such laser modification of HA coatings has not been performed previously.

Material and methods

Sample preparation

The samples were prepared from Ti grade 4 ASTM B348 EN10204/3.1. Disks with a diameter of 14 mm and a thickness of 2 mm were milled from Ti rods. The sample surface was sandblasted using alumina (Al_2O_3) with a particle size of 53–75 μm at a pressure of 4 atm.

Electrochemical deposition of hydroxyapatite

The HA coating was electrodeposited on a working electrode – commercially pure Ti disks with a diameter of 14 mm and a thickness of 2 mm, fused with the Ti rod. As a counter electrode, a 10 cm × 10 cm platinum (Pt) mesh was used. The electrolyte was composed of 4.16×10^{-4} M $CaCl_2$, 2.5×10^{-4} M NaH_2PO_4 and 0.2 M NaCl in

distilled water, with pH adjusted to 6.3. An Autolab potentiostat-galvanostat (PGSTAT302N; Metrohm Autolab, Utrecht, the Netherlands) with a 2-electrode system in a galvanostatic mode was used. The process was carried out in a 100 mL 3-neck flask, used as an electrochemical reactor, immersed in an oil bath at 105°C. Electrodeposition was conducted for 60 min with a current of 5 mA, 120 min with a current of 30 mA, 120 min with a current of 10 mA, and 60 min with a current of 30 mA for samples A–D, respectively.

The electrodeposition of HA on a Ti surface occurs according to the following chemical reaction: $10Ca^{2+} + 6PO_4^{3-} + 2OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2$. Such a reaction is triggered by the increased pH on the cathode surface, caused by the formation of hydroxide ions from the reduction of water $2H_20 + 2e \rightarrow 2OH^- + H_2$. The pH alteration generates a critical supersaturation status at the interface of the electrode and the electrolyte for the precipitation of HA, and triggers the nucleation and growth of the HA coating.[25]

Laser treatment

The laser experimental setup shown in Fig. 1 was used for the sintering of HA particles on a Ti substrate. A laser system (Pharos P-20; Light Conversion, Vilnius, Lithuania) providing femtosecond pulses (τ = 213 fs) at a central wavelength of 1,030 nm with a spectral width of 15 nm, $M^2 \simeq 1.1$, was used. Upon irradiation, the Ti samples were fixed on the Ti substrate in an air atmosphere, and the laser beam was focused on the Ti surface with a spot diameter of 10.4 µm (at level 1/e2). A scanning mode with various overlaps was used for irradiation.

The surface of each Ti disk was divided into 4 quadrants. Each quadrant was numbered from 1-4 to 4-4, and was modified according to the parameters presented in Table 1.

Surface analysis

A Hitachi S-3400N scanning electron microscope (SEM) (Hitachi High-Technologies Corporation, Tokyo, Japan) was used to obtain micrographs of the investigated samples. Two magnifications, ×1,000 and ×5,000, were used. Contact profilometry was performed to analyze surface roughness. A T8000 profilometer (Hommel-Etamic, Herrenberg, Germany) with the EVOVIS software was used. Each quadrant was analyzed on the 3 profiles. An experimental sector of 8.0 mm was selected in accordance with PN-EN ISO 4287. A total number of 48 analyses were performed. Parameters Ra, Rt and RSm were measured. The results were expressed as mean ± standard deviation (*M* ±*SD*).

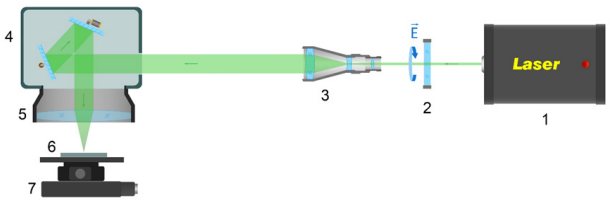

Fig. 1. Laser experimental setup

1 – laser; 2 – polarizer; 3 – telescope; 4 – galvanoscanner; 5 – lens; 6 – sample; 7 – coordinate table.

Table 1. Parameters for the laser modifications of the hydroxyapatite (HA)-coated titanium (Ti) samples

Quadrant	Modification	P [W]	V [m/s]	RR [kHz]	Steps per µm
1-4	LIPSS	0.33	1.000	500	5
	fusion	0.50	0.050	500	5
2-4	sintering	1.00	0.075	1,000	5
3-4	sintering	1.00	0.100	1,000	5
4-4	sintering	0.80	0.100	1,000	5

P – power; V – velocity; RR – frequency; LIPSS – laser-induced periodic surface structures.

Surface chemical composition

The chemical composition of the surface was determined using the energy-dispersive spectroscopy (EDS) analysis, the X-ray diffraction (XRD) analysis and the Raman spectroscopy analysis. The semi-quantitative chemical composition of the samples was determined using EDS (model No. 4481B-1UES-SN with the NSS Spectral Imaging System software; Thermo Fisher Scientific, Waltham, USA). The Raman spectroscopy analysis was conducted using the inVia™ Raman system (Renishaw, Wotton-under-Edge, UK). Raman spectra were collected in the spectral range of 200–4,000 cm^{-1}, using a 785 nm laser and a 1,200 l/mm diffraction grating. The XRD analysis was performed using an XRD diffractometer (D8 Advance; Bruker, Billerica, USA) with copper (Cu) lamp radiation. X-ray spectra were recorded in the angular range of 20–80° 2Θ, with a step size of 0.02° and a normalized count time of 2 s/step. The XRD analysis was conducted for each section of the disk. Lead (Pb) foil covered the 3 quadrants not being analyzed. The diffraction patterns in the angle range of HA reflexes were also performed. The identification of HA was based on the literature and the International Centre for Diffraction Data (ICDD 01-086-0740 data).[26–28]

Static contact angle studies

The contact angle of the sample surfaces was measured using a Theta Flex optical tensiometer (Biolin Scientific, Västra Frölunda, Göteborg, Sweden) at room temperature (22°C). The water contact angle of the 4 quadrants of each sample was evaluated by static contact angle measurements, utilizing the sessile drop method. A 1-microliter droplet of distilled water was placed on the dry

surface of each quadrant of the studied sample, and the image of the drop was recorded for 10 s. An average value of the contact angle was calculated based on at least 5 measurements. The static contact angle was then defined by fitting the Young–Laplace equation to the droplet. The results were expressed as $M \pm SD$.

Results

Surface analysis

Morphology and topography studies

The SEM images of the HA coating after electrodeposition and laser modification are presented in Fig. 2. The HA coating on sample A-4-4 presented a layer of spherical crystals with a diameter of less than 5 µm. Hydroxyapatite crystals did not completely cover the Ti surface. On sample B-4-4, the HA coating was composed of a continuous layer of needle-like crystals with a diameter of about 5 µm and a length of up to 20 µm. The HA coating was dense, with tightly packed crystals. The surface of sample C-4-4 was covered in short, needle-like crystals with a diameter of about 2 µm and a length of up to 5 µm. The crystals were not covering the Ti surface continuously. Sample D-4-4 was covered by a layer of short, needle-like crystals with a diameter of 1 µm and a length of 2 µm. The sample surface was not completely covered with crystals, and the packing of the crystals was not as dense as in the case of the B samples. On top of the layer of short crystals, foci of long, needle-like crystals with a diameter of 5 µm and length of up to 60 µm were present. Laser modifications from 1-4 up to 3-4 eliminated the needle-like shape of the crystals. Spherical structures were present after such modifications. Also, the Ti surface was modified. After laser modifications 1-4 to 3-4, the Ti surface exhibited parallel grooves with a width of about 2 µm. The depth of the grooves increased from 3-4 up to 1-4 modifications. Limited flat areas of the sample surfaces were not covered by grooves and presented spherical structures of about 1 µm in diameter.

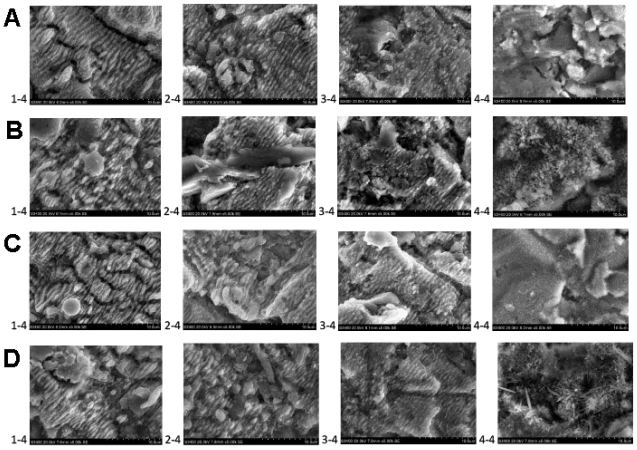

Fig. 2. Scanning electron microscopy (SEM) images of the electrodeposited hydroxyapatite (HA) after laser modifications, quadrants 1-4 to 4-4 magnification ×5,000.

Contact profilometry

The roughness of the analyzed surfaces expressed as Ra and Rt is presented in Table 2. The Ra values, which are the arithmetic means of the absolute values of the roughness profile ordinates, ranged from 1.32 µm for C-4-4 up to 1.85 µm for A-1-4. For samples B and C, the 2-4 type of laser conditioning resulted in the highest Ra parameters, whereas for samples A and D, the highest Ra parameters were found in conditions 1-4 and 3-4, respectively. The parameter Rt, defined as the total height of the profile, is the vertical distance between the maximum profile peak height and the maximum profile valley depth along the evaluation length. The Rt values for the analyzed samples ranged from 10.65 µm for sample C-4-4 up to 17.12 µm for sample C-2-4. The highest Rt values were noted for the 1-4 laser modification for samples A and B, and for the 2-4 and 4-4 modifications for samples C and D, respectively.

Surface chemical composition

Energy-dispersive spectroscopy (EDS) analysis

The EDS analysis revealed the peaks mainly from Ti, oxygen (O), Ca, phosphorus (P), and aluminum (Al) (Table 3).

Table 2. Surfaces roughness parameters (Ra and Rt) for 4 different laser modifications of the analyzed samples

Sample and quadrant	Ra [µm]	Rt [µm]
A-4-4	1.67 ±0.04	14.73 ±0.80
A-3-4	1.56 ±0.07	14.82 ±0.82
A-2-4	1.66 ±0.07	14.25 ±1.71
A-1-4	1.85 ±0.12	15.09 ±3.31
B-4-4	1.70 ±0.18	15.17 ±0.98
B-3-4	1.68 ±0.05	13.68 ±1.23
B-2-4	1.79 ±0.12	14.94 ±2.30
B-1-4	1.72 ±0.04	15.74 ±1.10
C-4-4	1.32 ±0.13	10.65 ±1.45
C-3-4	1.50 ±0.04	15.19 ±0.24
C-2-4	1.73 ±0.05	17.12 ±2.70
C-1-4	1.50 ±0.07	14.06 ±1.36
D-4-4	1.60 ±0.11	15.81 ±2.71
D-3-4	1.74 ±0.06	14.66 ±1.60
D-2-4	1.60 ±0.10	13.09 ±0.10
D-1-4	1.70 ±0.06	14.89 ±2.08

Data presented as mean ± standard deviation ($M \pm SD$).

Table 3. Energy-dispersive spectroscopy (EDS) analysis of the hydroxyapatite (HA) coating with laser sintering, concerning the oxygen (O), calcium (Ca), phosphorus (P), and carbon (C) concentrations, expressed as weight percent (wt%)

Sample and quadrant	Ti	O	Ca	P	C
A-4-4	54.76	32.45	0.47	0.31	1.08
A-3-4	61.19	22.67	0.03	0.04	0.83
A-2-4	71.21	27.93	0.01	0.04	0.77
A-1-4	69.63	18.99	0.02	0.05	1.27
B-4-4	39.38	36.39	9.01	5.04	1.91
B-3-4	57.33	32.32	0.61	0.40	0.63
B-2-4	73.78	18.87	1.71	1.06	1.12
B-1-4	77.16	19.79	0.55	0.36	2.14
C-4-4	52.75	33.77	1.84	1.07	1.51
C-3-4	61.47	30.78	0.24	0.16	0.50
C-2-4	64.69	24.50	0.07	0.09	0.72
C-1-4	81.01	13.41	0.07	0.05	0.48
D-4-4	45.95	34.67	2.72	1.86	1.47
D-3-4	63.00	23.23	0.05	0.10	1.06
D-2-4	63.57	21.07	0.04	0.09	1.43
D-1-4	73.92	17.05	0.13	0.10	0.98

The concentrations of Ca and P were the highest on the 4-4 quadrants. Among those quadrants, the Ca and P concentrations were the highest for sample B and the lowest for sample A. Laser processing 1-4 up to 3-4 resulted in a decrease in the Ca and P concentrations. In most cases, a decrease in the Ca and P concentrations could be observed with the change of laser modification from 3-4 to 1-4.

The O concentrations were the highest on the 4-4 quadrants. The level of oxygen decreased from quadrants 3-4 to 1-4.

For samples A and B, the highest C concentrations were observed for quadrants 1-4, and the lowest for quadrants 2-4 and 3-4, respectively. For samples C and D, the highest C concentrations were noted for quadrants 4-4, and the lowest for quadrants 1-4. For laser-sintered quadrants, the highest C concentrations were recorded for the 4-4 quadrants.

X-ray diffraction (XRD) analysis

Due to the shape of the samples, the XRD tests were carried out using Pb as a background (Pb gives reflections at known constant values of 2Θ). The diffractograms contained images of the tested samples and Pb. For the modified Ti surfaces, reflections from HA and Ti could be observed. The HA reflex at an angle of 25.8° 2Θ matching the (002) plane was recorded for almost all the samples (Fig. 3). The (113) plane matching the reflex at an angle of 35.4° 2Θ was overlapped by the reflexes from the Ti base, which may be attributed to a thin HA layer. Hydroxyapatite was not detected on quadrants A-4-4, C-4-4 and D-3-4. Based on the intensity of diffraction peaks for HA, we can order the quadrants as follows: C-2-4 > 1-4 > 3-4 > 4-4; B-1-4 > 4-4 > 3-4 > 2-4; D-1-4 > 4-4 > 2-4 > 3-4; A-1-4 > 2-4 > 3-4 >4-4, with the greatest concentration of HA on sample C.

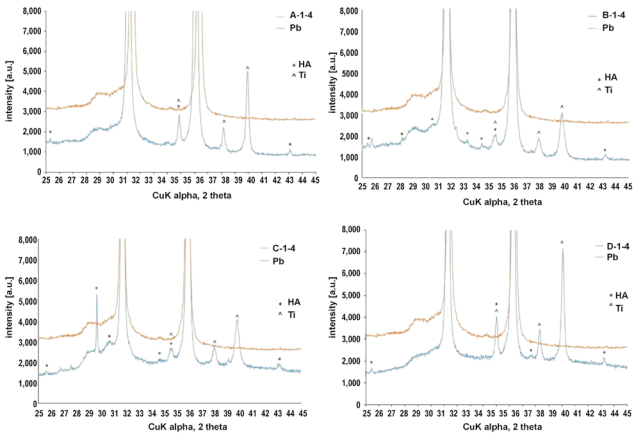

Fig. 3. X-ray diffraction (XRD) patterns in the angle range of the hydroxyapatite (HA) reflexes on one quadrant of each sample with the most intense HA peaks

Raman spectroscopy analysis

The Raman spectroscopy analysis confirmed the presence of HA on the 4th quadrant of all samples (Fig. 4). The presence of HA is manifested by the 4 bands appearing in the range 400–470 cm^{-1}, 550–650 cm^{-1}, 930–990 cm^{-1}, and 1,010–1,070 cm^{-1}, corresponding to the vibration in phosphate groups.[29,30] The single, most intensive and narrow band occurring at 960 cm^{-1} with a full width at half maximum equal to 15 cm^{-1} confirmed the presence of HA without substitution by other ions in the crystal lattice.[31] No other forms of calcium phosphates were detected. Raman spectroscopy did not identify HA on quadrants 1-4 to 3-4.

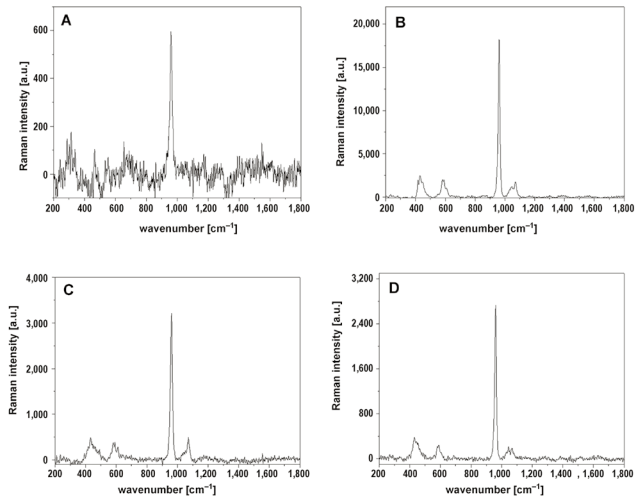

Fig. 4. Raman spectra of quadrants 4-4 for samples A–D

Contact angle studies

The contact angle analysis displayed a range of results from 20.51° up to 161.5° (Table 4). The most hydrophilic samples were B and C, with contact angles of 20.51° and 23.84°, respectively. These results were obtained for the 1-4 laser surface modification. The contact angles for samples A and D were greater than those of samples C and B. Laser modification 4-4 resulted in the highest contact angle for samples B and D.

Table 4. Contact angle of the hydroxyapatite (HA) coating after laser modification, quadrants 1-4 to 4-4

Sample and quadrant	Contact angle [°]
A-1-4	146.30 ±2.072
A-2-4	126.40 ±2.725
A-3-4	137.80 ±1.318
A-4-4	144.80 ±2.290
B-1-4	20.51 ±2.925
B-2-4	55.53 ±4.657
B-3-4	45.28 ± 3.110
B-4-4	56.27 ±5.523
C-1-4	23.84 ±6.031
C-2-4	53.30 ±4.362
C-3-4	85.84 ±0.952
C-4-4	36.89 ±5.965
D-1-4	153.20 ±0.426
D-2-4	145.30 ±2.133
D-3-4	136.40 ±0.289
D-4-4	161.50 ±1.033

Data presented as *M* ±*SD*.

Discussion

In the present study, 4 different techniques of HA laser modification on a Ti substrate were used. For the 1-4 laser modification, a combination of LIPSS and fusion was used, while for modifications 2-4 to 4-4, a sintering process with different parameters was implemented. The result of laser surface treatment depends on power density, which is correlated with the number of pulses and the speed of beam movement.

The 4-4 surface modification did not alter the crystal structure of the HA coating, as shown in the SEM images. The characteristics of the electrodeposited HA coating without laser modifications are discussed in our previous study.[17] On the other hand, modifications 1-4 up to 3-4 eliminated the crystal structure of HA, leaving spherical residues, as well as caused alterations in the Ti surface, leaving a microgroove pattern. In those surface modifications, the effects of laser melting and vaporization can be observed, since the crystal structures were transformed into spherical structures. Parallel grooves indicating LIPSS were also present on quadrants 2-4 and 3-4. The depth of the grooves which occurred after modifications 1-4 to 3-4 was the highest for the LIPSS and fusion modification and the lowest for the 3-4 modification procedure. The 1-4 up to 3-4 laser modifications resulted in the femtosecond laser ablation processing of Ti, leaving a microstructured surface. Such processing can influence the tribological properties of the surface and its wetting properties.[32] The microstructure of the parallel groves also had a positive effect on the osteoblast reaction by enhancing surface hydrophilicity, serum protein adsorption and osteoblast maturation.[33] Parallel grooves also facilitate cells expansion along the direction of the grooves, resulting in better coverage on the implant surface.[34]

An increase of the laser power by 0.2 W changed the morphology of HA, but did not cause its chemical degradation; HA peaks were found in the XRD spectra of quadrants 2-4 and 3-4 modified by laser with the highest power density. The crystal structure of the HA coating present on the 4-4 laser-conditioned quadrants proved to be osteoconductive, as shown in our previous study.[17]

Surface roughness, expressed as Ra, ranged from 1.32 μm to 1.85 μm. For most of the surfaces, laser modifications 1-4 and 2-4 resulted in the highest roughness values. However, for laser modifications 3-4 and 4-4, this parameter was lower. The Rt parameter, defined as the total height of the profile, was the greatest for the 1-4 and 2-4 laser modifications, with the C-2-4 sample exhibiting the greatest height of 17.12 μm. High Rt values were also noted for the D-4-4 sample, but this was correlated with the structure of the electrodeposited HA (i.e., the foci of long, needle-like crystals). The roughness of all the samples presented in this study can be defined as moderate.[34] As reported by Wennerberg and Albrektsson in a review of over 100 publications, implant surfaces of such roughness parameters can facilitate bone reaction better than smoother or even rougher surfaces.[34] Also, our previous studies proved positive osteoblast reactions for moderately rough Ti surfaces, expressed as increased osteoblast viability and differentiation.[35,36]

The analysis of the chemical composition of the samples revealed the presence of HA, although there were some differences between the EDS, XRD and Raman spectroscopy results. The XRD did not detect the presence of HA on quadrants A-4-4 and C-4-4, while Raman spectroscopy confirmed its presence on those quadrants. Such XRD results could be caused by instrumental limitations. The radiation might not have fallen on the sample due to its small size, or might only have fallen on it in a small percentage, since the distribution of Ca and P on the sample surface was not uniform. On the other hand, Raman spectroscopy detected the presence of HA only on quadrants 4-4, which, according to the EDS analysis, were the richest in Ca and P. The EDS revealed the presence of Ca and P in each quadrant of each sample, predominantly on quad-

rants 4-4. Raman spectroscopy also revealed that the HA present in the analyzed samples was pure, without substitution by other ions in the crystal lattice. Based on the analysis of the band occurring at 960 cm^{-1}, it can be stated that no other forms of calcium phosphates, such as tricalcium phosphate or tetracalcium phosphate, were present.

The weight percentages of Ca and P, based on the EDS analysis, for quadrants 4-4 comply with the thickness of the HA coating observed on the SEM images. The highest Ca and P concentrations were noted for sample B, then for D and C, and the lowest concentrations were observed for sample A. While analyzing the weight percentages of Ca and P on the sample surface, a pattern was found. The quadrants with the 4-4 laser modification presented the highest Ca and P concentrations, whereas the lowest were noted for the laser-sintered and fused quadrants. The same pattern can be observed in the O weight percentage. A similar pattern is observed in the results of the roughness analysis. The roughness Ra values are the lowest on the 4-4 quadrants and the highest on the laser-sintered and fused quadrants. The LIPSS and fusion, as well as the 2-4 and 3-4 modification processes, seem to increase the surface roughness while decreasing the Ca, P and O concentrations on the sample surface. The O weight percentage in the 4-4 quadrants is comparable with the result obtained for the samples before laser modification. The 2 remaining modification processes and the LIPSS and fusion process resulted in a decreased O concentration, with a higher reduction observed for the LIPSS and fusion quadrants. In the case of sample B – the sample with the richest HA coating – the 2-4 laser modification resulted in higher Ca and P concentrations than the 3-4 laser modification. This situation was opposite to the other samples.

Calcium phosphates have been reported to possess osteoinductive and osteoconductive characteristics.[37–43] Osteoinduction is the ability to induce progenitor cells to differentiate into osteoblastic lineages, while osteoconduction is the ability to promote bone growth on the surface of materials.[37] Such surface properties increase cell adhesion, differentiation and proliferation.[44] Cell adhesion is correlated with the ability to adsorb extracellular matrix (ECM) proteins, which, in turn, is related to such surface features as roughness, energy, crystallinity, and solubility.[37,44] An increased protein adsorption and cell adhesion are reported on calcium phosphate-coated implants.[37,45,46] Calcium phosphates also aid in the osteogenic differentiation of mesenchymal stem cells (MSCs).[37,47,48] The improved bone reaction of calcium phosphate-coated implants is also related to the entrapment of blood platelets on such surfaces and their activation. The activated platelets release biomolecules that can be retained by the fibrin matrix on the surface to facilitate the regeneration of the surrounding tissues.[40] Due to such surface features, calcium phosphate-coated implants are reported to present improved osseointegration.[17,38]

Quadrants 4-4 are the quadrants presenting the highest concentrations of Ca and P, with sample B exhibiting the highest concentrations of all of the samples. Based on the Ca and P concentrations, the B-4-4 quadrant should present the highest osteoinductive and osteoconductive properties, since calcium phosphates have been documented to possess biocompatible and osteoconductive properties.[16–18,36]

The presence of C on the analyzed sample surfaces can be associated with the presence of carbon dioxide (CO_2) and other organic molecules from the air.[49] Such carbon contamination, apart from roughness-induced hydrophobicity, is known to cause higher contact angles and lower surface energy, resulting in a surface with hydrophobic properties.[49–51] Rupp et al. reported a decreased water contact angle in samples after a 50% carbon reduction.[49] The reduction was from about 35 at% for the samples with a water contact angle of 122.40°–139.88° to the level of 15 at%, resulting in a water contact angle of 0°.[49] Hotchkiss et al. reported hydrophobic surface properties at a carbon concentration of ~43–44%.[51] In their study, a carbon level of ~30% resulted in a low contact angle and hydrophilic surface properties.[51] In the present study, the C concentration ranges from 0.48 wt% to 2.12 wt%, as revealed by the EDS analysis. The carbon levels are not reflected in the water contact angle results, which, according to the Rupp et al.'s study, should be at the level of 0°.[49] The C concentration in the present study is not correlated with the hydrophilicity of the surface. The highest C concentrations were reported for sample B, the richest in Ca and P. Lower carbon concentrations were noted for sample D, which also comes second according to the Ca and P concentrations. No correlation could be found between the C concentration and the type of laser conditioning.

Contact angle studies revealed a division of samples into 2 groups. A group of contact angles ranging from 20.51° to 85.84° consisted of samples B and C. Those samples can be classified as samples with hydrophilic surfaces. Samples A and D presented contact angles in the range of 126.4°–161.5° and could be classified as samples with hydrophobic surfaces. For the group of hydrophilic surfaces, the lowest result in both cases was noted for the 1-4 quadrants. Those quadrants also presented lower O concentrations, and are also the quadrants with the most intensive ablation. The correlation between the surface roughness and the surface wettability could not be established. While evaluating all 4 samples, the highest contact angle results were for quadrants 3-4 and 4-4.

The contact angle studies of the HA-coated, LIPSS plus fusion-modified quadrants of samples B and C revealed improved hydrophilic properties as compared to the LIPSS surfaces of uncoated Ti alloy studied by Schweitzer et al.[10] The contact angles of the LIPSS-modified surfaces reported by Schweitzer were similar to those of the HA-coated and sintered quadrants of the samples B and C.[10]

The enhanced surface hydrophilicity of the implant surface can result in the shortening of the osseointegration period, and increase the bond strength of the implant to bone tissue.[2] On the cellular level, hydrophilicity enhances protein adsorption, platelet aggregation, and monocyte and macrophage adhesion, increases the fibroblastic cell response, as well as the adhesion, differentiation and proliferation of osteoblasts.[37,52–54] Those events result in the acceleration of the healing process and bone formation in the initial period of osseointegration.[2,55–58] Hydrophilic surfaces can also increase the levels of alkaline phosphates, osteocalcin and bone sialoproteins, which are the differentiation markers of osteoblasts, indicating the osteoconductive potential of such surfaces.[2,54,59,60] The surface texture with parallel microgrooves was also reported to increase the surface hydrophilicity, along with serum protein adsorption and osteoblast maturation.[32,33] The phenomenon of increased hydrophilicity of micro-grooved Ti surfaces is related to altered surface chemistry.[32] A hydrophilic microrough Ti surface topography also elicits a macrophage phenotype associated with reduced inflammation and enhanced pro-osteogenic signaling.[61]

The laser modification of the HA coating resulted in 2 kinds of surfaces. Laser-induced periodic surface structure texturing could be observed on quadrants 1-4 to 3-4, with parallel grooves, and HA melted and sintered into spherical structures. The chemical composition of those quadrants had decreased Ca, P, O, and C concentrations. The 4-4 laser surface conditioning did not alter the needle-like morphology of the electrochemically deposited HA coating and caused only minor changes in the chemical composition of the surface. The contact angle studies revealed the presence of 2 groups: the hydrophilic group (B and C samples); and the hydrophobic group (A and D samples). The LIPSS–fusion modification decreased the water contact angle of the hydrophilic samples.

The surface with the most promising osteoconductive properties seems to be the B-1-4 sample with the optimal hydrophilic properties, LIPSS and the most intensive HA peaks based on the XRD analysis. Sample B-2-4 also presents favorable osteoconductive characteristics, such as high Ca and P concentrations, as shown by the EDS analysis, and LIPSS.

Further biological analyses are necessary to evaluate the cell and tissue response to such laser modifications of the HA coating on Ti. Also, testing the material toxicity, and the loading time and conditions would be beneficial for the analysis of the implant's dynamic integration with bone.[62–64]

Conclusions

The ultrashort-pulsed laser modification of the HA coating for regimes 1-4 to 3-4 resulted in the LIPSS texturing of the Ti surface with HA sinterization.

Further biological analyses are necessary to evaluate the cell and tissue response to such laser-modified HA coating on Ti.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are openly available in Zenodo at https://doi.org/10.5281/zenodo.8398379.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Brånemark PI, Hansson BO, Adel R, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand J Plast Reconstr Surg*. 1977;16:1–132. PMID:356184.
2. Souza JC, Sordi MB, Kanazawa M, et al. Nano-scale modification of titanium implant surfaces to enhance osseointegration. *Acta Biomater*. 2019;94:112–131. doi:10.1016/j.actbio.2019.05.045
3. Lee JWY, Bance ML. Physiology of osseointegration. *Otolaryngol Clin North Am*. 2019;52(2):231–242. doi:10.1016/j.otc.2018.11.004
4. Günay-Bulutsuz A, Berrak Ö, Yeprem HA, Arisan ED, Yurci ME. Biological responses of ultrafine grained pure titanium and their sand blasted surfaces. *Mater Sci Eng C Mater Biol Appl*. 2018;91:382–388. doi:10.1016/j.msec.2018.05.056
5. Granato R, Bonfante EA, Castellano A, et al. Osteointegrative and microgeometric comparison between micro-blasted and alumina blasting/acid etching on grade II and V titanium alloys (Ti-6Al-4V). *J Mech Behav Biomed Mater*. 2019;97:288–295. doi:10.1016/j.jmbbm.2019.05.026
6. Bi Q, Song X, Chen Y, Zheng Y, Yin P, Lei T. Zn-HA/Bi-HA biphasic coatings on titanium: Fabrication, characterization, antibacterial and biological activity. *Colloids Surf B Biointerfaces*. 2020;189:110813. doi:10.1016/j.colsurfb.2020.110813
7. Lu M, Chen H, Yuan B, et al. Electrochemical deposition of nanostructured hydroxyapatite coating on titanium with enhanced early stage osteogenic activity and osseointegration. *Int J Nanomedicine*. 2020;15:6605–6618. doi:10.2147/IJN.S268372
8. Ogawa T. Ultraviolet photofunctionalization of titanium implants. *Int J Oral Maxillofac Implants*. 2014;29(1):e95–e102. doi:10.11607/jomi.te47
9. Schnell G, Jagow C, Springer A, Frank M, Seitz H. Time-dependent anisotropic wetting behavior of deterministic structures of different strut widths on Ti6Al4V. *Metals*. 2019;9(9):938. doi:10.3390/met9090938

10. Schweitzer L, Cunka A, Pereira T, et al. Preclinical in vitro assessment of submicron-scale laser surface texturing on Ti6Al4V. *Materials (Basel)*.2020;13(23):5342. doi:10.3390/ma13235342
11. Gaggl A, Schultes G, Müller WD, Kächer H. Scanning electron microscopical analysis of laser-treated titanium implant surfaces – a comparative study. *Biomaterials*. 2000;21(10):1067–1073. doi:10.1016/s0142-9612(00)00002-8
12. Schnell G, Duenow U, Seitz H. Effect of laser pulse overlap and scanning line overlap on femtosecond laser-structured Ti6Al4V surfaces. *Materials (Basel)*. 2020;13(4):969. doi:10.3390/ma13040969
13. Bonse J, Höhm S, Rosenfeld A, Krüger J. Sub-100-nm laser-induced periodic surface structures upon irradiation of titanium by Ti: Sapphire femtosecond laser pulses in air. *Appl Phys A*. 2013;110:547–551. doi:10.1007/s00339-012-7140-y
14. Uskoković V, Uskoković DP. Nanosized hydroxyapatite and other calcium phosphates: Chemistry of formation and application as drug and gene delivery agents. *J Biomed Mater Res B Appl Biomater*. 2011;96(1):152–191. doi:10.1002/jbm.b.31746
15. Koutsopoulos S. Synthesis and characterization of hydroxyapatite crystals: A review study on the analytical methods. *J Biomed Mater Res*. 2002;62(4):600–612. doi:10.1002/jbm.10280
16. Rajesh P, Muraleedharan CV, Komath M, Varma H. Laser surface modification of titanium substrate for pulsed laser deposition of highly adherent hydroxyapatite. *J Mater Sci Mater Med*. 2011;22(7):1671–1679. doi:10.1007/s10856-011-4342-3
17. Łukaszewska-Kuska M, Krawczyk P, Martyla A, Hędzelek W, Dorocka-Bobkowska B. Hydroxyapatite coating on titanium endosseous implants for improved osseointegration: Physical and chemical considerations. *Adv Clin Exp Med*. 2018;27(8):1055–1059. doi:10.17219/acem/69084
18. Le Guéhennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. *Dent Mater*. 2007;23(7):844–854. doi:10.1016/j.dental.2006.06.025
19. Kim KH, Ramaswamy N. Electrochemical surface modification of titanium in dentistry. *Dent Mater J*. 2009;28(1):20–36. doi:10.4012/dmj.28.20
20. Ma M, Ye Wei, Wang XX. Effect of supersaturation on the morphology of hydroxyapatite crystals deposited by electrochemical deposition on titanium. *Mater Lett*. 2008;62(23):3875–3877. doi:10.1016/j.matlet.2008.05.009
21. Peng P, Kumar S, Voelcker NH, Szili E, Smart RStC, Griesser HJ. Thin calcium phosphate coatings on titanium by electrochemical deposition in modified simulated body fluid. *J Biomed Mat Res A*. 2006;76(2):347–355. doi:10.1002/jbm.a.30514
22. Kuo MC, Yen SK. The process of electrochemical deposited hydroxyapatite coatings on biomedical titanium at room temperature. *Mater Sci Eng C*. 2002;20(1–2):153–160. doi:10.1016/S0928-4931(02)00026-7
23. Maalouf M, Khali AA, Di Maio Y, et al. Polarization of femtosecond laser for titanium alloy nanopatterning influences osteoblastic differentiation. *Nanomaterials (Basel)*. 2022;12(10):1619. doi:10.3390/nano12101619
24. Cunha A, Zouani OF, Plawinski L, et al. Human mesenchymal stem cell behavior on femtosecond laser-textured Ti-6Al-4V surfaces. *Nanomedicine (Lond)*. 2015;10(5):725–739. doi:10.2217/nnm.15.19
25. Hu R, Lin CJ, Shi HY. A novel ordered nano hydroxyapatite coating electrochemically deposited on titanium substrate. *J Biomed Mater Res A*. 2007;80(3):687–692. doi:10.1002/jbm.a.30891
26. Ungureanu DN, Angelescu N, Ion RM, Stoian EV, Rizescu CZ. Synthesis and characterization of hydroxyapatite nanopowders by chemical precipitation. In: Bojkovic ZS, ed. *Recent Researches in Communications, Automation, Signal Processing, Nanotechnology, Astronomy and Nuclear Physics*. Conference proceedings. Cambridge, UK, February 20–22, 2011. WSEAS Press; 2011:296–301. https://www.researchgate.net/publication/262322346_Synthesis_and_characterization_of_hydroxyapatite_nanopowders_by_chemical_precipitation#full-text.
27. Lee SW, Hahn BD, Kang TY, et al. Hydroxyapatite and collagen combination-coated dental implants display better bone formation in the peri-implant area than the same combination plus bone morphogenetic protein-2-coated implants, hydroxyapatite only coated implants, and uncoated implants. *J Oral Maxillofac Surg*. 2014;72(1):53–60. doi:10.1016/j.joms.2013.08.031
28. Angelescu N, Ungureanu DN, Anghelina FV. Synthesis and characterization of hydroxyapatite obtained in different experimental conditions. *Sci Bull Valahia Univ Mater Mech*. 2011;9(6):15–18. https://www.researchgate.net/publication/281206966_Synthesis_and_characterization_of_hydroxyapatite_obtained_in_different_experimental_conditions#full-text
29. Buchwald T, Buchwald Z. Assessment of the Raman spectroscopy effectiveness in determining the early changes in human enamel caused by artificial caries. *Analyst*. 2019;144(4):1409–1419. doi:10.1039/c8an01494a
30. Buchwald T, Okulus Z, Szybowicz M. Raman spectroscopy as a tool of early dental caries detection – new insights. *J Raman Spectrosc*. 2017;48(8):1094–1102. doi:10.1002/jrs.5175
31. Okulus Z, Sandomierski M, Zielińska M, Buchwald T, Voelkel A. Zeolite fillers for resin-based composites with remineralizing potential. *Spectrochim Acta A Mol Biomol Spectrosc*. 2019;210:126–135. doi:10.1016/j.saa.2018.11.020
32. Raimbault O, Benayoun S, Anselme K, et al. The effects of femtosecond laser-textured Ti-6Al-4V on wettability and cell response. *Mater Sci Eng C Mater Biol Appl*. 2016;69:311–320. doi:10.1016/j.msec.2016.06.072
33. Lee SW, Lee MH, Oh N, Park JA, Leesungbok R, Ahn AJ. Correlation between surface hydrophilicity and osteoblastic differentiation on microgrooved titanium substrata. *J Oral Implantol*. 2012;38(1):11–19. doi:10.1563/AAID-JOI-D-09-00144.1
34. Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: A systematic review. *Clin Oral Implants Res*. 2009;20(Suppl 4):172–184. doi:10.1111/j.1600-0501.2009.01775.x
35. Lukaszewska-Kuska M, Wirstlein P, Majchrowski R, Dorocka-Bobkowska B. Osteoblastic cell behaviour on modified titanium surfaces. *Micron*. 2018;105:55–63. doi:10.1016/j.micron.2017.11.010
36. Lukaszewska-Kuska M, Wirstlein P, Majchrowski R, Dorocka-Bobkowska B. The effects of titanium topography and chemical composition on human osteoblast cell. *Physiol Res*. 2021;70(3):413–423. doi:10.33549/physiolres.934582
37. Jeong J, Kim JH, Shim JH, Hwang NS, Heo CY. Bioactive calcium phosphate materials and applications in bone regeneration. *Biomater Res*. 2019;23:4. doi:10.1186/s40824-018-0149-3
38. Nikolidakis D, van den Dolder, Wolke JG, Jansen JA. Effect of platelet-rich plasma on the early bone formation around Ca-P-coated and non-coated oral implants in cortical bone. *Clin Oral Implants Res*. 2008;19(2):207–213. doi:10.1111/j.1600-0501.2007.01456.x
39. Hayakawa T, Yoshinari M, Nemoto K, Wolke JG, Jansen JA. Effect of surface roughness and calcium phosphate coating on the implant/bone response. *Clin Oral Implants Res*. 2000;11(4):296–304. doi:10.1034/j.1600-0501.2000.011004296.x
40. Nakamura M, Aizawa H, Kawabata H, et al. Platelet adhesion on commercially pure titanium plates in vitro III: Effects of calcium phosphate-blasting on titanium plate biocompatibility. *Int J Implant Dent*. 2020;6(1):74. doi:10.1186/s40729-020-00270-2
41. Civantos A, Martínez-Campos E, Ramos V, Elvira C, Gallardo A, Abarrategi A. Titanium coatings and surface modifications: Toward clinically useful bioactive implants. *ACS Biomater Sci Eng*. 2017;3(7):1245–1261. doi:10.1021/acsbiomaterials.6b00604
42. de Groot K, Wolke JG, Jansen JA. Calcium phosphate coatings for medical implants. *Proc Inst Mech Eng H*. 1998;212(2):137–147. doi:10.1243/0954411981533917
43. Junker R, Dimakis A, Thoneick M, Jansen JA. Effects of implant surface coatings and composition on bone integration: A systematic review. *Clin Oral Implants Res*. 2009;20(Suppl 4):185–206. doi:10.1111/j.1600-0501.2009.01777.x
44. Samavedi S, Whittington AR, Goldstein AS. Calcium phosphate ceramics in bone tissue engineering: A review of properties and their influence on cell behavior. *Acta Biomater*. 2013;9(9):8037–8045. doi:10.1016/j.actbio.2013.06.014
45. Surmenev RA, Surmaneva MA, Ivanova AA. Significance of calcium phosphate coatings for the enhancement of new bone osteogenesis – a review. *Acta Biomater*. 2014;10(2):557–579. doi:10.1016/j.actbio.2013.10.036
46. Fujii E, Ohkubo M, Tsura K, et al. Selective protein adsorption property and characterization of nano-crystalline zinc-containing hydroxyapatite. *Acta Biomater*. 2006;2(1):69–74. doi:10.1016/j.actbio.2005.09.002

47. Orimo H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. *J Nippon Med Sch*. 2010;77(1):4–12. doi:10.1272/jnms.77.4
48. Komori T. Regulation of osteoblast differentiation by Runx2. *Adv Exp Med Biol*. 2010:658:43–49. doi:10.1007/978-1-4419-1050-9_5
49. Rupp F, Scheideler L, Olshanska N, de Wild M, Wieland M, Geis-Gerstorfer J. Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces. *J Biomed Mater Res A*. 2006;76(2):323–334. doi:10.1002/jbm.a.30518
50. Massaro C, Rotolo P, De Riccardis F, et al. Comparative investigation of the surface properties of commercial titanium dental implants. Part I: Chemical composition. *J Mater Sci Mater Med*. 2002;13(6):535–548. doi:10.1023/a:1015170625506
51. Hotchkiss KM, Ayad NB, Hyzy SL, Boyan BD, Olivares-Navarrete R. Dental implant surface chemistry and energy alter macrophage activation in vitro. *Clin Oral Implants Res*. 2017;28(4):414–423. doi:10.1111/clr.12814
52. Mendonça G, Mendonça DB, Aragão FJ, Cooper LF. Advancing dental implant surface technology – from micron- to nanotopography. *Biomaterials*. 2008;29(28):3822–3835. doi:10.1016/j.biomaterials.2008.05.012
53. Zhao G, Raines AL, Wieland M, Schwartz Z, Boyan BD. Requirement for both micron- and submicron scale structure for synergistic responses of osteoblasts to substrate surface energy and topography. *Biomaterials*. 2007;28(18):2821–2829. doi:10.1016/j.biomaterials.2007.02.024
54. Nagasawa M, Cooper LF, Ogino Y, et al. Topography influences adherent cell regulation of osteoclastogenesis. *J Dent Res*. 2016;95(3):319–326. doi:10.1177/0022034515616760
55. Chambrone L, Shibli JA, Mercúrio CE, Cardoso B, Preshaw PM. Efficacy of standard (SLA) and modified sandblasted and acid-etched (SLActive) dental implants in promoting immediate and/or early occlusal loading protocols: A systematic review of prospective studies. *Clin Oral Implants Res*. 2015;26(4):359–370. doi:10.1111/clr.12347
56. Kopf BS, Schipanski A, Rottmar M, Berne S, Maniura-Weber K. Enhanced differentiation of human osteoblasts on Ti surfaces pre-treated with human whole blood. *Acta Biomater*. 2015;19:180–190. doi:10.1016/j.actbio.2015.03.022
57. Wennerberg A, Jimbo R, Stübinger S, Obrecht M, Dard M, Berner S. Nanostructures and hydrophilicity influence osseointegration: A biomechanical study in the rabbit tibia. *Clin Oral Implants Res*. 2014;25(9):1041–1050. doi:10.1111/clr.12213
58. Philipp A, Duncan W, Roos M, Hämmerle CH, Attin T, Schmidlin PR. Comparison of SLA® or SLActive® implants placed in the maxillary sinus with or without synthetic bone graft materials – an animal study in sheep. *Clin Oral Implants Res*. 2014;25(10):1142–1148. doi:10.1111/clr.12255
59. Hyzy SL, Olivares-Navarrete R, Ortman S, Boyan BD, Schwartz Z. Bone morphogenetic protein 2 alters osteogenesis and anti-inflammatory profiles of mesenchymal stem cells induced by microtextured titanium in vitro. *Tissue Eng Part A*. 2017;23(19–20):1132–1141. doi:10.1089/ten.TEA.2017.0003
60. Lang NP, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Boosshardt DD. Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clin Oral Implants Res*. 2011;22(4):349–356. doi:10.1111/j.1600-0501.2011.02172.x
61. Hamlet SM, Lee RS, Moon HJ, Alfarsi MA, Ivanovski S. Hydrophilic titanium surface-induced macrophage modulation promotes pro-osteogenic signalling. *Clin Oral Implants Res*. 2019;30(11):1085–1096. doi:10.1111/clr.13522
62. Shamel M, Rady D, Al Ankily M. Evaluation of lingual mucosa toxicity and recovery follow-up in rats, following sub-chronic exposure to titanium dioxide nanoparticles. *Dent Med Probl*. 2022;59(3):427–435. doi:10.17219/dmp/137904
63. 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
64. Reda R, Zanza A, Galli M, De Biase A, Testarelli L, Di Nardo D. Applications and clinical behavior of BioHPP in prosthetic dentistry: A short review. *J Compos Sci*. 2022;6(3):90. doi:10.3390/jcs6030090

Original paper

Stevia vs. triple antibiotic paste: An intracanal battle?

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Abstract

Background. Intracanal medicaments are vital in treating the infections of the deciduous dentition due to the large percentage of accessory canals that hasten the microbial spread to the periradicular region. Though countless medicaments have been produced to reduce the microbial load and aid symptomatic relief, they still do not fulfill every function of an ideal medicament.

Objectives. The aim of the present study was to evaluate and compare the antimicrobial efficacy of *Stevia rebaudiana* (*S. rebaudiana*) and triple antibiotic paste (TAP) against *Enterococcus faecalis* (*E. faecalis*).

Material and methods. The present in vitro, parallel, double-blinded study had an equal allocation ratio. The specimens were prepared, randomly divided into 4 groups and inoculated with *E. faecalis* (ATCC35550). Following incubation, the first 3 groups were treated with *S. rebaudiana*, triple antibiotic therapy or carbopol gel, respectively, with the 4th negative control group left untreated. The microbial samples were collected before and after treatment, and the counts of colony-forming units (CFUs) were compared. The results were analyzed using the Kruskal–Wallis, Dunn's post-hoc and Wilcoxon's signed rank tests.

Results. The first 2 groups displayed a significant decrease in CFUs after drug application, while the carbopol and control groups showed an exponential increase. There was no statistically significant difference between the stevia and TAP groups ($p = 0.630$).

Conclusions. Stevia gel was comparable to TAP in terms of antimicrobial efficacy, and can therefore be considered a new alternative in intracanal treatment.

Keywords: triple antibiotic paste, intracanal medicament, stevia

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Introduction

The most important etiologic factors for pulp and periradicular diseases are pathogens, of which *Enterococcus faecalis* (*E. faecalis*) is of paramount importance. It is a facultative gram-positive anaerobe, occurring abundantly in failed endodontic canals.[1] Classical biomechanical preparations can help eliminate microorganisms while extirpating the infected tissue. However, tortuous roots and the complexity of accessory canals, as well as the ability of *E. faecalis* to exist independently as a biofilm without synergistic support, make it difficult to eliminate the possibility of reinfection after obturation. Therefore, better eradication of such microorganisms requires an additional disinfection approach with the use of irrigants and intracanal medications.

Intracanal medications aim to reduce bacterial colonies and prevent reinfection. Various drugs used to disinfect the canal include calcium hydroxide, Formocresol®, triple antibiotic paste (TAP), chlorhexidine digluconate, and Ledermix™, of which calcium hydroxide and TAP are most commonly used. Although their efficacy has been demonstrated in many studies, both have several disadvantages. Calcium hydroxide is less effective against *E. faecalis* and completely ineffective against yeast-like fungi,[2] which are the primary commensals of the infected ducts. In addition, allergic tissue reactions have been reported in numerous cases. Triple antibiotic paste (minocycline, ciprofloxacin, metronidazole), on the other hand, has been very successful in eliminating endodontic pathogens.[3] However, its use has resulted in coronal discoloration[4] and a reduction in dentin hardness.[5] Due to these drawbacks, there is a constant search for better novel materials.

Stevia rebaudiana (*S. rebaudiana*), also called candy leaf, is a flowering plant native to Paraguay that is cultivated for its sweet-tasting leaves. It is used by diabetics in a dried form as a calorie-free natural sweetener, and is rich in terpenes and flavonoids, which have good antibacterial and antifungal properties.[6] In dentistry, stevia was shown to inhibit plaque and gingivitis when used as a mouthwash in an in vivo study.[7] Stevia has been shown to be non-toxic,[8] and have therapeutic effects in treating cancer,[9] inflammation,[10] cysts,[11] and obesity.[12] Also, it can be safely consumed during pregnancy without a fear of teratogenicity.[8] Considering these benefits, the present study evaluated and compared the antimicrobial properties of stevia and TAP against *E. faecalis* when used as intracanal drugs.

Material and methods

Study design

The present study followed an in vitro design, and was planned and performed according to the Checklist for Reporting In-vitro Studies (CRIS) and Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Since the present study was conducted in vitro, it was exempted from the review by the institutional ethics committee.

Study setting

The study was conducted at the Department of Pediatric and Preventive Dentistry of AME's Dental College and Hospital, Raichur, India, in collaboration with the Department of Pharmaceutics of the V.L. College of Pharmacy, Raichur, India, and the Department of Microbiology of Raichur Institute of Medical Sciences.

Sample size

The sample size was estimated using the G*Power software, v. 3.1.9.4 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). Considering a power (1-β) of 80% and an α-error of 5%, the total sample size was set at 40 (10 × 4 groups = 40 samples).

Preparation of the teeth

The examined samples included freshly extracted premolars with intact crowns and fully formed root apices.[13] Teeth that were fractured, carious, previously restored, calcified, or with multiple canals or resorbed roots were excluded.[14]

The root length of the specimens was standardized by decoronating the teeth with a 0.1-millimeter-thick double-sided diamond disk (Dentorium Products, Farmingdale, USA). The roots were prepared to a working length of 14 mm, using K files with an apical file size 35–70 (Mani Medical India, New Delhi, India), with an increment of 1 mm to ensure the standardization of measurements. All canals were rinsed with 1 mL of 2% NaOCl (Vishal Dentocare, Ahmedabad, India) for 30 s between the instrumentation stages. A 3-milliliter preparation of 17% ethylenediaminetetraacetic acid (EDTA) (Dental Avenue India, Palghar, India) was administered and the canals were soaked for 5 min, followed by final irrigation with 5 mL of sterile saline. The apical foramen was sealed with light-cured composite resin and the outer surface of the root was made impermeable by coating it with 2 layers of epoxy resin (Fortune Chemie, Bangalore, India).[15] All specimens were autoclaved under standard conditions (121°C for 15 min) and stored in an ultraviolet (UV) chamber to ensure sterility.

Preparation of the drugs

Stevia extraction and formulations

A total of 40 g of dried powdered stevia (Stevia World Agrotech, Bangalore, India) was added to 200 mL

of absolute alcohol and boiled in a Soxhlet apparatus for 23 cycles.[16] The obtained extract was then reduced to 20 mL and formulated at different concentrations (0.1%, 0.2%, 0.6%, 0.7%, and 0.8%) to determine the minimal bactericidal concentration (MBC) (Table 1). The experimental formulations were based on random concentrations, and if positive for microbial inhibition, new formulations with adjacent lower concentrations were used. The MBC of stevia was determined using the spot inoculation technique,[17] in which 1 mL of activated *E. faecalis* suspension was combined with equal parts of different drug concentrations and dropped onto a blood agar plate divided into 5 compartments. The contaminated plate was then incubated at 37°C and 5% CO_2 for 24 h, and changes were noted. The minimum drug concentration at which the complete elimination of the organism occurred was 0.7%. The concentrations for different formulations were chosen randomly, and since 0.6% did not show any inhibition, there was no need for formulations at 0.3%, 0.4% or 0.5% concentrations.

Stevia gel preparation

Carbopol powder (50 mg) was added to 10 mL of distilled water and sonicated for 5 cycles, each lasting 2 min. After achieving a homogeneous solution, 0.7 mL of stevia from a freshly prepared extract was added. Triethanolamine (Qualigens Fine Chemicals, Mumbai, India) was then added dropwise and stirred until a gel-like consistency was obtained. Carbopol served as a thickening agent, while triethanolamine acted as a neutralizing agent.

Triple antibiotic gel preparation

Triple antibiotic gel (TAG) was formulated using Hashimoto's ratio, combining equal parts of minocycline (100 mg), ciprofloxacin (200 mg) and metronidazole (500 mg) (Lifecare Neuro Products, Baddi, India) with 50 mg of Carbopol®-934 (Rolex Pharmaceuticals Limited, Ahmedabad, India) and 10 mL of distilled water. Sonication was performed for 5 cycles. Triethanolamine (Qualigens Fine Chemicals) was then added dropwise and stirred until a gel-like consistency was reached.

Table 1. Different concentrations of the stevia extract formulated for checking the minimal bactericidal concentration (MBC)

No.	Formulation	Concentration [%]
1	0.1 mL of the extract brought up to 10 mL by the addition of ethanol yields 1.007 mg/mL	0.1
2	0.2 mL of the extract brought up to 10 mL by the addition of ethanol yields 2.014 mg/mL	0.2
3	0.6 mL of the extract brought up to 10 mL by the addition of ethanol yields 6.042 mg/mL	0.6
4	0.7 mL of the extract brought up to 10 mL by the addition of ethanol yields 7.049 mg/mL	0.7
5	0.8 mL of the extract brought up to 10 mL by the addition of ethanol yields 8.056 mg/mL	0.8

Inoculation

The samples were fixed in sterile Eppendorf tubes and stabilized on 5-milliliter tubes. A standard strain of *E. faecalis* was reactivated in brain–heart infusion (BHI) broth, and incubated at 37°C and 5% CO_2 for 24 h. After microbial growth, a suspension of the culture was prepared in a tube containing 10 mL of saline (0.9%) at a concentration of 2 on the McFarland scale, with 5 mL of the prepared suspension mixed with 5 mL of BHI broth in a test tube to obtain the final suspension. Then, 20 µL of the final suspension was introduced into the root canal with the use of a 0.3-milliliter insulin syringe, and a cotton pellet dipped in the suspension was inserted into the canal opening. Incubation continued for 21 days in a 5% CO_2 atmosphere at 37°C. The viability and purity of the microorganisms were assessed weekly by Gram staining.[15]

Microbiological examination

After 21 days, the microbial samples were collected by inserting absorbent paper cones into the canal for 1 min, which were then transferred to Eppendorf tubes containing 1 mL of saline and shaken in a tube shaker for 30 s. Serial dilutions of each suspension were prepared to a concentration of 10^{-5} mg/mL, and 0.1 mL of the suspension from each sample was seeded and streaked onto blood agar plates, and incubated at 37°C and 5% CO_2 for 24 h. Colony-forming units (CFUs) were then mechanically counted and the number of viable germs was calculated.[14] Saline irrigation was performed 3 times to remove the free-flowing detached bacteria, and the intracanal medication was then administered: 0.7% stevia gel (group 1); TAP (group 2); or carbopol gel (vehicle) (group 3). Group 4 (control) was left untreated.

Analysis of the root canal samples

After an incubation period of 7 days, the drug was flushed out by rinsing with saline. Absorbent paper cones were inserted and the procedure was repeated.[4] The count of CFUs was noted and compared (Fig. 1).

Statistical analysis

The quantitative data post triplicate experimentation was subjected to statistical analysis and tabulated. The intergroup analysis was performed using the Kruskal–Wallis test, whereas intragroup comparisons used the Wilcoxon test, with a p-value set at 0.05. Pairwise comparisons with Dunn's post-hoc test determined the significant intergroup differences.

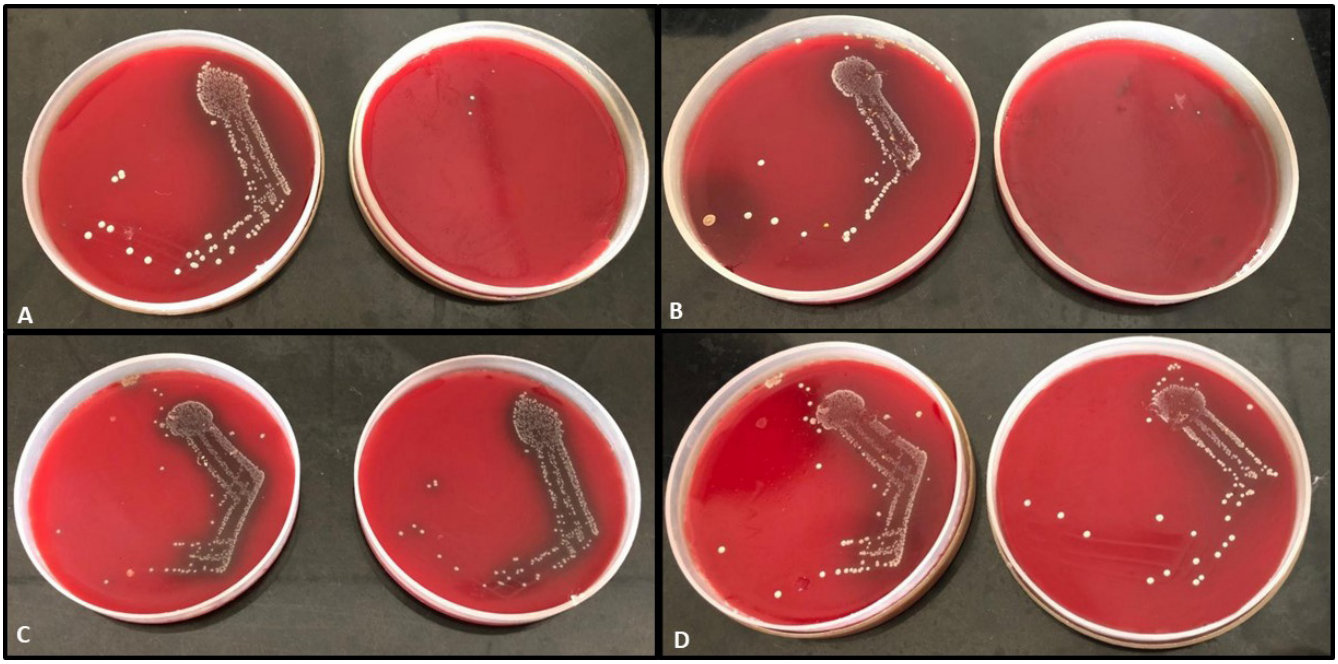

Fig. 1. Microbial colonies formed pre- and post-treatment with different drugs
A – stevia; B – triple antibiotic paste (TAP); C – carbopol; D – control group (untreated).

Results

Minimal bactericidal concentration

The MBC of stevia that totally inhibited the growth of *E. faecalis* was 0.7%.

Intergroup analysis

Pre-treatment

The mean count of CFUs of *E. faecalis* before treatment was 407.60 ±177.37 in the stevia group, 443.50 ±130.04 in the TAP group, 393.90 ±88.34 in the vehicle group, and 444.30 ±52.70 in the control group, with no significant differences between the 4 groups (p = 0.420) (Table 2, Fig. 2).

Post-treatment

The mean count of *E. faecalis* CFUs after treatment was 2.30 ±0.82 in the stevia group, 1.90 ±0.74 in the TAP group, 391.80 ±87.97 in the vehicle group, and 444.30 ±52.37 in the control group. There were significant differences in the *E. faecalis* CFUs between the 4 groups post-intervention (p < 0.001) (Table 2, Fig. 2).

Pairwise analysis

Multiple pairwise comparisons revealed that in groups 1 and 2, the mean count of CFUs was significantly smaller as compared to groups 4 and 5 ($p \leq 0.001$). However, no remarkable differences were observed between groups 1 and 2 (p = 0.630), and groups 3 and 4 (p = 0.400) (Table 3).

Table 2. Comparison of the mean count of colony-forming units (CFUs) of *Enterococcus faecalis* (*E. faecalis*) (×10^8) between the 4 groups pre- and post-treatment (Kruskal–Wallis test)

Group	CFUs of *E. faecalis* (×10^8)			
	pre-treatment	*p*-value	post-treatment	*p*-value
Stevia	407.60 ±177.37	0.420	2.30 ±0.82	<0.001*
TAP	443.50 ±130.04		1.90 ±0.74	
Carbopol	393.90 ±88.34		391.80 ±87.97	
Control	444.30 ±52.70		444.30 ±52.37	

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

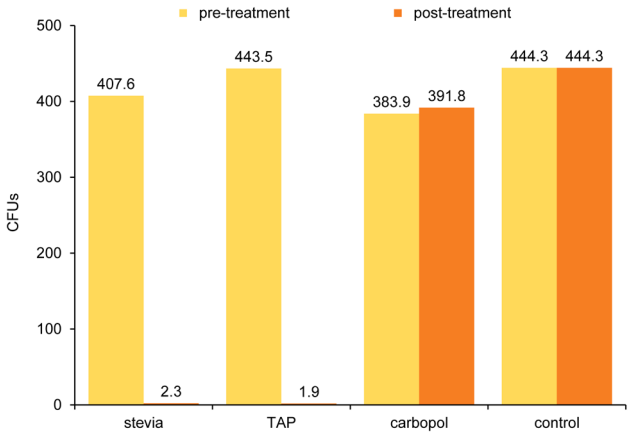

Fig. 2. Mean count of colony-forming units (CFUs) of *Enterococcus faecalis* (*E. faecalis*) before and after drug application

Table 3. Multiple pairwise comparisons of the mean differences in the count of colony-forming units (CFUs) of *Enterococcus faecalis* (*E. faecalis*) ($\times 10^8$) between the 4 groups post-treatment (Dunn's post-hoc test)

Group (A)	Group (B)	*MD* (A – B)	95% *CI* for *MD*		*p*-value
			lower	upper	
Stevia	TAP	0.40	–61.26	62.06	0.630
	carbopol	–389.50	–451.16	–327.84	0.001*
	control	–442.00	–503.66	–380.34	<0.001*
TAP	carbopol	–389.90	–451.56	–328.24	<0.001*
	control	–442.40	–504.06	–380.74	<0.001*
Carbopol	control	–52.50	–114.16	9.16	0.400

MD – mean difference; *CI* – confidence interval; * statistically significant.

Discussion

This study focused on the formulation of a drug that overcomes the disadvantages of commercially available preparations, but also leads the way in terms of antimicrobial properties. Stevia was the preferred alternative, as it has been shown historically to be superior to industrially marketed chlorhexidine mouthwashes in limiting plaque, gingivitis,[16] and the count of caries-forming organisms.[18] The steviol glycoside that the preparation contains also makes it palatable to children because of its sweet taste. The other group in this trial received TAP (in the present study, it was in fact TAG to distinguish the vehicle used), which causes coronal discoloration despite its good canal-disinfecting properties.[19] Also, the minocycline component in TAP has been associated with allergic reactions in many cases.[20] The available literature suggests that a biocompatible herbal drug with minimal/no side effects is preferable.

The overall performance of intracanal drugs depends on the type of carrier used. Gels are generally much less viscous than pastes, which improves penetration deep into the canal and collateral canals. In addition, pastes adversely affect the microhardness of dentin.[5] A study investigating the properties of different carrier materials demonstrated that macrogol and propylene glycol, the traditional carrier materials for TAP, had some antimicrobial effects, leading to some bias.[21] Considering all of the above factors, a carbopol gel-based vehicle was selected as the delivery system, as it has a neutral pH and is efficient in terms of permeability.[22]

Enterococcus faecalis naturally produces reactive oxygen species (ROS), and to combat them, antioxidants are produced simultaneously. This property makes the bacteria tolerant to acidic environments; nevertheless, TAP with its acidic pH (5.5) successfully limits the *E. faecalis* count, suggesting that both acidic and alkaline niches can affect the growth of *E. faecalis*.[23]

The results of the present study indicate that stevia acts very similarly to TAP; its antibacterial properties could result from oxygen radicals (hydroxyl, nitrate and peroxide) causing DNA mutations, base pair modification and intercellular protein cross-linking.[17] Carbopol is acidic in nature, with a pH of 2–2.5, but the pH rises to neutral by adding triethanolamine, which is both a gelling agent and a neutralizer.

Stevia is a novel product with very limited reports in the literature, and this study was conducted to explore the possibility of using stevia as a bactericidal/bacteriostatic agent. The exploratory nature of the study made it justifiable to use a relatively small sample size.

Conclusions

Stevia demonstrated a significant reduction in the count of *E. faecalis*, similar to TAP, when used as an intracanal medicament.

This study concluded that carbopol did not influence the antimicrobial efficacy of the experimental drug used as an intracanal medicament, which was comparable to that of the traditionally used TAP.

However, it should be noted that the oral cavity and the root canal system are inhabited by a variety of microflora, and the lack of substantial data on the antimicrobial efficacy of stevia against this microbiota suggests the need for further studies.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

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References

1. Aydın H, Er K, Kuştarcı A, et al. Antibacterial activity of silver nanoparticles activated by photodynamic therapy in infected root canals. *Dent Med Probl*. 2020;57(4):393–400. doi:10.17219/dmp/123615
2. Kim D, Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: A literature review – Part I. In vitro studies. *Restor Dent Endod*. 2014;39(4):241–252. doi:10.5395/rde.2014.39.4.241

3. Shaik J, Garlapati R, Nagesh B, Sujana V, Jayaprakash T, Naidu S. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste and calcium hydroxide using chitosan as carrier against *Candida albicans* and *Enterococcus faecalis*: An in vitro study. *J Conserv Dent*. 2014;17(4):335–339. doi:10.4103/0972-0707.136444
4. Venkataraman M, Singhal S, Tikku AP, Chandra A. Comparative analysis of tooth discoloration induced by conventional and modified triple antibiotic pastes used in regenerative endodontics. *Indian J Dent Res*. 2019;30(6):933–936. doi:10.4103/ijdr.IJDR_782_18
5. Parashar V, Khan SA, Singh P, Sharma S, Kumar A, Kumar A. Effect of intracanal medicaments (modified triple antibiotic paste, calcium hydroxide, and aloe vera) on microhardness of root dentine: An in vitro study. *J Contemp Dent Pract*. 2020;21(6):632–635. PMID:33025931.
6. Muradashvili M, Jabnidze N, Koiava L, et al. Antibacterial and antifungal activity of *Stevia rebaudiana* leaf extract in vitro condition. *Biol Forum*. 2019;11(1):212–216. https://www.researchtrend.net/bfij/pdf/Antibacterial%20and%20Antifungal%20Activity%20of%20Stevia%20rebaudiana%20(Asteraceae)%20Leaf%20Extract%20in%20vitro%20Condition%20M%20MURADASHVILI.pdf. Accessed February 12, 2022.
7. Vandana K, Reddy VC, Sudhir KM, Kumar K, Raju SH, Babu JN. Effectiveness of stevia as a mouthrinse among 12–15-year-old schoolchildren in Nellore district, Andhra Pradesh – a randomized controlled trial. *J Indian Soc Periodontol*. 2017;21(1):37–43. doi:10.4103/jisp.jisp_54_17
8. Momtazi-Borojeni AA, Esmaeili SA, Abdollahi E, Sahebkar A. A review on the pharmacology and toxicology of steviol glycosides extracted from *Stevia rebaudiana*. *Curr Pharm Des*. 2017;23(11):1616–1622. doi:10.2174/1381612822666161021142835
9. Konoshima T, Takasaki M. Cancer-chemopreventive effect of natural sweeteners and related compounds. *Pure Appl Chem*. 2002;74(7):1309–1316. doi:10.1351/pac200274071309
10. Fengyang L, Yunhe F, Bo L, et al. Stevioside suppressed inflammatory cytokine secretion by downregulation of NF-κB and MAPK signaling pathways in LPS-stimulated RAW264.7 cells. *Inflammation*. 2012;35(5):1669–1675. doi:10.1007/s10753-012-9483-0
11. Yuajit C, Homvisasevongsa S, Chatsudthipong L, Soodvilai S, Muanprasat C, Chatsudthipong V. Steviol reduces MDCK cyst formation and growth by inhibiting CFTR channel activity and promoting proteasome-mediated CFTR degradation. *PLoS One*. 2013;8(3):e58871. doi:10.1371/journal.pone.0058871
12. Abo Elnaga NI, Massoud MI, Yousef MI, Mohamed HH. Effect of stevia sweetener consumption as non-caloric sweetening on body weight gain and biochemical's parameters in overweight female rats. *Ann Agric Sci*. 2016;61(1):155–163. doi:10.1016/j.aoas.2015.11.008
13. Pandey S, Shekhar R, Paul R, et al. A comparative evaluation and effectiveness of different antimicrobial herbal extracts as endodontic irrigants against *Enterococcus faecalis* and *Candida albicans*. *University J Dent Scie*. 2018;4(2):75–81. https://old.amu.ac.in/pdf/dentjour/A%20COMPARATIVE%20EVALUATION.pdf. Accessed February 12, 2022.
14. Kharouf N, Pedullà E, La Rosa GR, et al. In vitro evaluation of different irrigation protocols on intracanal smear layer removal in teeth with or without pre-endodontic proximal wall restoration. *J Clin Med*. 2020;9(10):3325. doi:10.3390/jcm9103325
15. Hartmann RC, Neuvald L, Barth V Jr., et al. Antimicrobial efficacy of 0.5% peracetic acid and EDTA with passive ultrasonic or manual agitation in an *Enterococcus faecalis* biofilm model. *Aust Endod J*. 2019;45(1):57–63. doi:10.1111/aej.12279
16. Koseki S, Yoshida K, Kamitani Y, Itoh K. Influence of inoculation method, spot inoculation site, and inoculation size on the efficacy of acidic electrolyzed water against pathogens on lettuce. *J Food Prot*. 2003;66(11):2010–2016. doi:10.4315/0362-028x-66.11.2010
17. Ghosh S, Subudhi E, Nayak S. Antimicrobial assay of *Stevia rebaudiana* Bertoni leaf extracts against 10 pathogens. *Int J Integr Biol*. 2008;2(1):27–31.
18. Usha C, Ramarao S, John BM, Babu ME. Anticariogenicity of *Stevia rebaudiana* extract when used as a mouthwash in high caries risk patients: Randomized controlled clinical trial. *World J Dent*. 2017;8(5):364–369. doi:10.5005/jp-journals-10015-1466
19. Thomas MS. Crown discoloration due to the use of triple antibiotic paste as an endodontic intra-canal medicament. *Saudi Endod J*. 2014;4(1):32–35. doi:10.4103/1658-5984.127985
20. Mohammadi Z, Jafarzadeh H, Shalavi S, Yaripour S, Sharifi F, Kinoshita JI. A review on triple antibiotic paste as a suitable material used in regenerative endodontics. *Iran Endod J*. 2018;13(1):1–6. doi:10.22037/iej.v13i1.17941
21. Mandal SS, Margasahayam SV, Shenoy VU. A comparative evaluation of the influence of three different vehicles on the antimicrobial efficacy of triple antibiotic paste against *Enterococcus faecalis*: An in vitro study. *Contemp Clin Dent*. 2020;11(2):150–157. doi:10.4103/ccd.ccd_372_19
22. Lubrizol. Neutralizing Carbopol® and Pemulen™ polymers in aqueous and hydroalcoholic systems. 2009. https://studylib.net/doc/25287828/neutralizing-carbopol-and-pemulen-in-aqueous-and-hydroalc. Accessed February 12, 2022.
23. Szemes T, Vlkova B, Minarik G, et al. On the origin of reactive oxygen species and antioxidative mechanisms in *Enterococcus faecalis*. *Redox Rep*. 2010;15(5):202–206. doi:10.1179/135100010X12826446921581

Review

Most common congenital syndromes with facial asymmetry: A narrative review

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Abstract

Symmetry is present in various aspects of everyday life. A symmetrical face is considered attractive, whereas a lack of facial symmetry is regarded as a source of functional and aesthetic problems. Most of the people exhibit slight asymmetries, but some of them reveal severe asymmetries. Among patients presenting with severe facial asymmetries, there may be those with congenital defects. Congenital defects may manifest at the time of birth or be a result of birth trauma.

One of the most prevalent asymmetrical birth defects is cleft lip and/or cleft palate. Other congenital defects include craniofacial syndromes, such as Treacher Collins syndrome (TCS) and Goldenhar syndrome. Among the rare syndromes with facial asymmetries, Klippel–Feil syndrome (KFS), PHACE (posterior fossa brain malformation, hemangiomas, arterial anomalies, cardiac anomalies, and eye abnormalities) syndrome, plagiocephaly, and Parry–Romberg syndrome are worth noticing. The majority of craniofacial asymmetries require surgery to improve the patient's facial appearance. The treatment is multidisciplinary and long, and the most common procedures involve reparative and regenerative surgeries. The aim of this review was to present the most common congenital defects with facial asymmetry.

Keywords: congenital abnormalities, cleft lip, facial asymmetry, cleft palate, PHACE syndrome

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Introduction

Symmetry is a factor that influences our perception of beauty. It is present in various aspects of everyday life, including architecture and art. Symmetry is often equated with elegance.[1] An aesthetic smile is considered more attractive,[2] and the attractiveness of a smile is one of the key factors influencing the cooperation during the orthodontic and aesthetic dental treatments.[3] Teeth size and gingival margin symmetry contribute to the perceived attractiveness of a smile.[4] A symmetrical face is recognized as attractive, and a lack of symmetry may cause facial and functional problems.[5] Craniofacial asymmetry is a normal condition, especially when compared to orthodontic diagnosis. Asymmetries have a strong genetic background, as demonstrated by Babczyńska et al.[6] Additionally, this topic has been described in the context of other dental specialties.[7] Based on the analysis of the patients treated orthodontically in North Carolina, 74% of cases were observed in the lower third of the face, 36% in the middle part, and the least asymmetries (5%) were observed in the upper part of the face.[8] According to Kozanecka et al.,[9] bite asymmetries (including crossbites) are one of the most common causes for good patient cooperation during the orthodontic treatment, which supports the thesis that facial aesthetics is a crucial factor in this process. It is also worth mentioning that males tend to assess dental asymmetries with greater precision than females.[2]

However, there are certain conditions that are not caused by malocclusions but rather are the result of congenital syndromes. These conditions usually have a genetic background.[10] Nowadays, the use of distractors in the treatment of facial asymmetries is becoming increasingly popular. It may facilitate the treatment of asymmetries in hemifacial underdevelopment by elongating the mandible or rotating it in the gonial angle.[11] The main causes of facial asymmetry are congenital disorders and developmental deformities.[5]

The aim of this study was to present the most common birth defects associated with facial asymmetry and congenital diseases. This paper collates the most important data on prevalent congenital syndromes that are characterized by craniofacial asymmetry. Such conditions may manifest at the time of birth or be a result of birth trauma. The article presents recently collected data on congenital syndromes that have not been previously summarized.

Material and methods

For the purpose of this paper, the Scopus and PubMed databases were searched. The terms "congenital defect", "face" and "asymmetry" were used to determine the most common defects affecting the orofacial area. The study included articles from the last 10 years, as general knowledge about rare congenital diseases is increasing. The majority of the papers focused on cleft lip and cleft palate. Therefore, we incorporated this anomaly into the research. However, we excluded it from the search criteria to identify other articles about the facial asymmetry.

Cleft lip and/or cleft palate

Cleft lip and cleft palate are the most common congenital anomalies, occurring in 1:700–1:1,000 patients. The non-syndromic cleft occurs in the 5^{th}–12^{th} week in utero. It is usually a multifactorial condition, with a genetic background identified in approx. 20% of cases.[12] The lips and the nose (especially the philtrum, columella and the vermilion border of the upper lip) are the most asymmetrical regions. This condition is more prevalent before the surgical procedure of closing the cleft lip and improves after surgery. Unfortunately, growth is unfavorable in this case, resulting in pronounced facial assymetry as the scar on the clefted side develops.[13] The asymmetry is also more pronounced when a total cleft is present, when compared to an isolated cleft lip.[14] Patients with clefts present with asymmetry not only in the face but also in the occlusion and dental arch form. Asymmetry of the dental arch is observed in all cases. Malocclusions are also asymmetrical, as crossbites are the most common conditions in cleft patients.[15,16] Hereditary dental anomalies, such as hypodontia, hyperdontia or tooth impaction, are also common and occur more frequently in cleft patients than in the general population.[17] Cleft deformities have a strong genetic background. The genetic pattern is based on multiple genes and is strongly influenced by environmental factors, which makes this problem even more difficult to diagnose.[18]

Patients with clefts require a multidisciplinary approach that should be initiated at the neonatal period.[19] The approach depends on the type of cleft and the individual treatment needs. The procedures focus on the reconstruction and plastic surgery, but other aspects like speech therapy and orthodontic treatment are also very important. The most common procedures performed in patients with clefts are presented in Table 1.

The first procedures performed in patients with clefts concentrate on the presurgical preparation of the patient for lip and/or palate closure. The procedures involve a lip massage to lengthen soft tissues and reduce the pressure of the prospective scar. All presurgical actions are aimed at reducing the stigma associated with cleft lip and cleft palate. To reduce columella and mold the palate, nasoalveolar molding (NAM) plates, introduced by Grayson and Maull, can be used.[18,20] The NAM plate rotates the premaxilla and, therefore, reduces the cleft of the alveolus.

The first surgical procedure concerns the lip and/or palate closure, which is typically performed between the 3^{rd} and 6^{th} month of age.[21] The soft tissues are restored, but the fissure in the bone requires filling. This is achieved

Table 1. Scheme of treatment for a patient with a cleft deformity

Patient's age	Performed procedures
6 months	• presurgical orthodontic preparation (if necessary) • lip reconstruction • palatal reconstruction (in some cases) • nose correction
8–10 months	• evaluation of speech • hearing test (repeated every 6 months)
1.5–3 years	• dental check-up • orthodontic check-up (treatment needs) • speech training • palatal reconstruction (if not performed in the first months) • alveolar bone grafting (if the lip and palate were corrected simultaneously) • first aesthetic, plastic corrections
4–5 years	• nasal septum correction • active orthodontic treatment (1st phase of Hyrax screw appliance or removable appliance)
7–10 years	• orthodontic treatment • possible alveolar bone grafting • Furlow palatoplasty
>12 years	• orthodontic treatment with fixed appliances • traction of impacted teeth
>16 years	• plastic surgeries of the lip and nose • possible preparation for orthognathic surgery

through bone grafting, which is most frequently derived from the iliac crest.[22] The palate of the operated patient is shortened and has lower mobility. A number of procedures can be performed to restore the palate to its normal length. Of these, Furlow palatoplasty is the most commonly performed procedure. It is a simple Z-plasty surgery, in which the soft tissues are mobilized to lengthen the palate. The purpose of this procedure is to seal the oral cavity and prevent oronasal communication. Furlow palatoplasty is usually performed between the ages of 8 and 12 years.[23]

During the entire treatment period, the patient requires orthodontic care. The presence of soft tissue scars results in a repetitive narrowing of the arch. It is necessary to widen the upper arch of the patient, which may be achieved through the use of removable or fixed appliances. A face mask is worn for maxillary protraction. As the majority of patients present with crossbites and maxillary hypoplasia, it is important to determine whether the patient would benefit from orthognathic surgery in adulthood.[14,18]

Mandibular condyle ankylosis

Temporomandibular joint ankylosis is defined as a permanent constriction of the jaws and a limited mouth opening to a maximum of 30 mm (measured between the incisal edges). Due to the limited mandibular movement, the ability to chew, speak and swallow may be impaired.[24] Mandibular condyle ankylosis may result from injury at birth or complications during labor (17.8%). Temporomandibular joint ankylosis is a dangerous condition in children, as it may lead to facial asymmetry. This condition is mostly caused by trauma during the childhood (48.9%).[25] If left untreated, the ankylosis in a child will result in facial widening and crossbites. Additionally, severe class II and skeletal open bite may result from the impairment of potential growth caused by the growth cone on the mandibular head. This can lead to disturbances in both mandibular length and ramal height.[26,27] The limited mouth opening is comparable to that observed in rheumatoid arthritis and may impede proper oral hygiene, increasing the risk of caries and periodontal disease.[28,29] In addition to limiting mandibular movement, this condition does not influence muscular tonus and function.[30] To reduce the presented consequences of mandibular condyle fractures, early rehabilitation is essential.[30] Prompt management of mandibular fractures plays a key role in reducing growth disturbances in mandibular dimension and morphology.[25,26]

Unilateral congenital disturbances in facial development

Several disturbances in facial development are observed, and in the majority of cases, these are asymmetrical. The most common unilateral congenital anomalies affecting the face include hemifacial microsomia, Treacher Collins syndrome (TCS) and Goldenhar syndrome. These anomalies pertain to malformations of the 1st and 2nd pharyngeal arches.[31,32] Most of the symptoms manifest in the face and dental region. A common issue is the potential for a dentigerous cyst, which may cause disturbances in tooth eruption. The typical treatment for this condition is marsupialization or the extraction of the affected tooth.[33]

Hemifacial microsomia

Craniofacial microsomia (CFM) appears in 1:3,500 to 1:5,600 live births, which situates this malformation as the 3rd most common congenital craniofacial anomaly, preceded by cleft lip, cleft palate and craniosynostosis. Patients with CFM present with asymmetries in mandibular body and ramal lengths. This leads to the retrusion of the mandible. In addition, hypoplasia of the ear occurs in 66–99% of the individuals.[34] The full etiology of this condition remains unclear, but it manifests during embryonic development. The possible models for this condition include vascular abnormalities, hemorrhage or neurocristopathy among the nasal placode and the 1st and 2nd pharyngeal arches.[35,36] In rare instances, bilateral microsomia is observed, which is symmetrical (present in 5–15% of cases).[34] There is no specific diagnostic criterion for microstomia. However, most of the patients present

with the underdevelopment of half of the face, specifically the mandible, maxilla, facial soft tissues, ear, orbit and/or facial nerve. This might influence facial movements, food intake, breathing, and disrupt hearing. The facial appearance is interrupted and easily noticeable.[32] The treatment requires bone and soft tissue reconstruction, as well as the correction of auricular anomalies.[37] Despite extensive knowledge of the genetic basis of CFM, the definitive genetic background of this anomaly remains unclear.[38]

Treacher Collins syndrome

Treacher Collins syndrome, also known as Franceschetti syndrome or mandibulofacial dysostosis, is a genetically driven condition caused by abnormal differentiation of the 1st and 2nd pharyngeal arches. The deformity is observed in 1 in 50,000 live births with a strong hereditary background, with 40% of cases having a family history. Genetically, 4 variants of mutations within genes have been observed: *TCOF1*; *POLR1D*; *POLR1C*; and *POLR1B*.[39] It is probably the first and the most studied cranial neural crest anomaly.[40]

The facial features of TCS include hypoplasia of bones such as midface hypoplasia, microtia and hemifacial micrognathia. The other characteristics are conductive hearing loss and slanting palpebral fissures with the possibility of coloboma of the lateral part of the lower eyelid. On occasion, cardiovascular problems associated with cleft palate, esophageal and/or choanal atresia or stenosis are observed. Due to the severe craniofacial malformation and retruded mandible, nocturnal apnea may be observed, which could potentially be a life-threatening situation.[39,41] Additionally, the hairline is displaced.[40] In individuals with TCS, clockwise rotation of the mandible may result in an open bite and class II malocclusion, which may subsequently lead to temporomandibular joint disorders.[42] On cephalometric X-rays, a shortening of the posterior and anterior cranial bases can be observed.[43] Due to the hearing problems, most patients present with speech issues. Other senses are also affected, including impaired sight and feeding.[39,41] Due to the severe craniofacial malformations, patients require surgical and reconstructive treatment. These procedures involve soft and hard tissues, especially the orbit zygomatic and maxillary regions.[39,41] The malformations result in poor oral hygiene in individuals with TCS, increasing the risk of calculus and caries.[42]

Goldenhar syndrome

Goldenhar syndrome, also known as ocular-auricular-vertebral (OAV) syndrome, is a congenital condition resulting from a defect in the 1st and 2nd brachial arches. This anomaly is caused by a combination of genetic and environmental factors.[44,45] Lately, a candidate gene on the 22q chromosome has been found to play a crucial role in OAV syndrome.[46] Facial asymmetry, ear-eye abnormalities, congenital problems, vertebral anomalies, and severe obstructive sleep apnea and sialorrhea are typical manifestations of Goldenhar syndrome.[44] The clinical presentation of the condition may vary, from slight facial asymmetry to severe craniofacial deformities. There are no minimum inclusion criteria defined.[45,47] In 100% of cases, hemifacial hypotrophy is observed. Auricular anomalies are observed in 80% of individuals. In 90% of cases, there is unilateral ophthalmic involvement, with upper eyelid coloboma (75.76%), lipodermoid (54.55%) and limbal dermoid (30.3%) being the most common.[48] Individuals with Goldenhar syndrome often require surgical procedures, such as the repair of eyelid colobomas.[48] The potential vertebral anomalies and the limitations of head and neck movement present a challenge to the successful performance of laryngoscopy and intubation.[49]

Acrofacial dysostosis

Miller syndrome and Nager syndrome are the most common acrofacial dysostoses.[50] Nager syndrome is very similar to TCS and may be misdiagnosed. The syndrome manifests with preaxial limb defects, hypoplasia or the absence of the thumbs. Additionally, recent studies found mutations in the *SF3B4* gene in approx. 60% of cases.[50–52] Hearing loss, present in 45% of cases, is probably caused by defective middle ear ossicles.[53]

Another syndrome that belongs to this group of malformations is Miller syndrome. It is also referred to as post-acrofacial dysostosis (POADS), Wildervanck–Smith syndrome or Genée–Wiedemann syndrome. The presence of downward slanting of palpebral fissures, hypoplasia of the zygomatic complex, coloboma of the lower eyelid, microtia and micrognathia as well as hearing loss, presents a significant challenge in differentiating POADS from TCS. It was determined that the condition is caused by autosomal recessive or heterozygous mutations in dihydroorotate dehydrogenase (*DHODH*).[50,51] The gene encodes the enzyme in the pyrimidine de novo biosynthesis pathway. It has been identified in the mitochondrial intermembrane space.[54]

The differentiation of these syndromes may be challenging. In order to organize the data and features concerning the 4 clinical units, a summary of the facial features of the previously described congenital disorders is presented in Table 2.

PHACE syndrome (PHACE association)

PHACE syndrome is a rare congenital condition, occurring in less than 1 in 1,000,000 cases, that is characterized by the presence of large facial hemangiomas (sometimes

Table 2. Comparison of the clinical manifestations of hemifacial microsomia, Treacher Collins syndrome, Goldenhar syndrome, and acrofacial dysostosis

Possible features	Hemifacial microsomia	Treacher Collins syndrome	Goldenhar syndrome	Acrofacial dysostosis (Miller syndrome and Nager syndrome)
Bone hypoplasia	+++	+++	+++	+++
Auricular anomalies	+++	++	+++	++
Hearing loss	+	+++	+++	++
Ophthalmic anomalies (e.g., coloboma)	+	++	++	++
Cleft lip and/or cleft palate	++	+	++	++
Limb defects	+	+	–	+
Difficulties in breathing	+	+	+++	–

+++ – very likely; ++ – possible; + – rare.

also of head and neck) and other systemic malformations. The acronym stands for the association of the following syndromes: posterior fossa brain malformation; hemangiomas; arterial anomalies; cardiac anomalies; and eye abnormalities. In most cases, hemangiomas can be successfully treated with oral propranolol. Large infantile hemangioma is often correlated with malformations of the posterior cranial fossa. Additionally, midline or ventral anomalies are a common feature. Cerebrovascular involvement is present in 80% of cases. Other anomalies do not refer to the face and are instead associated with anomalies of the central nervous system, cardiac defects, endocrine problems (e.g., thyroid dysgenesis), coarctation of the aorta, and ocular abnormalities.[55–57] The etiology of PHACE is not fully understood. It has been observed that PHACE syndrome is more frequently observed among females.[58]

Parry–Romberg syndrome

Parry–Romberg syndrome is a rare condition that is characterized by progressive hemifacial atrophy. The etiology of the disorder remains unknown. The characteristic feature is the unilateral, gradual atrophy of the skin. The process may involve the underlying tissues, including fat, muscles and osseocartilaginous structures. This results in a severe facial asymmetry.[59] The syndrome typically affects patients under the age of 20 years, with a higher prevalence among females. The younger the patient, the more severe the course of the symptoms.[60] In more severe cases, the neck and the other half of the face may also be involved.[61] The most common features of Parry–Romberg syndrome are presented in Fig. 1.

The progress of the disease is rather slow, but it frequently becomes complicated by the involvement of other systems (e.g., neurological, ophthalmic).[60] The most common neurological complications, such as epilepsy, headaches and trigeminal neuralgia, are described.[61]

Following the stabilization of the disease, the reconstruction of lost tissues is performed to restore facial asymmetry. Fat grafting is usually a method of choice. It shows favorable results when the reconstruction requires mild to moderate soft tissue deficiency. In more severe cases, free tissue transfers are necessary.[59] When complicated with melasma, skin bleaching is also indicated for aesthetic reasons.[62]

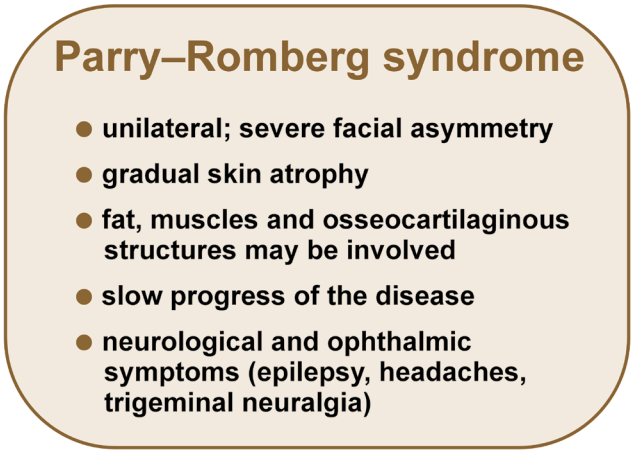

Fig. 1. Most common characteristics of Parry–Romberg syndrome

Craniosynostosis and plagiocephaly

Craniosynostosis is a condition that involves premature fusion of skull sutures.[63] It is typically an isolated condition, but in some cases it may be a feature of specific syndromes, such as Apert syndrome or Crouzon syndrome. In most cases, treatment is necessary to reduce the likelihood of developmental delay and other neurological complications, as well as abnormalities within the skull (e.g., facial, sensory, respiratory).[64] It is important to differentiate this condition from positional plagiocephaly, which is caused by an improper position of the fetus rather than the premature suture closure. It may be caused by injuries during birth, pregnancy and prematurity.[65] Craniosynostosial plagiocephaly has an influence on a child's development, and if not diagnosed promptly, may result in mental retardation.[66]

The term "plagiocephaly" is derived from Ancient Greek, where "plagios" means "oblique" and "kephalē" signifies "head". The typical presentation is asymmetry of the head due to unilateral flattening. Plagiocephaly is classified as a non-syndromic craniosynostosis, occurring at one of the sutures, specifically the coronal or lambdoid. It causes head and face asymmetry. When premature fusion concerns the coronal suture, the asymmetry manifests in the anterior region of the skull. Premature fusion of the lambdoid suture leads to posterior synostosis.[63] In cases of positional plagiocephaly, a conservative approach involving the use of a special helmet is recommended. If the condition is the result of premature suture closure, surgical procedure is the preferred method of treatment.[65]

Klippel–Feil syndrome

The defining characteristics of Klippel–Feil syndrome (KFS) arise from the fusion of cerebral vertebrae, most frequently C2 and C3 (more rarely, C5 and C6). In some cases, additional vertebrae are involved. Due to this condition, the neck is shortened and restricted in its mobility. Individuals with KFS also present with a low posterior hairline.[67] The latest report considered a possible fusion of C1 and C2 vertebrae, which results in limitations in head rotation.[68] Facial asymmetry is observed on the side of torticollis. The syndrome occurs with a prevalence of 1:42,000 births, and most of the cases concern females (60%).[69] The observed torticollis may "hide" the shortening of the neck.[70] The real prevalence is not known, although genetic factors (homeobox genes and differentiation factors, such as *MEOX1*, *GDF6* and *GDF3*) appear to play a crucial role in this syndrome.[69] Due to the severity of cervical vertebral fusion, Gunderson et al. divides KFS into 3 types: type I – fusion of multiple vertebrae, including the thoracic vertebrae; type II – fusion of 2 or 3 vertebrae; type III – fusion of both the cervical and lower thoracic or lumbar vertebrae.[48,71] Most of the patients receive non-surgical treatment. Only cases with severe neurological complications require surgical intervention.[72,73]

Conclusions

This study presents an overview of the most common congenital deformities characteristic of facial asymmetry, providing a summary of their most frequent features. Interestingly, the abovementioned deformities are associated with both maxillary and mandibular deformations. The emerging literature shows that this may lead to breathing problems, including obstructive sleep apnea.[74–77] This represents a potential area for future research, as, to the best of our knowledge, there is no existing data on this topic. It would be interesting to consider the potential benefits of splint therapy. This influences the position of the mandible and, in most cases, increases the volume of the airways.[78,79] One must, however, take into account that the properties of the materials used for splint preparation change with time, and the problems with durability and general structure may occur due to material aging and the influence of saliva.[80] Furthermore, teledentistry has emerged as a novel trend, especially during the coronavirus disease 2019 (COVID-19) pandemic.[81] Although teledentistry may offer certain advantages, the cooperation with a dental practitioner remains the most important aspect, given the necessity for precise diagnostic tools for the intraoral examination. In such cases, the appropriate treatment can be administered.[81–83]

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.

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References

1. Kobus K, Kobus-Zaleśna K. The treatment of facial asymmetry: Review. *Adv Clin Exp Med*. 2017;26(8):1301–1311. doi:10.17219/acem/68976
2. Chrapla P, Paradowska-Stolarz A, Skoskiewicz-Malinowska K. Subjective and objective evaluation of the symmetry of maxillary incisors among residents of Southwest Poland. *Symmetry*. 2022;14(6):1257. doi:10.3390/sym14061257
3. Sarul M, Antoszewska-Smith J, Park HS. Self-perception of smile attractiveness as a reliable predictor of increased patient compliance with an orthodontist. *Adv Clin Exp Med*. 2019;28(12):1633–1638. doi:10.17219/acem/110320
4. Pham TAV, Nguyen PA. Morphological features of smile attractiveness and related factors influence perception and gingival aesthetic parameters. *Int Dent J*. 2022;72(1):67–75. doi:10.1016/J.IDENTJ.2021.02.001
5. Cheong YW, Lo LJ. Facial asymmetry: Etiology, evaluation, and management. *Chang Gung Med J*. 2011;34(4):341–351. PMID:21880188.
6. Babczyńska A, Kawala B, Sarul M. Genetic factors that affect asymmetric mandibular growth – a systematic review. *Symmetry*. 2022;14(3):490. doi:10.3390/sym14030490
7. Minch L, Zielińska M, Kawala B. Perception of facial symmetry and attractiveness. *Dent Med Probl*. 2012;49(4):550–555. https://dmp.umw.edu.pl/en/article/2012/49/4/550. Accessed July 21, 2022.
8. Severt TR, Proffit WR. The prevalence of facial asymmetry in the dentofacial deformities population at the University of North Carolina. *Int J Adult Orthodon Orthognath Surg*. 1997;12(3):171–176. PMID:9511487.

9. Kozanecka A, Sarul M, Kawala B, Antoszewska-Smith J. Objectification of orthodontic treatment needs: Does the classification of malocclusions or a history of orthodontic treatment matter? *Adv Clin Exp Med*. 2016;25(6):1303–1312. doi:10.17219/acem/62828
10. Sahu A, Lall R, Nezam S, Singh R, Kumar SB, Ayub FB. Comparative assessment of facial asymmetry in malocclusion using posteroanterior view. *J Contemp Dent Pract*. 2018;19(6):712–718. PMID:29959301.
11. Fatima F, Jeelani W, Ahmed M. Current trends in craniofacial distraction: A literature review. *Dent Med Probl*. 2020;57(4):441–448. doi:10.17219/dmp/122579
12. Paradowska-Stolarz A. *MSX1* gene in the etiology orofacial deformities. *Postepy Hig Med Dosw (Online)*. 2015;69:1499–1504. PMID:27259221.
13. Al-Rudainy D, Ju X, Mehendale FV, Ayoub A. Longitudinal 3D assessment of facial asymmetry in unilateral cleft lip and palate. *Cleft Palate Craniofac J*. 2019;56(4):495–501. doi:10.1177/1055665618780108
14. Jodeh DS, Soni S, Cray JJ, Rottgers SA. Degree of asymmetry between patients with complete and incomplete cleft lips. *Cleft Palate Craniofac J*. 2021;58(5):539–545. doi:10.1177/1055665620962365
15. Pucciarelli MGR, Toyoshima GH, Cardoso JF, de Oliveira TM, Neppelenbroek KH, Soares S. Arch asymmetry in patients with cleft lip and palate after rehabilitation treatment using stereophotogrammetry. *J Craniofac Surg*. 2021;32(5):e501–e504. doi:10.1097/SCS.0000000000007460
16. Paradowska-Stolarz A, Kawala B. Occlusal disorders among patients with total clefts of lip, alveolar bone, and palate. *Biomed Res Int*. 2014;2014:583416. doi:10.1155/2014/583416
17. Pastuszak P, Dunin-Wilczyńska I, Lasota A. Frequency of additional congenital dental anomalies in children with cleft lip, alveolar and palate. *J Clin Med*. 2020;9(12):3813. doi:10.3390/jcm9123813
18. Paradowska-Stolarz A, Mikulewicz M, Duś-Ilnicka I. Current concepts and challenges in the treatment of cleft lip and palate patients – a comprehensive review. *J Pers Med*. 2022;12(12):2089. doi:10.3390/jpm12122089
19. Roguzińska S, Pelc A, Mikulewicz M. Orthodontic-care burden for patients with unilateral and bilateral cleft lip and palate. *Dent Med Probl*. 2020;57(4):411–416. doi:10.17219/dmp/125874
20. Grayson BH, Maull D. Nasoalveolar molding for infants born with clefts of the lip, alveolus, and palate. *Clin Plast Surg*. 2004;31(2):149–158. doi:10.1016/S0094-1298(03)00140-8
21. Raghavan U, Vijayadev V, Rao D, Ullas G. Postoperative management of cleft lip and palate surgery. *Facial Plast Surg*. 2018;34(6):605–611. doi:10.1055/s-0038-1676381
22. Dowgierd K, Pokrowiecki R, Borowiec M, et al. Protocol and evaluation of 3D-planned microsurgical and dental implant reconstruction of maxillary cleft critical size defects in adolescents and young adults. *J Clin Med*. 2021;10(11):2267. doi:10.3390/jcm10112267
23. Abdel-Aziz M, Nassar A, Rashed M, Naguib N, El-Tahan AR. Furlow palatoplasty for previously repaired cleft palate with velopharyngeal insufficiency. *Int J Pediatr Otorhinolaryngol*. 2015;79(10):1748–1751. doi:10.1016/j.ijporl.2015.07.044
24. Bénateau H, Chatellier A, Caillot A, Diep D, Kün-Darbois JD, Veyssière A. Temporo-mandibular ankylosis [in French]. *Rev Stomatol Chir Maxillofac Chir Orale*. 2016;117(4):245–255. doi:10.1016/j.revsto.2016.07.001
25. Ko EWC, Huang CS, Chen YR, Figueroa AA. Cephalometric craniofacial characteristics in patients with temporomandibular joint ankylosis. *Chang Gung Med J*. 2005;28(7):456–466. PMID:16231529.
26. de Andrade LHR, Cavalcante MAA, Raymundo R, Ribeiro de Souza IP. Temporomandibular joint ankylosis in children. *J Dent Child (Chic)*. 2009;76(1):41–45. PMID:19341578.
27. Roychoudhury A, Yadav P, Bhutia O, Kaur K, Dekyi T, Pandey RM. Growth outcome and jaw functions are better after gap arthroplasty plus costochondral graft reconstruction than gap arthroplasty alone in pediatric temporomandibular joint ankylosis patients: A cluster randomized controlled trial. *J Oral Maxillofac Surg*. 2021;79(12):2548–2561. doi:10.1016/j.joms.2021.08.164
28. Radwan-Oczko M, Duś-Ilnicka I, Richards P, Thomsen AM, Rasmussen C. Evaluation of oral health status and oral care of patients with rheumatoid arthritis. *Int J Dent*. 2020;2020:8896766. doi:10.1155/2020/8896766
29. Radwan-Oczko M, Duś-Ilnicka I, Richards P, Thomsen AM, Rasmussen C. Rheumatoid arthritis patients' oral health and disease activity. *Int J Rheum Dis*. 2019;22(8):1538–1543. doi:10.1111/1756-185X.13590
30. Wieckiewicz M, Smardz J, Martynowicz H, Wojakowska A, Mazur G, Winocur E. Distribution of temporomandibular disorders among sleep bruxers and non-bruxers – a polysomnographic study. *J Oral Rehabil*. 2020;47(7):820–826. doi:10.1111/joor.12955
31. Rzewuska A, Kijak E, Halczy-Kowalik L. Rehabilitation in the treatment of mandibular condyle fractures. *Dent Med Probl*. 2021;58(1):89–96. doi:10.17219/dmp/128092
32. Birgfeld C, Heike C. Craniofacial microsomia. *Clin Plast Surg*. 2019;46(2):207–221. doi:10.1016/j.cps.2018.12.001
33. Nahajowski M, Hnitecka S, Antoszewska-Smith J, Rumin K, Dubowik M, Sarul M. Factors influencing an eruption of teeth associated with a dentigerous cyst: A systematic review and meta-analysis. *BMC Oral Health*. 2021;21(1):180. doi:10.1186/s12903-021-01542-y
34. Chen YF, Baan F, Bruggink R, Bronkhorst E, Liao YF, Ongkosuwito E. Three-dimensional characterization of mandibular asymmetry in craniofacial microsomia. *Clin Oral Investig*. 2020;24(12):4363–4372. doi:10.1007/s00784-020-03302-8
35. Hartsfield JK. Review of the etiologic heterogeneity of the oculo-auriculo-vertebral spectrum (hemifacial microsomia). *Orthod Craniofac Res*. 2007;10(3):121–128. doi:10.1111/j.1601-6343.2007.00391.x
36. Chen Q, Zhao Y, Shen G, Dai J. Etiology and pathogenesis of hemifacial microsomia. *J Dent Res*. 2018;97(12):1297–1305. doi:10.1177/0022034518795609
37. Brandstetter KA, Patel KG. Craniofacial microsomia. *Facial Plast Surg Clin North Am*. 2016;24(4):495–515. doi:10.1016/j.fsc.2016.06.006
38. Timberlake AT, Griffin C, Heike CL, et al. Haploinsufficiency of SF3B2 causes craniofacial microsomia. *Nat Commun*. 2021;12(1):4680. doi:10.1038/s41467-021-24852-9
39. Marszałek-Kruk BA, Wójcicki P, Dowgierd K, Śmigiel R. Treacher Collins syndrome: Genetics, clinical features and management. *Genes (Basel)*. 2021;12(9):1392. doi:10.3390/genes12091392
40. Siismets EM, Hatch NE. Cranial neural crest cells and their role in the pathogenesis of craniofacial anomalies and coronal craniosynostosis. *J Dev Biol*. 2020;8(3):18. doi:10.3390/jdb8030018
41. Aljerian A, Gilardino MS. Treacher Collins syndrome. *Clin Plast Surg*. 2019;46(2):197–205. doi:10.1016/j.cps.2018.11.005
42. Cabanillas-Aquino AG, Rojas-Yauri MC, Atoche-Socola KJ, Arriola-Guillén LE. Assessment of craniofacial and dental characteristics in individuals with Treacher Collins syndrome. A review. *J Stomatol Oral Maxillofac Surg*. 2021;122(5):511–515. doi:10.1016/j.jormas.2020.10.011
43. Kantaputra PN, Tripuwabhrut K, Intachai W, et al. Treacher Collins syndrome: A novel TCOF1 mutation and monopodial stapes. *Clin Otolaryngol*. 2020;45(5):695–702. doi:10.1111/coa.13560
44. Marvulli R, Gallo GA, Mastromauro L, et al. Efficacy and non invasive treatment of sialorrhea in the Goldenhar syndrome. *Endocr Metab Immune Disord Drug Targets*. 2018;18(4):397–400. doi:10.2174/1871530318666180328122035
45. Bogusiak K, Puch A, Arkuszewski P. Goldenhar syndrome: Current perspectives. *World J Pediatr*. 2017;13(5):405–415. doi:10.1007/s12519-017-0048-z
46. Glaeser AB, Santos AS, Diniz Lixinski B, Deconte D, Rosa RFM, Zen PRG. Candidate genes of oculo-auriculo-vertebral spectrum in 22q region: A systematic review. *Am J Med Genet A*. 2020;182(11):2624–2631. doi:10.1002/ajmg.a.61841
47. Bragagnolo S, Colovati MES, Souza MZ, et al. Clinical and cytogenomic findings in OAV spectrum. *Am J Med Genet A*. 2018;176(3):638–648. doi:10.1002/ajmg.a.38576
48. Singh M, Kaur M, Grewal AM, et al. Ophthalmic features and management outcomes of 30 children having Goldenhar syndrome. *Int Ophthalmol*. 2020;40(3):667–675. doi:10.1007/s10792-019-01227-0
49. Khan WA, Salim B, Khan AA, Chughtai S. Anaesthetic management in a child with Goldenhar syndrome. *J Coll Physicians Surg Pak*. 2017;27(3):S6–S7. PMID:28302228.
50. Terrazas K, Dixon J, Trainor PA, Dixon MJ. Rare syndromes of the head and face: Mandibulofacial and acrofacial dysostoses. *Wiley Interdiscip Rev Dev Biol*. 2017;6(3):10.1002/wdev.263. doi:10.1002/wdev.263

51. Bukowska-Olech E, Materna-Kiryluk A, Walczak-Sztulpa J, et al. Targeted next-generation sequencing in the diagnosis of facial dysostoses. *Front Genet*. 2020;11:580477. doi:10.3389/fgene.2020.580477
52. Cassina M, Cerqua C, Rossi S, et al. A synonymous splicing mutation in the *SF3B4* gene segregates in a family with highly variable Nager syndrome. *Eur J Hum Genet*. 2017;25(3):371–375. doi:10.1038/ejhg.2016.176
53. Maharana SK, Saint-Jeannet JP. Molecular mechanisms of hearing loss in Nager syndrome. *Dev Biol*. 2021;476:200–208. doi:10.1016/j.ydbio.2021.04.002
54. Fang JX, Uchiumi T, Yagi M, et al. Protein instability and functional defects caused by mutations of dihydro-orotate dehydrogenase in Miller syndrome patients. *Biosci Rep*. 2012;32(6):631–639. doi:10.1042/BSR20120046
55. Disse SC, Toelle SP, Schroeder S, et al. Epidemiology, clinical features, and use of early supportive measures in PHACE syndrome: A European multinational observational study. *Neuroepidemiology*. 2020;54(5):383–391. doi:10.1159/000508187
56. Rotter A, Samorano LP, Rivitti-Machado MC, Oliveira ZNP, Gontijo B. PHACE syndrome: Clinical manifestations, diagnostic criteria, and management. *An Bras Dermatol*. 2018;93(3):405–411. doi:10.1590/abd1806-4841.20187693
57. Bongsebandhu-Phubhakdi C, Tempark T, Supornsilchai V. Endocrine manifestations of PHACE syndrome. *J Pediatr Endocrinol Metab*. 2019;32(8):797–802. doi:10.1515/jpem-2019-0126
58. Heyer GL. PHACE(S) syndrome. *Handb Clin Neurol*. 2015;132:169–183. doi:10.1016/B978-0-444-62702-5.00012-3
59. Schultz KP, Dong E, Truong TA, Maricevich RS. Parry Romberg syndrome. *Clin Plast Surg*. 2019;46(2):231–237. doi:10.1016/j.cps.2018.11.007
60. Arif T, Fatima R, Sami M. Parry-Romberg syndrome: A mini review. *Acta Dermatovenerol Alp Pannonica Adriat*. 2020;29(4):193–199. PMID:33348939.
61. Khanduri S, Singh A, Agrawal S, Katyal G. Parry Romberg syndrome. *Indian J Otolaryngol Head Neck Surg*. 2019;71(Suppl 1):42–44. doi:10.1007/s12070-016-0981-5
62. Liapakis IE, Tzouganakis AC, Paschalis EI, et al. Parry-Romberg syndrome treatment with fat transfer and a new bleaching formula. *J Cosmet Dermatol*. 2019;18(5):1424–1429. doi:10.1111/jocd.12819
63. Unnithan AKA, De Jesus O. *Plagiocephaly*. Treasure Island, FL: StatPearls Publishing; 2023. PMID:33232004.
64. Kajdic N, Spazzapan P, Velnar T. Craniosynostosis – recognition, clinical characteristics, and treatment. *Bosn J Basic Med Sci*. 2018;18(2):110–116. doi:10.17305/bjbms.2017.2083
65. Jung BK, Yun IS. Diagnosis and treatment of positional plagiocephaly. *Arch Craniofac Surg*. 2020;21(2):80–86. doi:10.7181/acfs.2020.00059
66. Martiniuk AL, Vujovich-Dunn C, Park M, Yu W, Lucas BR. Plagiocephaly and developmental delay: A systematic review. *J Dev Behav Pediatr*. 2017;38(1):67–78. doi:10.1097/DBP.0000000000000376
67. Patel K, Evans H, Sommaruga S, et al. Characteristics and management of pain in patients with Klippel-Feil syndrome: Analysis of a global patient-reported registry. *J Neurosurg Spine*. 2019:32(4):578–583. doi:10.3171/2019.9.SPINE19820
68. Leggio A, Gallieni M, Puzo P, Introna F, Sablone S. Klippel–Feil syndrome: The curious case of the skeleton of a young slavic soldier who died in 1946. *Forensic Sci*. 2022;2(1):155–162. doi:10.3390/forensicsci2010012
69. Paradowska A, Szelag J, Sławecki K. Klippel-Feil syndrome – review of the literature. *Dent Med Probl*. 2007;44(4):491–494. https://www.dbc.wroc.pl/Content/2169/PDF/x11-parad.pdf. Accessed July 21, 2022.
70. Agarwal AK, Goel M, Bajpai J, Shukla S, Sachdeva N. Klippel Feil syndrome: A rare case report. *J Orthop Case Rep*. 2014;4(3):53–55. doi:10.13107/jocr.2250-0685.197
71. Gunderson CH, Greenspan RH, Glaser GH, Lubs HA. The Klippel-Feil syndrome: Genetic and clinical reevaluation of cervical fusion. *Medicine (Baltimore)*. 1967;46(6):491–512. doi:10.1097/00005792-196711000-00003
72. Tracy MR, Dormans JP, Kusumi K. Klippel-Feil syndrome: Clinical features and current understanding of etiology. *Clin Orthop Relat Res*. 2004;(424):183–190. PMID:15241163.
73. Nagib MG, Maxwell RE, Chou SN. Identification and management of high-risk patients with Klippel-Feil syndrome. *J Neurosurg*. 1984;61(3):523–530. doi:10.3171/jns.1984.61.3.0523
74. Tofangchiha M, Esfehani M, Eftetahi L, Mirzadeh M, Reda R, Testarelli L. Comparison of the pharyngeal airway in snoring and non-snoring patients based on the lateral cephalometric study: A case–control study. *Dent Med Probl*. 2023;60(1):121–126. doi:10.17219/dmp/154776
75. Tahmasbi S, Seifi M, Soleymani AA, Mohamadian F, Alam M. Comparative study of changes in the airway dimensions following the treatment of Class II malocclusion patients with the twin-block and Seifi appliances. *Dent Med Probl*. 2023;60(2):247–254. doi:10.17219/dmp/142292
76. Dastan F, Ghaffari H, Hamidi Shishvan H, Zareiyan M, Akhlaghian M, Shahab S. Correlation between the upper airway volume and the hyoid bone position, palatal depth, nasal septum deviation, and concha bullosa in different types of malocclusion: A retrospective cone-beam computed tomography study. *Dent Med Probl*. 2021;58(4):509–514. doi:10.17219/dmp/130099
77. Smardz J, Wieckiewicz M, Wojakowska A, et al. Incidence of sleep bruxism in different phenotypes of obstructive sleep apnea. *J Clin Med*. 2022;11(14):4091. doi:10.3390/jcm11144091
78. Pihut M, Gorecka M, Ceranowicz P, Wieckiewicz M. The efficiency of anterior repositioning splints in the management of pain related to temporomandibular joint disc displacement with reduction. *Pain Res Manag*. 2018;2018:9089286. doi:10.1155/2018/9089286
79. Minervini G, Lucchese A, Perillo L, Serpico R, Minervini G. Unilateral superior condylar neck fracture with dislocation in a child treated with an acrylic splint in the upper arch for functional repositioning of the mandible. *Cranio*. 2017;35(5):337–341. doi:10.1080/08869634.2016.1203560
80. Paradowska-Stolarz A, Wezgowiec J, Malysa A, Wieckiewicz M. Effects of polishing and artificial aging on mechanical properties of Dental LT Clear® resin. *J Funct Biomater*. 2023;14(6):295. doi:10.3390/jfb14060295
81. Minervini G, Russo D, Herford AS, et al. Teledentistry in the management of patients with dental and temporomandibular disorders. *Biomed Res Int*. 2022;2022:7091153. doi:10.1155/2022/7091153
82. Boening K, Wieckiewicz M, Paradowska-Stolarz A, Wiland P, Shiau YY. Temporomandibular disorders and oral parafunctions: Mechanism, diagnostics, and therapy. *Biomed Res Int*. 2015;2015:354759. doi:10.1155/2015/354759
83. Minervini G, Fiorillo L, Russo D, et al. Prosthodontic treatment in patients with temporomandibular disorders and orofacial pain and/or bruxism: A review of the literature. *Prosthesis*. 2022;4(2):253–262. doi:10.3390/prosthesis4020025

Review

Advancements in alveolar bone reconstruction: A systematic review of bone block utilization in dental practice

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Abstract

Alveolar reconstructive surgery employs a variety of surgical techniques and biomaterials, with a particular focus on bone blocks as a crucial methodology for restoring and augmenting deficient bone structures. Bone blocks are often employed to support periodontal health or as a foundation for future prosthetic rehabilitation with dental implants. This systematic review investigated recent advances in bone blocks for alveolar bone reconstruction, comparing autologous, allogeneic and xenogeneic types. A search of PubMed identified 56 records, of which 21 were included in the qualitative analysis. The studies involved 685 patients in total. Bone blocks are pivotal for three-dimensional bone regeneration, providing a stable scaffold for achieving the desired bone volume during healing. Autologous bone, harvested from the patient, boasts high biocompatibility, excellent osteogenic properties and minimal immunologic risks. However, its drawbacks include the need for an additional surgical site and extended procedural times. Allogeneic bone blocks involve transferring bone between individuals, offering increased graft availability and customization options without requiring a second surgical site. However, they exhibit moderate resorption rates and carry a heightened risk of immunologic reactions and disease transmission. Innovative techniques, such as tunneling, laser osteotomy, graft customization, and platelet-rich fibrin (PRF) application on wound during surgical treatment show promise in enhancing alveolar bone reconstruction efficacy. In conclusion, despite the traditional preference for autologous bone, the review suggests that alternative materials, particularly individualized allogeneic bone blocks, coupled with modern techniques, could emerge as a standard procedure for regenerating alveolar bone defects due to their satisfactory results and potential advantages.

Keywords: alveolar bone loss, allografts, bone regeneration, bone block, reconstruction

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Introduction

The jawbones, including the mandible and maxilla, may be affected by a number of conditions. Such conditions can be extensive, as in the case of trauma or infection, or localized, as is the case with tumors and cysts. Iatrogenic defects may develop as a consequence of applied treatments, such as radiotherapy of malignant lesions.[1] The jawbones can be affected in the course of chronic and general diseases, including osteoporosis and osteomyelitis. Congenital causes include developmental anomalies that may impact the normal growth and formation of the jawbones.[2] The frequency of jawbone-related issues in clinical dental practice varies based on patient demographics, oral hygiene practices and general health. The prevalence of bone defects is considerable, reaching 91%, and underscores the significance of research in the field of alveolar bone reconstruction.[3] Treatment of bone defects is also differentiated. It may include surgical augmentations to protect against additional bone loss and to secure the capacity for future implantation.[2]

Bone blocks play a key role in alveolar bone reconstruction, providing a reliable methodology for the restoration and augmentation of deficient bone structures.[4] Bone blocks have gained significant attention and popularity due to their ability to overcome donor site morbidity and achieve high survival rates. Regenerated tissues play a crucial role in achieving stable, long-term implant rehabilitation, enhancing bone remodeling, and minimizing factors such as early marginal bone loss and inflammation.[5,6] Different regenerative methods can be used for alveolar ridge or bone reconstructions, ranging from minor augmentations with bone or bone substitute particles to extensive reconstructions with microsurgical free flaps.[7,8] A variety of surgical techniques and biomaterials are employed in alveolar reconstructive surgery.[9] The majority of augmentations are performed to maintain the condition of the periodontium (e.g., augmentation of periodontal defects, guided bone regeneration) or to prepare a future prosthetic base for rehabilitation with the use of dental implants.[8] In cases of severe sagittal discrepancies between the maxilla and mandible resulting in bone defects, orthodontic treatment alone may not be sufficient.[10] Reconstructing the vertical dimensions of the teeth area in patients with preserved dentition to prevent progressive loss of tooth support structures poses its own set of challenges.[11] A multidisciplinary approach, involving a surgical, orthodontic and periodontal team is essential for the customized treatment of such cases apart from the application of standard treatment methods. When selecting a surgical technique and material, several factors should be considered. These include the location and size of the defect, the properties of the biomaterial and the ease of obtaining it. Additionally, cost, ease of use, stability, and maintenance of the recipient site are crucial considerations. It is important to be aware of the potential complications associated with selecting a specific treatment method. Moreover, ssessing the long-term effects of the chosen surgical approach and biomaterial is essential. A comprehensive evaluation of these factors is necessary to make an informed decision in clinical practice.

The regeneration of vertical bone defects caused by periodontitis is typically straightforward and tends to yield predictable outcomes. Similarly, augmenting a post-extraction socket or addressing a small defect after removing an osteolytic lesion is not demanding.[12,13] However, addressing advanced three-dimensional defects poses a significant challenge. Horizontal regeneration in such cases is frequently unpredictable, and attempts at vertical reconstruction often result in less satisfactory outcomes.[10] The process of three-dimensional bone regeneration relies on establishing a stable scaffold to achieve the desired bone volume during the healing phase.[14] Clinicians commonly employ barrier membranes, including stiffer, non-absorbable, personalized membranes[9] or the increasingly popular bone blocks for this purpose. The implementation of these techniques involves the use of pins or mesh for fixation, and in many instances, biomaterial granules are applied to fill the voids.[15,16]

Bone blocks have a long history of use, and the existing literature contains numerous reports detailing their indications, methods of use and effects.[17,18] These involve the use of various graft types, including autologous, allogeneic, xenogeneic, and synthetic bone substitutes. These grafts act as scaffolds for new bone formation, promoting osteoinduction, osteoconduction and osteogenesis to restore natural bone structures.[19] The biomaterial market has experienced significant growth, offering surgeons a range of bone substitutes with similar properties. Despite the potential to choose and combine these substitutes for effective reconstructions with minimal morbidity and rapid healing, variations exist among the most commonly used substitutes in terms of their chemical, physical and morphological features.[20] While undoubtedly serving as an excellent scaffold for bone reconstruction, the selection of this reconstructive technique should consider factors such as the choice of biomaterial and other potential aspects to enhance the treatment process and achieve the best possible outcome.[10] This systematic review aimed to investigate recent advances in the use of bone blocks in oral surgery. Qualitative data synthesis was used to compare different types of bone blocks: autologous, allogeneic and xenogeneic. Furthermore, the objective was to explore contemporary methods designed to enhance the effectiveness of these procedures.

Material and methods

A systematic search was conducted in PubMed, and a manual search of review papers identified during the search was also performed. The search was conducted in accordance with the Preferred Reporting Items for

Systematic Reviews and Meta-Analyses (PRISMA) statement,[21] on November 17, 2023. The electronic search was constructed using the Medical Subject Headings (MeSH) term "Alveolar Bone Loss/Surgery" and the text word "bone block". The search was limited to studies involving adult participants and articles published in English. However, no restrictions were imposed on geographical scope. The inclusion and exclusion criteria were developed in accordance with the Population, Intervention, Comparison, Outcomes, and Study Design (PICOS) framework.[22] All eligibility criteria are outlined in Table 1.

Two reviewers were involved in the screening process, and any discrepancies were resolved through mutual agreement. In cases where a consensus could not be reached, a third independent reviewer was consulted to make the final decision. The data extraction process was carried out by a single reviewer, and then cross-verified by a second reviewer.

Results

Characteristics of patients and study procedure

The PubMed search identified 56 records, of which 21 were included in the qualitative analysis. The studies were relatively small, with sample sizes ranging from 8 to 101 participants, and involved a total of 685 patients. The majority of studies (n = 9) were conducted in Italy. Two studies each originated from Brazil, China, Sweden, and Israel, while 1 study each came from Spain, Portugal, Egypt, and the Netherlands. The study selection process is presented in Fig. 1.

The studies were categorized according to the type of bone biomaterial into autologous,[23–38] allogeneic[39–41] and xenogeneic[26,31,33,34,42] groups to facilitate the description of each type. It should be noted that a single study may investigate more than 1 type, which allows for a comprehensive description of each. Figure 2 depicts the characteristics of different types of bone blocks.

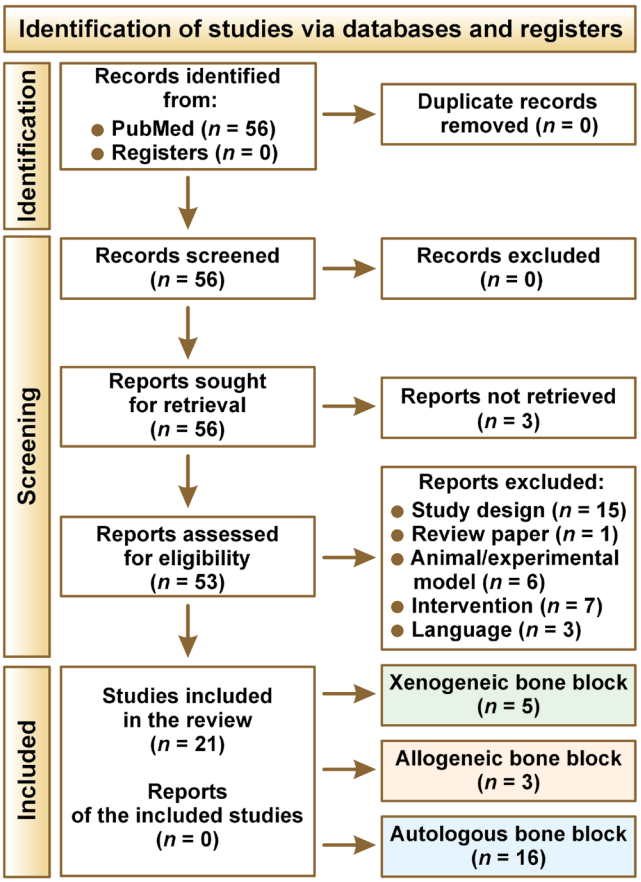

Fig. 1. Flow chart of the study in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[21]

Autologous bone blocks

Autologous bone refers to bone tissue harvested from the same patient. The procedure for bone reconstruction using autologous bone blocks involves harvesting the block, shaping or fitting it, and placing and fixing it in the defect in a single operation. Autologous grafts for the reconstruction of the jawbones can be obtained from intraoral or extraoral donor sites.[2,27] Intraoral sites are considered more suitable for graft harvesting due to the absence of scarring on the skin and reduced graft resorption, attributed to the similarity in embryological origin and microarchitecture.

Table 1. Eligibility criteria according to the Population, Intervention, Comparison, Outcomes, and Study Design (PICOS) framework[22]

PICOS	Inclusion criteria	Exclusion criteria
Population	– otherwise healthy individuals with alveolar bone deficiency that does not allow placement of dental implants – adults (≥18 years of age)	– patients with chronic diseases
Intervention	alveolar ridge augmentation/reconstruction with a bone block graft	–
Comparison	different types of bone block grafts with the focus on the source of the graft material, i.e., autologous, allogeneic and xenogeneic grafts	–
Outcomes	– efficacy outcomes – safety outcomes	–
Study design	– comparative study – cohort study	– experimental/animal study – case report – proof-of-concept study – review

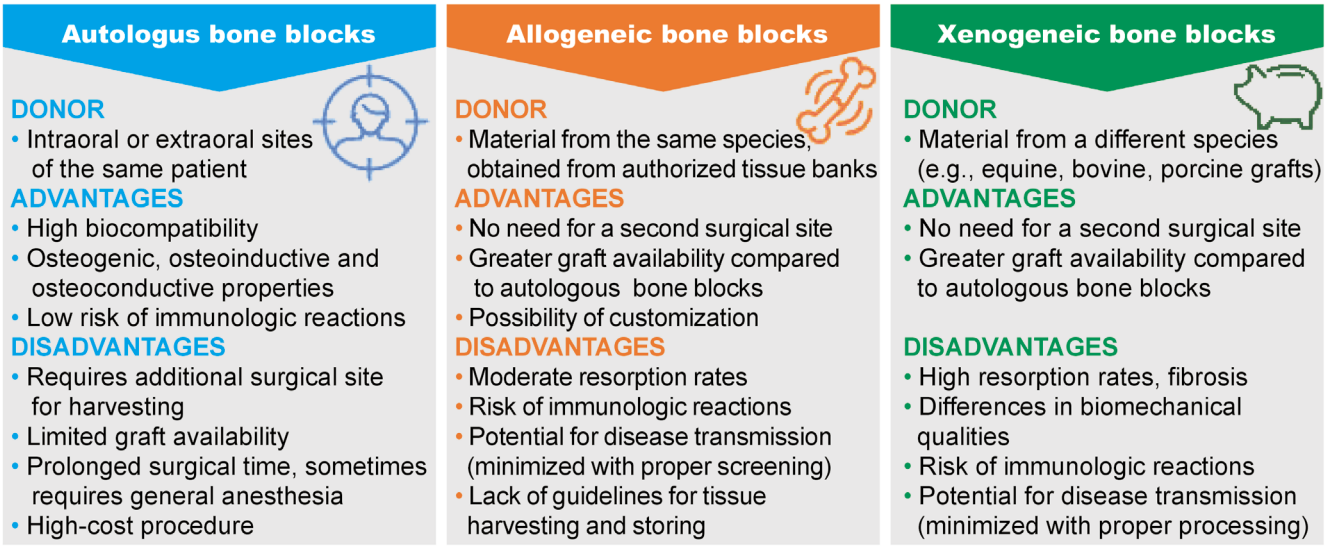

Fig. 2. Characteristics of bone blocks according to the biomaterial type

With regard to extraoral donor sites, bone can be harvested from the calvarium, anterior iliac crest, tibia, fibula, rib, and olecranon (proximal ulna).[26,28,30–33,35] Intraoral donor sites encompass the mandible (chin/symphysis, ramus, retromolar area), zygoma and maxilla (tuberosity).[23–25,29,34,36–38,43,44] With regard to one of the most common donor sites, the mandible, the utilization of the ramus as a donor site, as opposed to the symphysis, has been associated with several advantages. These include increased postoperative comfort and a lower risk of paresthesias, pulp necrosis of the inferior incisors, and labial ptosis.

The key factors for the successful incorporation of a bone block include the preparation of the recipient site and the effective adaptation of the bone blocks.[31] Autologous bone blocks are typically harvested subperiosteally with sufficient visualization of the donor site. This involves identifying crucial nearby anatomic structures, particularly neurovascular bundles and dental roots, and the implementation of adequate protection measures for these structures, in conjunction with the surrounding soft tissue. Subsequently, the osteotomy process, which involves cutting the bone, is performed, followed by the release of the graft. In order to ensure proper contouring of the graft, its size should be 2 mm larger than the size of the defect. Graft osteotomies are commonly executed using saw disks. The osteotomies are then connected, and the graft is elevated using a chisel and hammer.[38] Subsequently, the block must be shaped and contoured under abundant irrigation. Round fissure burs are commonly employed for this purpose, facilitating the removal of all sharp edges. The prepared graft is then stored in a cold, sterile aqueous solution of 0.9% sodium chloride until the recipient site is ready. It is crucial to consistently monitor the fit of the block and its adhesion to the bone surface at the recipient site. If needed, reshaping may be necessary. The adapted block is secured with bicortical titanium screws, which are applied using either a hammer or a screwdriver.[37,40] An alternative method involves the use of resorbable pins in the BoneWelding® technique. In this method, a resorbable pin is applied using ultrasound and heating during insertion into the drill hole. The pin penetrates the drill hole and subsequently melts laterally into the spongy bone structures beneath the cortical bone layer.[45]

Advantages and disadvantages of autologous bone blocks

The primary advantage of autologous bone blocks is a diminished risk of immune rejection, as the graft material originates from the same individual. This results in the graft material possessing good osteogenic, osteoinductive and osteoconductive properties.[2] Autologous bone blocks are considered a safe and reliable method, offering good long-term stability with minimal resorption and donor-site morbidity. The vital properties and the ability of the bone block to function as a scaffold for neoangiogenesis and tissue ingrowth, in addition to providing immediate mechanical stability, contribute to the smooth incorporation, healing and success of the bone graft.[29,34]

Autologous block grafts, sourced from the patient's own bone, typically exhibit lower resorption rates in comparison to allogeneic and xenogeneic grafts.[26] However, it is important to note that higher rates of resorption have been observed with autologous bone blocks derived from the iliac crest.[33] Nevertheless, some researchers have reported similar rates of volume gain regardless of the donor site.[36] Harvesting autologous bone carries a certain risk of donor site morbidity, which is applicable to both extraoral and intraoral donor sites. The complication rates are comparable across different donor sites, and in the majority of patients, healing proceeds uneventfully.[28] While patients generally experience infrequent and minor side effects, they can be as high as 35.7% for calvarial grafts and 33.3% for iliac crest grafts.[46] Bone harvesting from an intraoral site may lead to numbness of the teeth,

neurosensory disturbances, postoperative discomfort, and aesthetic issues such as contour changes and soft tissue recession. On the other hand, bone harvesting from an extraoral site is associated with a number of complications, including scarring, postoperative pain, hematomas, delayed muscle motility, the risk of cutaneous nerve injury, and higher hospitalization costs.[28,47–49] Furthermore, general anesthesia, particularly when grafts are harvested from calvarial donor sites,[30,32] may result in increased stress for patients, leading to increased postoperative pain and an extended hospital stay.[42]

Differences between cancellous and cortical autografts should be considered in the decision-making process for managing bone augmentation. The process of molding can pose a challenge when using autologous bone grafts, particularly with cortical autografts, which are less vascularized and more rigid. This characteristic increases the risk of cracking or fracturing the bone graft. However, this risk can be mitigated by the use of custom-made guides.[30]

Allogeneic bone blocks

Allogeneic bone blocks entail the transfer of bone from one individual to another, which involves an exchange of genetic material between different people. Allografts contain numerous chemical domains, endothelial cells and growth factors within the bone matrix released during resorption by osteoclasts. Additionally, allograft bone contains a small amount of bone morphogenic protein with osteoinductive properties.[50] As demonstrated by scanning electron microscopy (SEM), the morphology of the material surface can vary depending on the biobank. Materials sourced from cancellous bone exhibit a spongy structure with holes ranging from 100 to 350 μm in diameter. The surface is smooth, without collagen fibers. In the material sourced from cortical bone, small osteocyte canaliculi holes with an average diameter of 38 μm occur. The bone surface surrounding these holes is smooth, predominantly consisting of strongly bonded collagen fibers, with microcracks and layered particles across the entire surface.[20] The potential for antigenicity in allografts may not be entirely eliminated, as the formation of alloantibodies can complicate bone transplantation. Nevertheless, the quantification of major histocompatibility complex (MHC) molecules in various allogeneic bone grafting materials for alveolar ridge reconstruction revealed trace amounts of MHC molecules. These quantities are considered clinically irrelevant, and there is no evidence of late complications or rejections in clinical practice.[51]

Despite the relatively low risk of antigenicity and potential disease transmission, the significance of allografts increases due to constraints in the size of autologous block grafts from intraoral and extraoral sites. The associated morbidity with graft harvesting often restricts the range of treatment options and may influence patient acceptance.[41,50,52]

In terms of the efficacy of allogeneic bone blocks, while autologous bone block grafts are considered the gold standard in oral surgery, bone substitutes like bone allografts demonstrate comparable effectiveness. There were no significant differences observed in the rate of bone formation between allogeneic materials and autologous bone in maxillary sinus lift procedures.[53] Stability for subsequent fixed prosthetic rehabilitation was ensured when utilizing fresh-frozen iliac crest allografts for augmenting the atrophic maxilla. In addition, allogeneic bone grafts exhibited low resorption rates at 5 months.[41] The maintenance of consistent histological, histomorphometric and immunohistochemical features, along with the preservation of good vascularization, was observed in several studies.[39–41] Finally, allografts represent the optimal choice in terms of safety, as the use of allogeneic bone blocks eliminates donor site morbidity and allows for the acquisition of bone material from tissue banks.[41]

Xenogeneic bone blocks

Bones from various animal species, known as xenogeneic grafts, have been explored as an alternative to allografts due to the financial implications associated with the latter. However, they are used infrequently due to high immunogenicity, inadequate biomechanical qualities and the occurrence of foreign body reactions.[50] In contrast to human bone, the SEM images of animal-bone-derived material reveal a rough surface characterized by statically aggregated particles arranged in a two-stage structure. The first stage comprises particles with an average diameter of 0.353 μm, while the second stage involves larger particles with an average size of 1.395 μm. The material displays particle holes and pores, which increases its overall surface area.[20]

Xenogeneic bone blocks exhibit lower efficiency than other types of bone blocks. In an experimental model, after a 6-month healing period, the alveolar ridge was integrated into the target area.[54] However, significant peripheral resorption was observed, resulting in approx. 30% height and 50% length replacement with connective tissue. Furthermore, grafts containing a cancellous bovine bone mineral scaffold maintained their dimensions, with only moderate new bone formation observed at the graft base.[54] However, some researchers have reported favorable outcomes with the use of xenogeneic bone blocks. In a study involving 20 subjects, the success rate of the interpositional technique using cancellous equine bone blocks appeared to be higher than that of autologous onlay blocks, with an overall success rate of 93.8% for the interpositional technique compared to 82.4% for the onlay technique.[26] In another small study involving 15 patients with single or multiple tooth gaps and severe horizontal collapse of the alveolar ridge, a novel collagenated xenogeneic bone block demonstrated substantial gains in horizontal crestal width. However, this approach was associated with an increased risk of soft tissue dehiscence and early implant loss.[55]

Discussion

Grafting bone blocks is a novel technique with a limited number of large-scale studies. In our review, we identified several proof-of-concept studies and case reports. These preliminary investigations were designed to demonstrate the feasibility and viability of specific methods of block bone grafting and provide evidence that such methodologies are safe and effective in alveolar bone augmentation. Such studies are frequently conducted in the early stages of research to assess the potential efficacy of the treatment of bone defects. Furthermore, case reports were equally prevalent, indicating that considerable research is currently in the pilot stages of bone grafting. Our systematic review was intentionally focused, with the search limited to a single MeSH term. We aimed to identify a comprehensive range of alveolar bone reconstruction methodologies over time while limiting the inclusion of papers that repeatedly evaluated similar approaches.

The feasibility and safety demonstrated in the preliminary investigations of grafting bone blocks from various sources suggest potential advancements in alveolar bone augmentation, with significant implications for clinical practice. It is recommended that clinicians adopt a cautious approach to these emerging technologies, anticipating further research to establish their efficacy and broader applicability in routine clinical settings. Alongside advancements in bone grafting, new techniques are being developed to enhance their effectiveness. This review will discuss tunneling techniques, Er:YAG laser osteotomy, customization, and the supplementary use of platelet-rich fibrin (PRF). The ease of implementation and benefits for patients will be highlighted. These techniques were partially employed by the authors of the identified studies.

Tunneling techniques

Tunneling techniques have been used to increase the effectiveness of bone augmentation procedures conducted with diverse bone sources. This approach minimizes the necessity for extensive soft tissue reflection, potentially reducing surgical trauma and promoting faster healing. The technique involves creating a tunnel or channel in the recipient site's bone without fully exposing it, and then passing the bone graft material through this tunnel to the desired location.[29] The data suggests that employing a tunneling technique enhances bone formation in the context of xenogeneic bone block placement for vertical ridge augmentation. A study comparing flap and tunneling procedures for vertical ridge augmentation using xenogeneic bone blocks in a canine mandible model revealed that the tunneling group exhibited significantly greater new bone formation within the graft sites (46.6 ±23.4%) compared to the flap group (15.3 ±6.6%).[56] In clinical settings, the management of alveolar crest vertical defects in 10 patients using the tunneling technique and autologous bone blocks before the implant resulted in all individuals healing without complications. The study demonstrated a mean overall vertical bone remodeling of 0.55 ±0.49 mm (8.4%) after 8 months, thereby confirming the efficacy of this minimally invasive approach for bone regeneration in vertical defects.[29]

Er:YAG laser osteotomy

In the regeneration of alveolar bone using autologous bone blocks, the harvesting technique is of paramount importance. Inappropriate osteotomy techniques may result in mechanical and thermal damage, impacting the bone's vital potential. While standard methods involving saws, drills and burs are associated with disadvantages such as a limited cut geometry and a risk of soft tissue injury, laser ablation presents advantages like unconstrained positioning, allowing for precise osteotomy without mechanical pressure or stress on the bone. The potential benefits of laser ablation in overcoming limitations associated with traditional osteotomy methods in oral surgery translate into improved efficiency in clinical practice.[57] A pilot study evaluated the feasibility, benefits and limitations of using a variable square pulse Er:YAG laser for harvesting intraoral bone grafts. The results demonstrated excellent cutting efficiency with minimal damage to adjacent soft tissues and no impairment of wound healing. However, limitations, such as the difficulty in achieving a well-defined osteotomy line without irregularities and the necessity for careful laser beam positioning, suggest that the use of an Er:YAG laser may be most appropriate for regions where safe and fixed guidance of the laser beam is feasible. A meta-analysis was conducted to evaluate complications and donor site morbidity, which confirmed the growing utilization of Er:YAG lasers. Patients expressed satisfaction with the graft harvesting method, with higher acceptance reported for procedures involving harvesting from the ascending mandibular ramus.[58]

Customization

In the customization of the bone augmentation procedure for a single-tooth restoration, advanced backward planning can be used, involving preprosthetic bone and soft tissue augmentation. The treatment plan involves manufacturing an allogeneic bone block, which is a collaborative effort between the dentist, the implantologist and the dental laboratory. The optimal implant position and necessary block volume were determined using cone-beam computed tomography (CBCT) data and three-dimensional planning tools. A customized block graft, comprising processed freeze-dried cancellous bone from living donors, was obtained during arthroplasty surgery. The procedure can be supported by soft tissue optimization and tunneling of the recipient gingiva during implantation.[59] In terms of treatment expenses, both the use of stereolithographic models and computer-aided design (CAD) have been shown to improve individualization and increase costs. However, these

additional costs can be balanced by reduced surgery time. It should be noted that while there will be an increase in material expenses, when compared to autologous bone blocks harvested from extraoral donor sites, the overall treatment costs may appear significantly lower. Additionally, the surgical procedure for using customized allogeneic bone blocks might be simpler than trimming and adapting autologous bone blocks.

Platelet-rich fibrin

Autologous PRF is widely utilized in oral surgery. This is a blood-derived material, processed from whole blood containing high platelet and growth factor concentrations.[60] While primarily employed to alleviate pain, reduce edema and expedite healing after tooth extractions,[61] researchers are exploring its potential in reconstructive surgery.[62] Notably, key features of PRF include enhanced healing, improved graft stability, and acting as a natural scaffold, facilitating bone graft integration and improving the condition of adjacent tissues. These properties, coupled with PRF's ability to reduce inflammation, increase vascularization and potentially enhance bone density, make it a promising material for alveolar bone reconstruction and augmentation.[26,63]

Conclusions

Autologous bone has traditionally been considered the gold standard due to its inherent properties. However, the need for a second surgical site, increased discomfort, potential complications, intraoperative shaping, and extended surgical time raise the question of whether alternative materials could offer a better solution. Allogeneic blocks lack osteogenic properties, yet their final treatment results are often satisfactory. Overcoming the drawbacks associated with autologous blocks, such as low patient comfort and prolonged procedure time through modern techniques for individualizing blocks, raises the question of whether individualized allogeneic bone blocks could become the new gold standard.

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.

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References

1. Zehr LJ, Cooper JS. *Mandible Osteoradionecrosis*. Treasure Island, FL: StatPearls Publishing; 2023. PMID:28613667.
2. Bernardi S, Macchiarelli G, Bianchi S. Autologous materials in regenerative dentistry: Harvested bone, platelet concentrates and dentin derivates. *Molecules*. 2020;25(22):5330. doi:10.3390/molecules25225330
3. Dominiak M, Hnitecka S, Olchowy C, Olchowy A, Gedrange T. Analysis of alveolar ridge width in an area of central lower incisor using cone-beam computed tomography in vivo. *Ann Anat*. 2021;236:151699. doi:10.1016/j.aanat.2021.151699
4. Tolstunov L. Surgical algorithm for alveolar bone augmentation in implant dentistry. *Oral Maxillofac Surg Clin North Am*. 2019;31(2):155–161. doi:10.1016/j.coms.2019.01.001
5. Guarnieri R, Reda R, Di Nardo D, Miccoli G, Zanza A, Testarelli L. In vitro direct and indirect cytotoxicity comparative analysis of one pre-hydrated versus one dried acellular porcine dermal matrix. *Materials (Basel)*. 2022;15(5):1937. doi:10.3390/ma15051937
6. Guarnieri R, Reda R, Zanza A, Miccoli G, Nardo DD, Testarelli L. Can peri-implant marginal bone loss progression and a-MMP-8 be considered indicators of the subsequent onset of peri-implantitis? A 5-year study. *Diagnostics (Basel)*. 2022;12(11):2599. doi:10.3390/diagnostics12112599
7. Stahl A, Yang YP. Regenerative approaches for the treatment of large bone defects. *Tissue Eng Part B Rev*. 2021;27(6):539–547. doi:10.1089/ten.TEB.2020.0281
8. Lee J, Park D, Koo KT, Seol YJ, Lee YM. Validity of a regenerative procedure for a minor bone defect with immediate implant placement: A systematic review and meta-analysis. *Acta Odontol Scand*. 2019;77(2):99–106. doi:10.1080/00016357.2018.1508743
9. Tomas M, Čandrlić M, Juzbašić M, et al. Synthetic injectable biomaterials for alveolar bone regeneration in animal and human studies. *Materials (Basel)*. 2021;14(11):2858. doi:10.3390/ma14112858
10. Dominiak M, Dominiak S, Targonska S, Gedrange T. Three-dimensional bone block planning for mandibular sagittal bone defect reconstruction. *J Healthc Eng*. 2020;2020:8829288. doi:10.1155/2020/8829288
11. Dominiak M, Hnitecka S, Olchowy C, Dominiak S, Gedrange T. Possible treatment of severe bone dehiscences based on 3D bone reconstruction – A description of treatment methodology. *Appl Sci*. 2021;11(21):10299. doi:10.3390/app112110299
12. Baljoon M, Natto S, Bergstrom J. Occurrence of vertical bone defects in dentally aware individuals. *Acta Odontol Scand*. 2003;61(1):47–51. doi:10.1080/ode.61.1.47.51
13. El-Sioufi I, Oikonomou I, Koletsi D, Bobetsis YA, Madianos PN, Vassilopoulos S. Clinical evaluation of different alveolar ridge preservation techniques after tooth extraction: A randomized clinical trial. *Clin Oral Investig*. 2023;27(8):4471–4480. doi:10.1007/s00784-023-05068-1
14. Chinnasami H, Dey MK, Devireddy R. Three-dimensional scaffolds for bone tissue engineering. *Bioengineering (Basel)*. 2023;10(7):759. doi:10.3390/bioengineering10070759
15. Kurtiş B, Şahin S, Gürbüz S, et al. Vertical bone augmentation with customized CAD/CAM titanium mesh for severe alveolar ridge defect in the posterior mandible: A case letter. *J Oral Implantol*. 2023;49(2):147–156. doi:10.1563/aaid-joi-D-22-00094
16. Yazdanpanah Z, Johnston JD, Cooper DML, Chen X. 3D bioprinted scaffolds for bone tissue engineering: State-of-the-art and emerging technologies. *Front Bioeng Biotechnol*. 2022;10:824156. doi:10.3389/fbioe.2022.824156
17. Khoury F, Hanser T. Mandibular bone block harvesting from the retromolar region: A 10-year prospective clinical study. *Int J Oral Maxillofac Implants*. 2015;30(3):688–697. doi:10.11607/jomi.4117

18. Jacotti M, Wang HL, Fu JH, Zamboni G, Bernardello F. Ridge augmentation with mineralized block allografts: Clinical and histological evaluation of 8 cases treated with the 3-dimensional block technique. *Implant Dent*. 2012;21(6):444–448. doi:10.1097/ID.0b013e31826f7a67
19. Dorosz N, Dominiak M. Mandibular ridge reconstruction: A review of contemporary methods. *Adv Clin Exp Med*. 2018;27(8):1159–1168. doi:10.17219/acem/74054
20. Targonska S, Dominiak S, Wiglusz RJ, Dominiak M. Investigation of different types of micro- and nanostructured materials for bone grafting application. *Nanomaterials (Basel)*. 2022;12(21):3752. doi:10.3390/nano12213752
21. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
22. Amir-Behghadami M, Janati A. Population, Intervention, Comparison, Outcomes and Study (PICOS) design as a framework to formulate eligibility criteria in systematic reviews. *Emerg Med J*. 2020;37(6):387. doi:10.1136/emermed-2020-209567
23. Acocella A, Bertolai R, Colafranceschi M, Sacco R. Clinical, histological and histomorphometric evaluation of the healing of mandibular ramus bone block grafts for alveolar ridge augmentation before implant placement. *J Craniomaxillofac Surg*. 2010;38(3):222–230. doi:10.1016/j.jcms.2009.07.004
24. Alérico FA, Bernardes SR, Gasparini Kiatake Fontão FN, Diez GF, Alérico JHS, Claudino M. Prospective tomographic evaluation of autogenous bone resorption harvested from mandibular ramus in atrophic maxilla. *J Craniofac Surg*. 2014;25(6):e543–e546. doi:10.1097/SCS.0000000000001045
25. Anitua E, Murias-Freijo A, Alkhraisat MH. Implant site under-preparation to compensate the remodeling of an autologous bone block graft. *J Craniofac Surg*. 2015;26(5):e374–e377. doi:10.1097/SCS.0000000000001839
26. Barone A, Toti P, Menchini-Fabris GB, Felice P, Marchionni S, Covani U. Early volumetric changes after vertical augmentation of the atrophic posterior mandible with interpositional block graft versus onlay bone graft: A retrospective radiological study. *J Craniomaxillofac Surg*. 2017;45(9):1438–1447. doi:10.1016/j.jcms.2017.01.018
27. Chiapasco M, Abati S, Romeo E, Vogel G. Clinical outcome of autogenous bone blocks or guided bone regeneration with e-PTFE membranes for the reconstruction of narrow edentulous ridges. *Clin Oral Implants Res*. 1999;10(4):278–288. doi:10.1034/j.1600-0501.1999.100404.x
28. Chiapasco M, Tommasato G, Palombo D, Scarnò D, Zaniboni M, Del Fabbro M. Dental implants placed in severely atrophic jaws reconstructed with autogenous calvarium, bovine bone mineral, and collagen membranes: A 3- to 19-year retrospective follow-up study. *Clin Oral Implants Res*. 2018;29(7):725–740. doi:10.1111/clr.13281
29. De Stavola L, Tunkel J. Results of vertical bone augmentation with autogenous bone block grafts and the tunnel technique: A clinical prospective study of 10 consecutively treated patients. *Int J Periodontics Restorative Dent*. 2013;33(5):651–659. doi:10.11607/prd.0932
30. Elhadidi M, Aldahouk A, Shawky M, Elbehairy MS, Atef M, El-Gengehi M. Computer-guided calvarial mono-cortical bone blocks harvest: A novel approach for three-dimensional alveolar reconstruction of atrophic maxilla. *Clin Implant Dent Relat Res*. 2019;21(1):85–93. doi:10.1111/cid.12714
31. Felice P, Soardi E, Pellegrino G, et al. Treatment of the atrophic edentulous maxilla: Short implants versus bone augmentation for placing longer implants. Five-month post-loading results of a pilot randomised controlled trial. *Eur J Oral Implantol*. 2011;4(3):191–202. PMID:22043464.
32. Hallman M, Mordenfeld A, Strandkvist T. A retrospective 5-year follow-up study of two different titanium implant surfaces used after interpositional bone grafting for reconstruction of the atrophic edentulous maxilla. *Clin Implant Dent Relat Res*. 2005;7(3):121–126. doi:10.1111/j.1708-8208.2005.tb00055.x
33. Pistilli R, Felice P, Piatelli M, Nisii A, Barausse C, Esposito M. Blocks of autogenous bone versus xenografts for the rehabilitation of atrophic jaws with dental implants: Preliminary data from a pilot randomised controlled trial. *Eur J Oral Implantol*. 2014;7(2):153–171. PMID:24977250.
34. Qiu L, Yu H. Onlay grafting with bovine bone mineral block for horizontal reconstruction of severely atrophic alveolar ridges in anterior maxillae: A 6-year prospective study. *J Craniomaxillofac Surg*. 2018;46(8):1199–1204. doi:10.1016/j.jcms.2018.04.004
35. Rasmusson L, Thor A, Sennerby L. Stability evaluation of implants integrated in grafted and nongrafted maxillary bone: A clinical study from implant placement to abutment connection. *Clin Implant Dent Relat Res*. 2012;14(1):61–66. doi:10.1111/j.1708-8208.2010.00239.x
36. Schwartz-Arad D, Levin L. Intraoral autogenous block onlay bone grafting for extensive reconstruction of atrophic maxillary alveolar ridges. *J Periodontol*. 2005;76(4):636–641. doi:10.1902/jop.2005.76.4.636
37. Yu H, Chen L, Zhu Y, Qiu L. Bilamina cortical tenting grafting technique for three-dimensional reconstruction of severely atrophic alveolar ridges in anterior maxillae: A 6-year prospective study. *J Craniomaxillofac Surg*. 2016;44(7):868–875. doi:10.1016/j.jcms.2016.04.018
38. Zerbo IR, de Lange GL, Joldersma M, Bronckers ALJJ, Burger EH. Fate of monocortical bone blocks grafted in the human maxilla: A histological and histomorphometric study. *Clin Oral Implants Res*. 2003;14(6):759–766. doi:10.1046/j.0905-7161.2003.00967.x
39. Chaushu G, Mardinger O, Peleg M, Ghelfan O, Nissan J. Analysis of complications following augmentation with cancellous block allografts. *J Periodontol*. 2010;81(12):1759–1764. doi:10.1902/jop.2010.100235
40. Deluiz D, Santos Oliveira L, Ramôa Pires F, et al. Incorporation and remodeling of bone block allografts in the maxillary reconstruction: A randomized clinical trial. *Clin Implant Dent Relat Res*. 2017;19(1):180–194. doi:10.1111/cid.12441
41. Pereira E, Messias A, Dias R, Judas F, Salvoni A, Guerra F. Horizontal resorption of fresh-frozen corticocancellous bone blocks in the reconstruction of the atrophic maxilla at 5 months. *Clin Implant Dent Relat Res*. 2015;17 Suppl 2(Suppl 2):e444–e458. doi:10.1111/cid.12268
42. Pistilli R, Felice P, Piattelli M, et al. Posterior atrophic jaws rehabilitated with prostheses supported by 5 × 5 mm implants with a novel nanostructured calcium-incorporated titanium surface or by longer implants in augmented bone. One-year results from a randomised controlled trial. *Eur J Oral Implantol*. 2013;6(4):343–357. PMID:24570980.
43. Reininger D, Cobo-Vázquez C, Rosenberg B, López-Quiles J. Alternative intraoral donor sites to the chin and mandibular body-ramus. *J Clin Exp Dent*. 2017;9(12):e1474–e1481. doi:10.4317/jced.54372
44. Kim YK, Ku JK. Ridge augmentation in implant dentistry. *J Korean Assoc Oral Maxillofac Surg*. 2020;46(3):211–217. doi:10.5125/jkaoms.2020.46.3.211
45. Gareb B, Roossien CC, van Bakelen NB, et al. Comparison of the mechanical properties of biodegradable and titanium osteosynthesis systems used in oral and maxillofacial surgery. *Sci Rep*. 2020;10(1):18143. doi:10.1038/s41598-020-75299-9
46. Mertens C, Decker C, Seeberger R, Hoffmann J, Sander A, Freier K. Early bone resorption after vertical bone augmentation – A comparison of calvarial and iliac grafts. *Clin Oral Implants Res*. 2013;24(7):820–825. doi:10.1111/j.1600-0501.2012.02463.x
47. Sanz-Sánchez I, Sanz-Martín I, Ortiz-Vigón A, Molina A, Sanz M. Complications in bone-grafting procedures: Classification and management. *Periodontol 2000*. 2022;88(1):86–102. doi:10.1111/prd.12413
48. Friberg B. Bone augmentation for single tooth implants: A review of the literature. *Eur J Oral Implantol*. 2016;9 Suppl 1:S123–S134. PMID:27314117.
49. Schaaf H, Lendeckel S, Howaldt HP, Streckbein P. Donor site morbidity after bone harvesting from the anterior iliac crest. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;109(1):52–58. doi:10.1016/j.tripleo.2009.08.023
50. Dinçel YM. Bone Graft Types. In: Kummoona R, ed. *Bone Grafting – Recent Advances with Special References to Cranio-Maxillofacial Surgery*. Rijeka, Croatia: IntechOpen; 2018. doi:10.5772/intechopen.79449
51. Solakoglu Ö, Götz W, Heydecke G, Schwarzenbach H. Histological and immunohistochemical comparison of two different allogeneic bone grafting materials for alveolar ridge reconstruction: A prospective randomized trial in humans. *Clin Implant Dent Relat Res*. 2019;21(5):1002–1016. doi:10.1111/cid.12824

52. Kouhestani F, Rad MR, Mohaghegh S, Motamedian SR. Effect of metformin on the behavior of dental pulp stem cells cultured on freeze-dried bone allografts. *Dent Med Probl*. 2021;58(3):343–349. doi:10.17219/dmp/131988
53. Schmitt CM, Doering H, Schmidt T, Lutz R, Neukam FW, Schlegel KA. Histological results after maxillary sinus augmentation with Straumann® BoneCeramic, Bio-Oss®, Puros®, and autologous bone. A randomized controlled clinical trial. *Clin Oral Implants Res*. 2013;24(5):576–585. doi:10.1111/j.1600-0501.2012.02431.x
54. Araújo MG, Sonohara M, Hayacibara R, Cardaropoli G, Lindhe J. Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. *J Clin Periodontol*. 2002;29(12):1122–1131. doi:10.1034/j.1600-051x.2002.291213.x
55. Ortiz-Vigón A, Suarez I, Martínez-Villa S, Sanz-Martín I, Bollain J, Sanz M. Safety and performance of a novel collagenated xenogeneic bone block for lateral alveolar crest augmentation for staged implant placement. *Clin Oral Implants Res*. 2018;29(1):36–45. doi:10.1111/clr.13036
56. Xuan F, Lee CU, Son JS, Fang Y, Jeong SM, Choi BH. Vertical ridge augmentation using xenogenous bone blocks: A comparison between the flap and tunneling procedures. *J Oral Maxillofac Surg*. 2014;72(9):1660–1670. doi:10.1016/j.joms.2014.04.003
57. Stübinger S, Nuss K, Landes C, von Rechenberg B, Sader R. Harvesting of intraoral autogenous block grafts from the chin and ramus region: Preliminary results with a variable square pulse Er:YAG laser. *Lasers Surg Med*. 2008;40(5):312–318. doi:10.1002/lsm.20639
58. Starch-Jensen T, Deluiz D, Deb S, Bruun NH, Tinoco EMB. Harvesting of autogenous bone graft from the ascending mandibular ramus compared with the chin region: A systematic review and meta-analysis focusing on complications and donor site morbidity. *J Oral Maxillofac Res*. 2020;11(3):e1. doi:10.5037/jomr.2020.11301
59. Nilius M, Nilius MH, Mueller C, et al. Customized allogeneic bone augmentation improves esthetic outcome in anteromaxillary dental implantation. *Case Rep Dent*. 2022;2022:6943930. doi:10.1155/2022/6943930
60. Egierska D, Perszke M, Mazur M, Duś-Ilnicka I. Platelet-rich plasma and platelet-rich fibrin in oral surgery: A narrative review. *Dent Med Probl*. 2023;60(1):177–186. doi:10.17219/dmp/147298
61. Ritto FG, Pimentel T, Canellas JVS, Junger B, Cruz M, Medeiros PJ. Randomized double-blind clinical trial evaluation of bone healing after third molar surgery with the use of leukocyte- and platelet-rich fibrin. *Int J Oral Maxillofac Surg*. 2019;48(8):1088–1093. doi:10.1016/j.ijom.2019.01.020
62. Simonpieri A, Del Corso M, Vervelle A, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 2: Bone graft, implant and reconstructive surgery. *Curr Pharm Biotechnol*. 2012;13(7):1231–1256. doi:10.2174/138920112800624472
63. Bhattacharya HS, Gummaluri SS, Rani A, Verma S, Bhattacharya P, Rayashettypura Gurushanth SM. Additional benefits of titanium platelet-rich fibrin (T-PRF) with a coronally advanced flap (CAF) for recession coverage: A case series. *Dent Med Probl*. 2023;60(2):279–285. doi:10.17219/dmp/141919

Review

Studies on the content of toxic metals in teeth: A narrative review of literature

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Abstract

The presence of toxic metals in the human environment can have detrimental effects on people's well-being. This literature review examines the ways in which various environmental and non-environmental factors can contribute to the accumulation of heavy metals in hard dental tissues. It is of the utmost importance to ensure the safety of the environment by restricting the presence of toxic metals originating from both industrial and non-industrial sources. The aim of this study is to analyze current research and identify the primary sources of heavy metal exposure and the mechanisms by which these metals are deposited in dental tissues. Moreover, the objective of this review is to synthesize data from various studies to determine the main environmental and non-environmental sources of toxic metal exposure that contribute to their presence in dental tissues, as well as the biological and chemical processes that are responsible for the deposition of heavy metals in hard dental tissues. Additionally, the review aims to assess the impact of heavy metal accumulation on dental health and its potential systemic effects on overall well-being. The accumulation of heavy metals in the teeth is influenced by a number of factors, such as age, systemic conditions, the nutritional status, and dental caries. The presence of supernumerary teeth results in altered levels of microelements, including an increase in cadmium (Cd) and copper (Cu). Additionally, smoking exacerbates toxic metal accumulation, especially Cd and lead (Pb), and disrupts the balance of essential minerals within the teeth. These findings underscore the impact of environmental pollution on dental health and highlight the potential of teeth as biomarkers of environmental exposure, emphasizing the need for continued research to address the health risks associated with environmental toxins.

Keywords: teeth, biomonitoring, toxic metals, bioelements

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Introduction

The environment in which humans reside and work is characterized by a notable and pervasive presence of heavy metals. This category encompasses a variety of elements, such as zinc (Zn), copper (Cu) and iron (Fe), that play crucial roles as micronutrients in the human body. It also includes metals that are not essential for life processes, such as cadmium (Cd), mercury (Hg) and lead (Pb). It is vital to carefully manage the levels of these metals as trace elements, since both deficiencies and excesses can have harmful effects on human health.[1–3]

The role of Zn in the body is crucial for the production of insulin, as well as the synthesis of proteins and nucleic acids. Insufficient levels of Zn within the body may contribute to the development of obesity and diabetes. The uneven distribution of Zn may impede the onset of metabolic disorders and diabetes by regulating several biological processes. The influence of Zn on a range of hormones, including testosterone, growth hormone and gonadotropins, is widely recognized. Zinc also takes part in the synthesis, storage and secretion of insulin. Additionally, it is a crucial component of thymulin, which is responsible for T cell maturation and differentiation. Recent studies have indicated that Zn is crucial in the development and maintenance of bone tissue homeostasis. The element plays an essential role in bone tissue, not only serving as a constituent but also facilitating the formation of the collagen matrix, the process of mineralization, and the turnover of bone. Zinc deficiency can lead to a gradual reduction in bone density. Bones that lack or have a severe deficiency of Zn are known to be thin, fragile and exhibit increased resorption.[4,5]

Copper is an essential element in numerous physiological processes. It plays a key role in the synthesis of hemoglobin, the formation of bones, the deposition of calcium (Ca) and phosphorus (P) in bones, the absorption of Fe, and the hardening of collagen. Additionally, Cu exhibits antiviral properties and aids in detoxification processes.[6,7] It functions as a coenzyme in a number of enzymes, such as cytochrome c oxidase, representing a crucial component in the process of cellular respiration. Furthermore, Cu participates in the process of keratinization of hair and coat. A deficiency in Cu results in the inhibition of melanin synthesis, a reduction in the immune response, an increased likelihood of infections, an elevated risk of cardiovascular disorders, and impaired cholesterol metabolism.[6,8]

Lipid metabolism is a complex process that involves various factors, one of which is Fe. Iron plays a crucial role in oxidation processes and serves as a constituent of important molecules such as cytochromes, hemoglobin and myoglobin.[9,10] One of its primary functions is to facilitate the transportation of oxygen, storage of transitional tissues and cellular utilization of oxygen. Additionally, it is equally significant in the cytochromes that are housed within the mitochondria. Iron is involved in the transfer of electrons in the electron transport chain.[6] It is stored in the liver, spleen and bone marrow through proteins called ferritin and hemosiderin, both of which have a strong affinity for the element. Additionally, Fe plays a role in erythropoiesis, the formation of leukocytes and immune reactions, impacting both cellular and humoral immunity. Furthermore, the presence of Fe is of the utmost importance for the optimal performance of osteoblasts, which are the cells responsible for bone development.[11,12]

Nevertheless, it is important to acknowledge the presence of heavy metals under specific circumstances. The elements that occur naturally within the Earth's crust play a role in various natural phenomena, including the erosion of rocks, oceanic evaporation, the formation of soil, and volcanic eruptions.[13] The human body can be exposed to heavy metals through various means, including air, water and soil. Soil contamination can occur due to the presence of fertilizers, plant protection products, industrial dust, and sewage. The absorption of water by plants from contaminated soil results in the uptake of toxic metals, which subsequently accumulate in their tissues. However, it is worth noting that the inhalation route provides the most accessible means of absorption. A number of industrial sectors serve as the primary sources of environmental contamination. These include metallurgy, electrotechnology and the chemical industry, as well as the production of fertilizers, paints and solvents. Metals are also heavily present in car exhaust emissions. Additionally, cigarette smoking is a significant non-industrial factor that exposes individuals to high levels of heavy metals. It is crucial to take into account the presence of toxic metals in amalgam fillings and the alloys utilized in prosthetics and orthodontics. Furthermore, the presence of heavy metals has been identified in a range of cosmetics and dietary supplements.[1–3]

Metal toxicity operates through various mechanisms, which can be classified into 3 primary categories. These classifications encompass the obstruction of vital functional groups within proteins, the displacement of metal ions that act as cofactors for enzymes and other functional proteins, and the modification of the spatial arrangement of proteins.[14] A wide array of illnesses can be attributed to the presence of heavy metals. The manifestation of the harmful impact of toxic metals on the human body is contingent upon a number of factors, including the specific type of metal, the dosage, the method of exposure, the duration of exposure, and the individual's personal susceptibility. Heavy metals have an impact on various cellular organelles and components within biological systems. These include the cell membrane, mitochondria, lysosomes, endoplasmic reticulum, nuclei, and certain enzymes that are critical for metabolic processes, detoxification and the repair of damage.[15] Toxic metals, in addition to their toxic properties, possess the capability to accumulate within human parenchymal tissues. Once they

enter the body, these metals tend to accumulate in the kidneys, liver and pancreas.[16] Additionally, heavy metals accumulate in the hard tissues of teeth.[1–3] The gradual deterioration of physical, muscular and neurological capabilities, which resembles the symptoms of diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, and muscular dystrophy, can be attributed to extended contact with specific metals and their compounds. Some metals possess properties that have been linked to the development of birth defects and cancer. The progression of cancers can be influenced by certain heavy metals, which stimulate cancer cells through various pathogenic connections and may also decrease their receptiveness to treatment.[1,2,17]

The significance of biomonitoring in the context of health and the environment is gradually increasing. As a component of environmental dentistry, biomonitoring encompasses a range of actions aimed at evaluating the condition of the environment through the use of biomonitors. These biomonitors, which are utilized to assess the exposure to toxic metals in humans, are known as non-invasive matrices. Examples of such matrices include hair, nails, urine, saliva, and teeth. Among these, deciduous teeth are particularly convenient for obtaining biological material, resulting in a wealth of studies that analyze the levels of toxic metals present in their structure.[1,3] The teeth of an individual are an enduring testimony to their lifestyle within a specific environment and an indicator of the influence of environmental pollutants. During the process of mineralization, noxious metal cations become incorporated into the crystalline framework of hydroxyapatites. These detrimental substances gain access to the tooth tissues via the bloodstream.[18,19]

It is important to acknowledge that chelation therapy has been the main approach to the management of cases of heavy metal poisoning. This method employs the use of chelating agents to form chelates, which are complex ring-like structures that trap metal ions and facilitate their removal from the body. However, the use of metal chelators has its limitations. Metal chelators have the potential to facilitate the migration of heavy metals from other regions of the body to the brain, which can escalate the neurotoxicity of the individual in question. Moreover, the use of these chelators may lead to the depletion of important metals, including Zn and Cu, resulting in serious side effects, such as liver damage.[20,21]

The content of toxic metals in dental tissues is influenced by both industrial and non-industrial factors. The following paragraphs will present a comprehensive analysis of this dichotomy, offering a more exhaustive investigation and inquiry. Within the discourse, certain publications that explore the composition of heavy metals within teeth and the various factors that contribute to their presence will be discussed.

This review offers a novel perspective by examining the interplay between environmental and non-environmental factors in the accumulation of heavy metals in dental tissues. It integrates recent research to identify key sources of toxic metal exposure, elucidate the biological mechanisms of metal deposition in teeth, and assess the broader health implications of this accumulation. The aim of this review is to enhance understanding of the potential of teeth as biomarkers for environmental exposure, to assess the impact of environmental factors on both dental and systemic health, and to underscore the importance of developing diagnostic and preventive strategies. Furthermore, the study emphasizes the need for improved environmental safety measures.

Material and methods

Literature search strategy

A comprehensive literature search was conducted using the following databases: PubMed; Google Scholar; Polska Bibliografia Lekarska; and Web of Science. The search was limited to articles published between 1978 and 2023. The keywords and phrases used in the search included "heavy metals in teeth," "toxic metals and dental health" and "smoking impact on metal accumulation." Boolean operators AND and OR were employed to refine the search results. The search was limited to articles published in English and Polish.

Inclusion and exclusion criteria

The articles included in the review examined the impact of heavy metals on dental health or associated environmental factors, were peer-reviewed and focused on human subjects. Non-peer-reviewed articles, articles not available in full text, and studies with an unrelated focus were excluded from consideration.

Selection process

The articles were subjected to a screening process based on their titles and abstracts. Full-text articles were reviewed for relevance, resulting in the inclusion of 83 studies. Duplicate articles were excluded during the screening process. The methodology is presented in Fig. 1.

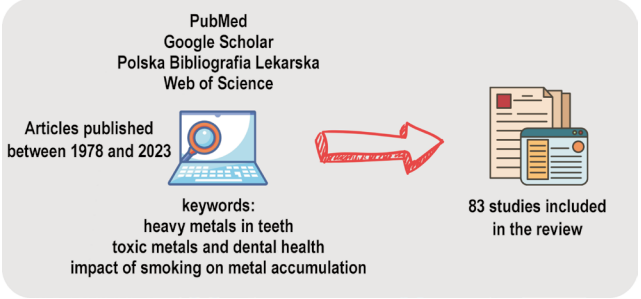

Fig. 1. Methodology of the selection process

Data extraction and analysis

Data extraction involved reviewing abstracts and full-text articles to identify relevant studies. The extracted information included study design, sample size, metal types examined, and key findings related to heavy metal impact on dental health. We employed a narrative synthesis approach to integrate and summarize findings across studies. This involved categorizing results according to themes such as types of metals, mechanisms of impact, and health outcomes.

Quality assessment

The quality of the included studies was evaluated based on the study design, the sample size and the methodological rigor. No specific assessment tools were employed; rather, a critical appraisal was conducted to evaluate the reliability and relevance of each study.

Results

Age and sex

The levels of toxic metals found within human teeth can be influenced by age. Fischer and Wiechuła conducted a study with the objective of analyzing the accumulation of Pb in the calcified tissue of permanent teeth.[22] Atomic absorption spectroscopy (AAS) was employed to measure the concentration of Pb in teeth samples from a particular group of Polish individuals. The research aimed to determine the accumulation of Pb in the human body based on the changes in Pb concentration in teeth from individuals aged 13–84 years. The results of the study showed that the concentration of Pb increased with age in calcified tooth tissues, and this was a statistically significant process. The research also revealed that subjects over the age of 60, born in the 1930s, had a lower concentration of Pb compared to those born in the 1950s. The concentration of Pb in the teeth of younger individuals (<60 years) was observed to increase. The analysis of changes in Pb levels revealed that even low exposure can result in a relatively high accumulation of Pb concentration in calcified tooth tissues. Fischer et al. have analyzed the changes in the concentrations of various elements, including manganese (Mn), Fe, magnesium (Mg), Cu, potassium (K), chromium (Cr), Pb, Cd, and Ca, in deciduous teeth.[23] The study aimed to investigate whether metal concentration changes with age and to describe changes in mineral composition. The researchers obtained deciduous teeth samples from children aged 5–14 years living in southern Poland through non-invasive physiological replacement. Metal concentration was determined via AAS. The results demonstrated a significant decrease in the concentration of the analyzed elements in the deciduous teeth of older children, when compared to those of younger children. However, no significant correlation was observed between the total metal concentration and the Ca content in relation to age.[23]

Chromium is a vital element for the human body. Although it has a significant physiological function, it can also be harmful, with potential carcinogenic, mutagenic, embryotoxic, and teratogenic effects, which depend on its valence state. Malara et al. conducted research into the various factors that influence the presence of Cr in teeth.[24] The objective of the research was to evaluate the influence of tooth type, age and sex on the level of Cr present in the tooth structure. The study sample consisted of permanent teeth sourced from individuals aged between 20 and 68 years residing in Ruda Śląska, Poland. Atomic absorption spectrometry was employed to measure the concentration of Cr in the teeth. The type of tooth and the sex of the donor had no significant influence on the level of Cr in the tooth tissue. Furthermore, there was no meaningful correlation between the Cr contents in teeth and the age of the donors.

Weight

Eating disorders represent a prevalent social phenomenon that may manifest in weight loss and weight gain. Malnutrition, whether due to an excess or deficiency of nutrients, can result in severe health consequences. The study conducted by Fischer and Wiechuła aimed to determine the levels of certain elements, including Cr, Ca, Cu, Fe, and Mn, in the deciduous teeth of children in relation to their body weight.[25] Although there were no significant differences in the concentration of the metals between children with normal and abnormal body weight, the correlation between the metals in teeth varied according to the children's weight.[25] This observation may indicate fluctuations in the mineral composition of tissues that are associated with metabolic disorders.

Systemic diseases, neurodevelopmental disorders and food allergies

The assessment of element levels in teeth, like in other bodily tissues, has the potential to be valuable in the diagnosis of diseases. The concentrations of heavy metals in both the tissue and serum have been linked to a wide range of illnesses, making them potentially valuable biomarkers for early disease detection.

Orzechowska-Wylęgała et al. investigated the presence of Cd and Pb in the teeth of children from the Upper Silesia region of Poland suffering from celiac disease or food allergies.[26] This particular region is known for its prevalent heavy industries, including coal mining, which have led to considerable environmental contamination. As all the children who participated in the study resided in the same location, their exposure to environmental pollution was consistent across the studied groups. While several factors

can impact the levels of heavy metals in dental tissues, the focus of their research was on systemic illnesses that necessitate long-term adherence to restricted diets.[26] The most commonly observed illnesses in pediatric patients include celiac disease and food allergies. The prolonged implementation of restricted diets has been associated with mineral deficiencies in children, as well as quantitative and qualitative changes in the mineral composition of bones and dental tissues. Such alterations may lead to an increased accumulation of toxic metals within the body, such as Pb, Cd, strontium (Sr), Hg, and arsenic (As). The study revealed that children with celiac disease and food allergies have a higher tendency for the accumulation of certain metals in their deciduous teeth, in comparison to healthy individuals. Moreover, the Pb to Ca and Cd to Ca ratios were observed to be elevated in children with celiac disease and food allergies, providing additional evidence of the presence of these harmful metals in the body.[26] The results of this study suggest that children who undergo restrictive diets may require adjustments to their dietary intake, with a particular focus on increasing the consumption of proteins and sulfur amino acids.

In a study conducted by Yalçin et al., the teeth and blood samples of both healthy children and those with congenital heart disease (CHD) were examined.[27] The study included 39 children with CHD and 42 healthy children. The researchers used inductively coupled plasma mass spectrometry to evaluate the levels of 13 different elements, namely Mg, P, Ca, Cr, Mn, Fe, Cu, Zn, Sr, Cd, Pb, Hg, and molybdenum (Mo). After adjusting for potential confounding variables, it was observed that children with cyanotic and acyanotic CHD exhibited significantly lower levels of tooth Ca and a lower Ca:P ratio in comparison to the control group. Furthermore, children with acyanotic CHD exhibited markedly elevated levels of Cu in their teeth, along with increased levels of Mo in their blood and reduced levels of Mg in their blood, when compared to the control group, which consisted of healthy children.[27]

The research conducted by Sitarik et al. investigated the correlation between in utero and postnatal levels of Pb, which were measured using deciduous baby teeth, and the bacterial and fungal gut microbiota of infants in their first year of life.[28] The discovered associations between Pb exposure and gut microbiota could potentially affect the development of a child.[28] However, since there is a lack of research that investigates these correlations in humans, particularly with regards to fungal microbiota, further examination is necessary.

The study conducted by Abdullah et al. aimed to investigate the potential correlation between the presence of heavy metals in children's tooth enamel and the occurrence of autism and disruptive behaviors.[29] The researchers analyzed the concentrations of Pb, Hg and Mn in both prenatal and postnatal enamel regions of deciduous teeth from children diagnosed with Autism Spectrum Disorder (ASD), those exhibiting high levels of disruptive behavior, and typically developing children. The usage of laser ablation inductively coupled plasma mass spectrometry revealed no statistically significant disparities in the levels of these neurotoxicants between children with ASD and typically developing children.[29]

The variations in sex regarding ASD diagnosis and the mutagenic influence of toxic exposures suggest that these factors might have a significant impact on the causal relationships observed in any potential associations.[30]

In a study conducted by Adams et al., the levels of Hg, Pb and Zn in the baby teeth of children with autism were compared to those of a control group.[31] The results showed that children with autism exhibited significantly elevated levels of Hg, while the levels of Pb and Zn were comparable to those observed in the control group. This study indicates that children with autism may have had a greater accumulation of Hg in their bodies during fetal and infant development. The researchers also suggest that the increased use of oral antibiotics in children with autism could have hindered their ability to eliminate Hg, thereby contributing to the higher levels of the element observed in their baby teeth.[31]

The study conducted by Figueroa-Romero et al. aimed to investigate the potential dysregulation of metal uptake during childhood in individuals who were later diagnosed with amyotrophic lateral sclerosis.[32] By examining the co-exposure to different elements, the researchers found a strong association between childhood metal dysregulation and the development of amyotrophic lateral sclerosis.[32]

The impact of childhood exposure to low levels of Pb was assessed by Needleman et al.[33] The study revealed a strong correlation between elevated Pb levels during childhood and lower social standing during high school, increased rates of absenteeism, diminished language skills and logical thinking abilities, decreased hand-eye coordination, delayed reaction times, and reduced finger tapping speed.[33]

The study by Haavikko et al. investigated the concentrations of Zn and Cu in the deciduous teeth of Finnish children and adolescents.[34] The study focused on ways in which these mineral levels could serve as indicators of potential atherosclerosis precursors and systemic diseases. By analyzing the mineral content in dental tissues, the study aimed to understand the relationship between these trace elements and the risk of developing atherosclerosis and other systemic health issues.[34]

Although Mn is a necessary component for growth and development, elevated Mn levels have been associated with neurobehavioral impairment in children. The aim of the study conducted by Bauer et al. was to determine whether there is a correlation between visuospatial learning and memory test results and prenatal or postnatal Mn levels, as measured in deciduous teeth.[35] The deciduous teeth were collected from 142 participants who resided in areas with varying ferromanganese industries in Italy. The prenatal and postnatal tooth regions were analyzed for Mn concentrations using laser ablation inductively coupled

plasma mass spectrometry. The results of the study indicate that the prenatal period could be a crucial timeframe for the influence of environmental Mn on executive function and visuospatial ability, particularly in females.[35]

In a study conducted by Gunier et al., the relationship between Mn levels in teeth and neurodevelopment was analyzed in young Mexican-American children.[36] The researchers used laser ablation inductively coupled plasma mass spectroscopy to measure Mn levels in both prenatal and postnatal dentin from children's shed teeth. The children's scores on the Mental Development Index (MDI) and Psychomotor Development Index (PDI) on the Bayley Scales of Infant Development at 6, 12 and 24 months were examined and compared to Mn levels. A unique biomarker was used to measure prenatal and early postnatal Mn levels in tooth dentin. The findings revealed a negative, temporary correlation between postnatal Mn levels and early neurodevelopment, with sex-specific modifications and prenatal hemoglobin interactions.[36]

Horton et al. investigated the relationships between dentin biomarkers of Pb, Zn and Mn and behaviors observed in later childhood.[37] The behaviors exhibited during childhood could reveal postnatal periods of vulnerability to both individual and combined metal levels found in deciduous teeth. While Mn present in prenatal dentin may offer protective effects, elevated levels of Mn during early postnatal development may increase the risk of negative behaviors. Furthermore, the simultaneous presence of higher concentrations of Mn, Zn and Pb may adversely affect behavior, with Pb specifically associated with an increase in anxiety symptoms.[37]

Mora et al. analyzed the levels of Mn in the dentin of shed teeth during both prenatal and early postnatal stages.[38] The teeth were collected from children residing near agricultural fields that had been treated with Mn-containing fungicides in California, USA. The study aimed to examine the relationship between Mn levels and various aspects of behavior, cognition, memory, and motor functioning in children. The results revealed a significant association between higher levels of prenatal and early postnatal Mn in deciduous teeth and adverse behavioral outcomes, including internalizing, externalizing and hyperactivity problems, in both boys and girls.[38]

By utilizing tooth-matrix biomarkers and examining detailed temporal patterns of exposure, scientists have identified specific periods of development during which Mn is linked to visual-spatial skills. The findings indicate that the associations between Mn and cognitive abilities are significantly influenced by the timing of exposure, with positive effects observed for prenatal levels and detrimental effects observed for postnatal levels.[39]

Environmental pollution

It is crucial to underscore the significant impact of environmental pollution, such as that of the air, soil and water, on the accumulation of toxic metals in the human body, including the teeth. There are a number of methods that can be used to evaluate the extent of heavy metal exposure in the environment. One of these methods is the analysis of various biological samples. Specifically, human teeth are a particularly suitable option because they possess a stable elemental composition. This stability allows for the comparison of the effects of long-term exposure to heavy metals among individuals inhabiting regions with varying degrees of pollution. It should be noted that the placenta is a crucial organ that receives considerable attention during pregnancy. Unfortunately, this organ provides an inadequate barrier against the transfer of dangerous heavy metals, especially Pb, to the developing fetus. The presence of Pb in this organ poses a serious environmental threat to the well-being of future generations. Hormonal fluctuations during pregnancy result in the release of Pb from long-term deposits in bones and teeth into the mother's bloodstream, which can have harmful effects due to exposure to a contaminated environment.[40]

In order to assess early-life metal exposure in a community that had expressed concerns about previous exposures, Friedman et al. conducted a study using deciduous teeth.[41] The teeth were collected from children who had lived in Holliston, Massachusetts (USA) from the time of conception. By analyzing naturally shed teeth, the researchers were able to obtain detailed information about the timing and dosage of metal exposure during early life. This study effectively demonstrates the usefulness of deciduous teeth in community-based research, particularly in cases with a history of water contamination.[41]

Anttila and Anttila investigated the absolute concentrations of Mn, Fe, Ni, Cu, Zn, Sr, and Pb in whole enamel, as well as in the labial and lingual surface enamel of deciduous incisors, using proton-induced X-ray emission.[42] The mean concentration values derived from the 19 samples collected in the urban area did not exhibit significant differences when compared to the 9 samples obtained from the rural region of Finland.[42]

The study by Anttila et al. focused on the Pb content in the enamel of deciduous teeth from an area with high radon (Rn) levels.[43] The study aimed to assess the concentration of Pb in the enamel of teeth from children living in a region with elevated Rn exposure and to investigate any potential relationship between Rn exposure and Pb levels in the teeth. The average Pb concentration in the enamel was comparable to previous measurements of Pb in other regions of Finland, indicating that Rn decay did not cause a notable rise in Pb levels in the teeth.[43]

In a study conducted by Järvinen et al., the levels of Pb in the enamel of deciduous molars were analyzed using proton-induced X-ray emission.[44] The results suggest that the general population of Finland is not currently subjected to significantly elevated levels of artificially introduced environmental Pb, whether in urban or rural settings. Naturally occurring environmental Pb remains a critical factor in cumulative long-term exposure experienced in Finland.[44]

Fosse and Justesen conducted an investigation into the levels of Pb present in the deciduous teeth of Norwegian children.[45] The study aimed to assess the concentration of Pb in dental tissues in order to understand the extent of Pb exposure among children in Norway. The research sought to identify potential sources of Pb exposure and to evaluate the impact of environmental factors on Pb accumulation in teeth. The deciduous teeth were obtained from a variety of Norwegian counties, including urban centers, industrial zones, and rural as well as fishing communities. The findings indicated that both urbanization and industrialization resulted in increased Pb absorption. However, the average level of Pb detected in Norway was significantly lower than the levels typically observed in other nations. Additionally, automobile exhaust was dismissed as a major contributor to excessive Pb absorption.[45]

The assessment conducted by Arora et al. focused on examining the distribution of Pb in primary teeth as a means of assessing Pb exposure during the pre- and neonatal stages.[46] The researchers employed laser ablation inductively coupled plasma mass spectrometry to measure the presence of Pb in both the enamel and dentin of 10 primary teeth. The study illustrates a valuable methodology for obtaining temporal data on environmental Pb exposure during the pre- and neonatal periods by analyzing the spatial distribution of Pb in the dentin of primary teeth.[46]

Modern human populations are increasingly exposed to chronic environmental heavy metal contamination due to rapid urbanization and extensive industrial activities. A bioindicator for elemental uptake is found in tooth dentin, where the absorption occurs during the processes of mineralization and formation, resulting in significant storage over many years. This uptake encompasses essential elements, primarily sourced from geogenic diets, along with non-essential elements introduced through environmental exposure. Asaduzzaman et al. examined 50 human teeth from different ethnic groups.[47] It has been noted that concentrations of heavy metals tend to increase with age. A comparison of ethnic groups revealed that the teeth of ethnic Chinese individuals exhibited slightly higher metal concentrations than those of Malays and Indians. Additionally, female dentin demonstrated greater levels of metal concentrations than male dentin. The molars contained higher concentrations of Hg, Cu and tin (Sn), whereas the incisors showed elevated levels of Pb, Sr, antimony (Sb), and Zn. The increased levels of heavy metals in tooth dentin indicate pollution originating from industrial emissions and urbanization. This demonstrates that human tooth dentin serves as a reliable bioindicator of environmental pollution and can provide chronological data on exposure.[47]

Wychowanski and Malkiewicz conducted a study on residents of Central Poland (Mazowieckie province) to measure the concentration of metal ions present in the hard tissues of their teeth.[48] They collected samples of enamel and dentin from participants living in urban and agricultural areas. The researchers used graphite furnace atomic absorption spectrometry to determine the concentration of Mn, Pb, Cd, and Cr in the enamel and dentin samples from retained teeth. A comparative analysis of the data revealed that the enamel and dentin of individuals living in industrialized areas exhibited significantly higher levels of Pb and Cd compared to those residing in agricultural areas. However, both groups exhibited comparable levels of Mn and Cr in hard tooth tissues. The results of this study confirm that the likelihood of exposure to heavy metals is dependent on both the place of residence and the extent of environmental pollution in that area.[48]

Nowak analyzed the presence of sodium (Na), Ca, K, and heavy metals in human teeth.[49] The analysis was conducted in Katowice and Istebna in Poland between 1992 and 1993. The measurements were taken to determine the levels of heavy metals and Na, Ca and K in the teeth of individuals. Notably, in the relative unpolluted southern region of Poland, namely Istebna, the concentration of heavy metals in the teeth of the local population was significantly lower than in Katowice, an industrial hub in Silesia. The ratios of Pb:Fe and Pb:Mn detected in teeth could potentially serve as a measure of pollution. The analysis of the metal concentration levels in the teeth of individuals residing in Katowice and Istebna revealed significant differences in the mean concentrations of Pb, nickel (Ni), Cd, Zn, cobalt (Co), Mn, Cr, Na, and Ca.[49]

The focus of the study conducted by Nowak and Chmielnicka was to examine the correlation between Pb and Cd and essential elements present in the hair, teeth and nails of individuals living in the environmentally exposed Katowice District in Poland.[50] The investigation aimed to assess the extent of environmental exposure to Pb and Cd between 1990 and 1997 in the residents of this district, which is known for its high levels of environmental exposure to these toxic metals. Additionally, the study examined the exposure to Fe, Zn, Cu, Mn, Ni, Cr, Ca, Na, and K based on the concentration levels found in hair, teeth and nails. The investigation aimed to ascertain whether the accumulation of Pb and Cd could have an impact on the concentration of essential metals, including Fe, Zn, Cu, and Ca. Additionally, a control group was included in the study, consisting of residents from the Beskid area in Poland. The aforementioned elements were analyzed with regard to sex, age and tooth type. Atomic absorption spectroscopy was utilized to determine the concentrations of the elements present in the media under examination. Notably, teeth samples obtained from individuals residing in the Katowice District exhibited elevated levels of Ni, Cr and Mn.

The purpose of the study conducted by Fischer et al. was to analyze the Pb content in various groups of teeth among inhabitants of the Silesian region in Poland.[51] The highest concentration of Pb was identified in canines, while molars exhibited the lowest concentration of Pb among all groups of teeth. The study demonstrated that the accumulation of Pb in the hard tissues of the body increases with age. Additionally, individuals exposed to Pb

emissions from industrial sources were found to have significantly higher levels of Pb present in their teeth.[51]

In their study, Malara et al. aimed to establish whether impacted mandibular teeth and the adjacent bones could serve as a form of biomonitoring media for evaluating the exposure to heavy metals.[52] The research involved the utilization of impacted lower third molars and fragments of the cortical bone, which were extracted during the removal of wisdom teeth. The research participants were chosen from 2 locations: the relatively polluted Ruda Śląska region in Poland; and the Bielsko-Biała region in Poland. Atomic absorption spectrometry with flame atomization was used to determine the concentrations of Cd, Cr, Cu, Fe, Pb, Mn, and Zn in the samples. The inhabitants of the Ruda Śląska region exhibited significantly higher concentrations of Cd and Pb in their impacted third molars and the surrounding bones compared to those living in the Bielsko-Biała region. Furthermore, a significant positive correlation was observed between the concentrations of Cd in the impacted teeth and the surrounding bones. These findings suggest that the levels of Cd and Pb in the environment may be reflected in the impacted mandibular teeth and the surrounding bones of individuals.

A study that explored the concentration of toxic metals in impacted third molars and surrounding bone tissue was carried out by Bryła et al.[3] The study was similar to a previous investigation, but the samples were collected from 2 distinct groups: residents of the Wroclaw district; and residents of Wroclaw city in Poland. The objective of the study was to determine the concentrations of several elements, including Pb, Cd, Cr, Ni, Fe, Mn, Cu, and Zn, in a portion of bone covering the third molars and in the teeth themselves. The levels of Cd, Pb and Mn in the samples demonstrated an increase in correlation with the patient's age. The levels of Cd and Pb were found to be higher in the residents of Wroclaw, as compared to other locations. Additionally, the residents of Wroclaw exhibited higher levels of Cu in their teeth. It was observed that the concentrations of Cr in the teeth was 33% lower than those in the mandibular bone, and that the concentration of Ni decreased with age. In all hard tissues of the tooth and bone, Pb and Cd aggregates were identified, in contrast to bioelements, which demonstrated a greater tendency to aggregate, primarily within the dentin.

The primary source of Cr in the human body is dietary consumption. However, in cases where there is occupational exposure or residing in areas with high levels of Cr in the air, the absorption of Cr through the lungs may be greater than through ingestion. Fischer et al. conducted a study to investigate the correlation between Cr and other selected elements in impacted wisdom teeth.[53] The objective of the study was to determine the levels of Cr, as well as other elements such as Fe, boron (B), Co, Cu, Zn, selenium (Se), Mo, and barium (Ba) in the mineralized tissues of impacted teeth in a population exposed to elevated levels of Cr in the air. The level of exposure to Cr compounds can be determined through the use of impacted wisdom teeth as a diagnostic tool. A comparison between the Cr content in impacted third molars and erupted permanent teeth reveals a lower amount of Cr in the former, indicating that the primary source of Cr in embedded teeth is the bloodstream. The concentration of Cr in hydroxyapatites of impacted wisdom teeth was found to be mainly influenced by the levels of Fe, B, Cr, Co, Se, and Mo.[53]

Rayad et al. conducted an in vitro assessment to measure the concentration of toxic metals in third molars obtained from residents of the Legnica–Głogów Copper District in Poland.[1] The objective of the study was to identify the concentration of toxic metals, which included Mn, Cr, Ni, Cu, Fe, Cd, Pb, and Zn, in the extracted third molars of patients from the Legnica–Głogów Copper District, as well as to ascertain the risk factors that determine the accumulation of these metals. The patients were divided into 2 groups based on their place of residence: residents of the Legnica–Głogów Copper District; and a control group of residents from Wroclaw. The SpectraAA atomic absorption spectrometer was used to determine the concentrations of Pb, Cd, Cr, Ni, Fe, Mn, Cu, and Zn in an air-acetylene flame. The analysis of third molars among inhabitants of the Legnica–Głogów Copper District revealed elevated levels of Fe and Pb. The identification of major risk factors that could lead to the accumulation of harmful metals in the teeth has been established. The study demonstrated a strong correlation between the concentration levels of Cr, Cu and Zn and a person's age. Additionally, a correlation was identified between the amount of Cr present and the concentration of vitamin D3 found in the bloodstream.[1]

In their research, Rayad et al. conducted an analysis of the Hg levels in impacted wisdom teeth obtained from individuals residing in the Legnica–Głogów Copper District.[2] The primary objective of the study was to emphasize the impact of environmental pollution on the human body by determining the amount of Hg present in the impacted third molars of residents in this area. To ascertain the levels of Hg in the samples, AAS was employed. The accumulation of Hg in the teeth of individuals in the control group located in Wroclaw was also studied, with a focus on identifying the risk factors that contribute to this phenomenon. The final model examined a number of factors, including thyroid and parathyroid gland ailments, cardiac ailments, and interval-scale vitamin D3 concentration. Among these factors, the presence of cardiac diseases was found to be statistically significant in relation to an increase in Hg concentration in third molars. The concentration of Hg was found to increase with age and the duration of residence in the Legnica–Głogów Copper District.[2]

The impact of environmental pollution in the Legnica–Głogów Copper District on the incidence of tooth count disorders in adolescents residing in that region was investigated by Rzepnicka et al.[54] The primary objective

of their research was to ascertain the degree of hypodontia in adolescents from educational institutions in Legnica (Poland). The dental health of students from secondary schools in Legnica was evaluated. Of the 1,000 students evaluated, 7.7% had hypodontia. This finding is higher than in other parts of Poland, which could be a result of environmental pollution in the Copper Basin of Lublin, the Głogów Region of Poland.[54]

The study conducted by Malara et al. aimed to investigate the impact of environmental exposure on the coexistence of Cd and Zn in teeth.[55] Specifically, the objective of their research was to determine whether the exposure to heavy metals in the environment affects the coexistence of Cd and Zn in human teeth. The study utilized a sample size of permanent teeth, with teeth sourced from residents of Bielsko-Biała and Ruda Śląska in Poland. The levels of Cd and Zn in the teeth were determined through the use of AAS. A negative correlation was observed between Cd and Zn levels in the teeth of residents from Bielsko-Biała, whereas a positive correlation was noted between the 2 metals in the teeth of residents from Ruda Śląska. The research findings indicated a notable discrepancy between the levels of Zn and Cd present in the teeth of residents from Ruda Śląska, in comparison to those from Bielsko-Biała. Additionally, the exposure to environmental sources of these metals influenced their interaction within the human body.[55]

The assessment of the exposure of a population to heavy metals from the environment can be determined through the analysis of biological samples. Teeth, in particular, can be utilized to estimate long-term environmental exposure due to the stability of their elemental composition. The objective of the study conducted by Malara and Kwapuliński was to identify the presence of physiological and toxic metals in the teeth of individuals residing in Ruda Ślaska and Bielsko-Biała in Poland.[56] Additionally, the study aimed to explore the potential impact of environmental exposure to heavy metals on the occurrence and coexistence of these metals in teeth. The research material for this study consisted of the permanent teeth obtained from individuals between the ages of 20 and 68 from both cities. The levels of Cd, Cr, Cu, Fe, Mn, Pb, Zn, K, Na, Ca, and Mg were measured using AAS with flame atomization. The teeth of individuals residing in Ruda Śląska showed a significant increase in the levels of Cd, Cr, Cu, Fe, Mn, Pb, and Zn, accompanied by a decrease in the levels of Ca and Mg and elevated Pb:Ca and Pb:Mn ratios. An examination of the correlation matrix revealed differences in the co-occurrence of metals in the teeth of the 2 studied groups. The study confirmed that the exposure to heavy metals in the environment has a significant impact on the presence and coexistence of these elements in individuals' teeth.[56] Malara et al. conducted similar research on the effect of Pb on the content of other metals to investigate their interdependence.[57] The permanent teeth extracted from the inhabitants of Ruda Śląska were analyzed. The content of Pb, Fe, Mn, K, Ca, and Mg was determined through the use of AAS with flame atomization. Furthermore, Pearson's product moment correlation analysis demonstrated that Pb exerted an adverse effect on the content of Ca, Mn, Fe, and Mg, while having no impact on the content of Na and K. The tooth structure showed a strong interdependence between Ca, Fe and Mn.[57] Studies have identified an inverse correlation between the presence of Pb and these 3 vital elements, which may be attributed to the process of substitution.

In their investigation, Fischer et al. conducted a detailed analysis of the concentration of Ba in the facial bones, deciduous teeth and impacted teeth of individuals residing in Poland.[58] The research involved both children and adults (aged from 6 to 78 years) from an industrialized region. The condition of the teeth was assessed holistically, without any distinction between the dentin and enamel. To prepare the facial bones and teeth, a sequence of procedures was undertaken, including rinsing, desiccation, crushing in a ceramic mortar, measurement of the sample (approx. 0.2 g), and microwave mineralization in pure nitric acid with a spectral dimension. The concentration of Ba increased with age in bone tissue and in impacted teeth. In contrast, the level of Ba was observed to decline with age in the deciduous teeth.[58]

The study conducted by Fischer et al. involved an evaluation of the Se content in retained wisdom teeth.[59] Specifically, the researchers analyzed the Se content in the teeth of 10 individuals residing in the Upper Silesian Industrial Region in Poland. The results indicated an average Se content of 0.77 ±0.25 μg/g in the teeth, which was notably lower in comparison to the concentration of Se found in other hard tissues, as reported by prior researchers. Notably, a high correlation was observed between the Se content and the content of other elements, such as As, Fe, Sb, and Hg, which suggests that the industrial emissions in the area were the primary source of these elements found in the teeth.[59]

Piekut et al. conducted an evaluation to determine the potential of primary teeth as an indicator of children's environmental exposure to heavy metals.[60] The objective of their study was to ascertain whether primary teeth could serve as an effective environmental indicator of Cd, Pb and Zn exposure in children residing in Bytom, Poland. The study involved an examination of the primary teeth of children between the ages of 2 and 14. Before the analysis, the teeth underwent a mineralization process. The concentration of Cd, Pb and Zn in the samples was determined using AAS. The results of the analysis demonstrated variability in the levels of Cd, Pb and Zn within the primary teeth. Younger children have been found to have higher levels of Cd and Pb than their older counterparts. This indicates that these metals may have been present during prenatal development. Additionally, the concentration of heavy metals identified in primary teeth differs between sexes, with boys showing higher levels

of Pb and Zn than girls. The study conducted in Bytom analyzed primary teeth of children and found limited potential for these teeth to serve as an indicator of exposure to Cd, Pb and Zn in the environment. It appears that prenatal exposure may be a complicating factor in assessing the exposure to these metals in children.[60]

Wiechuła et al. performed a statistical examination of quantities of metals found in the teeth of individuals residing in the Silesian region of southern Poland.[61] Specifically, the concentrations of 11 different metals, namely Cd, Cr, Cu, Fe, Mn, Pb, Zn, Na, K, Ca, and Mg, were measured in the teeth of 2 different groups of people residing in the Silesian region. The first group consisted of individuals living in the town of Katowice-Szopienice, situated in the center of the Upper Silesian Industrial Region, in close proximity to a Pb plant. The second group was comprised of residents of Strumień, an agricultural community. The results of the analysis showed that the residents of Katowice-Szopienice exhibited higher levels of trace metals in their teeth.[61]

Fischer et al. evaluated metal concentrations (Cd, Pb, Mn, Cu, Cr, Fe, Zn, Na, K, Mg, and Ca) in deciduous and permanent human teeth, specifically in the maxilla and mandible.[62] The results showed significantly higher concentrations of metals in the deciduous teeth in comparison to the permanent teeth. Regression and principal component analyses revealed that the process of binding elements by hydroxyapatite in deciduous teeth is becoming more dynamic. Furthermore, the concentration of metals was observed to be higher in the permanent and deciduous teeth of the maxilla compared to those in the mandible.[62]

It is important to acknowledge that the exposure to toxic metals in the workplace leads to their accumulation in the human body. In a study conducted by Malara et al., the presence of certain metals in the teeth of coal miners was investigated in relation to age and duration of employment.[63] The research consisted of an evaluation of Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, K, Na, Ca, and Mg concentrations in different types of teeth, including incisors, canines, premolars, and molars. Atomic absorption spectroscopy was utilized to measure the concentration levels of these metals. The results showed that the concentration of Pb in molars had a positive correlation with the age of the coal miners, whereas the concentration of Na and K demonstrated a positive correlation with the duration of employment.[63] Kwapuliński et al. also conducted a study on the prevalence of selected metals in the teeth of coal miners.[64] The study aimed to investigate the metal concentrations in the teeth of coal miners and non-miners in the town of Ruda Śląska, which is situated in the Upper Silesian Industrial Region in Poland. The study subjects consisted of men between the ages of 20 and 50. The results of the study provide valuable insights into the prevalence of metals in the teeth of individuals exposed to environmental contaminants in both occupational and non-occupational settings. The findings revealed that the concentrations of Pb, Cr, Fe, Na, Ca, and Mg in the teeth varied depending on the tooth type. Although there were no statistically significant differences in the concentrations of elements present in the teeth of miners and non-miners, some isolated differences were observed in the context of specific elements and teeth, such as elevated levels of K in incisors, Pb in premolars, and Cr, Zn and Mg in molars.[64]

Poczatek et al. performed an investigation to assess the potential risks to workers in the industry by evaluating the levels of particular elements in their teeth and bodily fluids.[65] The study involved measuring the levels of certain elements in the teeth and body fluids of Polish workers across 3 distinct industries with established production profiles: Rail Rolling Stock Repair Workshops, which specializes in repairing rail vehicles; Philips Lighting Poland, which produces lighting systems; and Metal-Plast, a factory that is involved in building and furnishing. As part of the study, the teeth were extracted, and the samples of various bodily fluids, including urine, blood and saliva, were collected during routine health check-ups. The study evaluated the levels of various elements, including Ca, Mg, fluorine (F), P (in the form of phosphates), K, Na, Fe, Zn, Cu, Cd, and Pb. To measure the elements, AAS was utilized. The study demonstrated that the concentrations of these elements present in the teeth and body fluids of the subjects varied across the industries. Significant differences were identified in the levels of Mg, phosphates, Zn, Na, and K in the teeth.[65]

Johnston et al. evaluated Pb and As in the shed deciduous teeth of children living near a lead-acid battery smelter.[66] The assessment of prenatal and initial stage exposure to toxic elements can be achieved through the use of shed deciduous teeth to measure Pb and As. A community-based research approach was taken to analyze 50 shed deciduous teeth from 43 children who have lived within a 2-mile radius of a smelter for the entirety of their lives. Laser ablation inductively coupled plasma mass spectrometry was used for the evaluation of the concentrations of Pb and As in the teeth. In order to determine the concentration of Pb in the soil, the researchers utilized spatial kriging to analyze the soil's surface. Findings of the research imply that the exposure to toxic metals during the prenatal and early life stages is linked to soil contamination caused by past industrial activities in an urban community located near a smelter.[66]

The detonation of bombs, bullets and other forms of ammunition in areas of conflict results in the release of several neurotoxicants into the environment. Currently, the Middle East is facing a considerable degree of environmental degradation as a result of extensive bombardments. Savabieasfahani et al. developed a method for elemental bioimaging to examine trace elements in the deciduous teeth of children from Iraq who were born with defects.[67] Additionally, the authors analyzed naturally shed teeth from Lebanon and Iran for trace elements. The research confirmed that elevated levels of metal in deciduous teeth are correlated with increased levels of war activity.[67]

Haavikko et al. employed the proton-induced X-ray emission technique to assess Pb levels in the enamel and dentin of deciduous teeth of children from 2 Finnish towns.[68] It was presumed that Helsinki, the capital, would indicate high Pb exposure, while the rural town of Kuopio in central Finland would represent low to moderate Pb exposure. In nearly all cases, with the exception of 2 teeth, the enamel exhibited greater Pb concentrations than the dentin. The ratio of Pb concentration between enamel and dentin was not consistent and showed significant variability.[68]

The study conducted by Shishniashvili et al. focused on the use of primary teeth and hair as reliable markers for measuring environmental pollution.[69] The results of their study revealed that in areas with unfavorable environmental conditions, the levels of toxic elements such as Pb, Hg, Sn, and titanium (Ti) were significantly higher compared to areas with more favorable conditions. Furthermore, their research demonstrated that dental hard tissues in polluted regions of Tbilisi (Georgia) exhibited higher concentrations of toxic elements and lower levels of essential elements when compared to less polluted areas.[69]

Tsuji et al. reported increased levels of Pb in the dentin of deciduous teeth obtained from isolated First Nations communities in the western James Bay region of northern Ontario, Canada.[70] The research findings indicated that the average concentration of Pb in shed teeth from this remote area was comparable to the levels observed in children living in urban environments or in proximity to smelting facilities. Furthermore, a notable percentage of the children exhibited elevated Pb levels in their dentin.[70]

van Wyk and Grobler assessed the Pb concentrations in the shed deciduous teeth from children residing in 2 selected urban areas of the Cape Peninsula.[71] The children residing in proximity to 2 major industrial facilities exhibited the following average Pb levels: whole teeth at 20,419 ppm; enamel at 10,952 ppm; and dentin at 22,733 ppm. In contrast, the children living near light industrial facilities exhibited Pb levels of 16,556 ppm in whole teeth, 2,919 ppm in enamel and 19,926 ppm in dentin. These variations were statistically significant at the 1% level for teeth and enamel, and at the 5% level for dentin.[71]

Lyngbye et al. conducted a study of Pb exposure in children. In a low-exposure area, the researchers explored potential predictors of Pb burden in children.[72] A total of 1,302 first-form school children from the municipality of Aarhus, Denmark, donated their deciduous teeth for the measurement of Pb concentration in the circumpulpal dentin. Families were interviewed regarding possible sources of Pb exposure. Children with a high Pb burden resided significantly more often in heavily-travelled streets than those with a lower burden, but only during their first 3 years of life. The relationship between traffic intensity and the risk of a high Pb burden was evident in a dose-response manner.[72]

Gomes et al. assessed the levels of Pb present in the superficial enamel of deciduous teeth in children aged 4 to 5 years.[73] The findings revealed that children residing in industrial regions exhibited considerably higher concentrations of Pb in their enamel compared to those living in areas distant from industrial influences.[73]

It is important to highlight that the dental environment is becoming increasingly contaminated with metals from dental materials, primarily due to procedures that generate aerosols, which could compromise the long-term health of dentists, dental students and dental staff. The current pollution in dentistry includes metallic nanoparticles that are highly reactive and can easily become airborne, particularly those that detach from the bulk composition. Additionally, dental amalgam may release liquid Hg or Hg vapors. These issues give rise to concerns within the dental community.[74]

Smoking

The deposition of tar and toxic metals in the body represents a significant health concern associated with smoking. Any form of smoking, whether active or passive, can result in the accumulation of toxic metals in one's teeth. In their analysis, Malara et al.[75] evaluated the impact of smoking on the concentration of Pb and Cd in the hard tissue of teeth. The study was conducted on permanent teeth obtained from both smokers and non-smokers residing in Ruda Śląska in Poland. Atomic absorption spectrophotometry was used to determine the concentration of Cd and Pb. To gain further insight into the accumulation of these harmful metals, the Ca levels in the tooth samples were assessed, resulting in the calculation of the Cd:Ca and Pb:Ca quotients. The act of smoking cigarettes has been observed to affect the concentration of Pb and Cd present in the teeth of individuals. The teeth of smokers contained higher levels of these elements in comparison to non-smokers, with the discrepancy in Cd content being statistically significant.[75] Additionally, studies have shown that the extent of the increase in Cd and Pb levels due to smoking is more pronounced in males than in females.[75,76] Inhalation of cigarette smoke represents a significant source of exposure to heavy metals. Passive exposure to cigarette smoke can also result in the accumulation of these substances. Deciduous teeth, with their stable chemical composition, are often utilized as an indicator of heavy metal exposure in children. In a study conducted by Malara et al., the impact of passive smoking on the concentration of specific metals in deciduous teeth was examined.[76] The study utilized deciduous teeth as its primary research material, with some samples sourced from children exposed to cigarette smoke in their apartments. The levels of various elements, including Cd, Cu, Fe, Mn, Pb, Zn, Ca, and Mg, were measured using AAS with flame atomization. The results indicate that the exposure to cigarette smoke in children can lead to changes in the levels of both toxic and essential elements found in deciduous teeth. Specifically, higher levels of Cd, Cu, Pb,

and Zn, which are permanent constituents of cigarette smoke, were observed, along with lower levels of Mn, Ca and Mg. Additionally, it was noted that children exposed to cigarette smoke in their apartments exhibited a disturbed gradient of Pb levels depending on the type of tooth.[76] Malara et al. also evaluated the occurrence of Pb and Cd in deciduous teeth of children exposed to cigarette smoke in apartments.[77] The study utilized shed deciduous teeth from children between the ages of 6 and 13 who had been exposed to tobacco smoke in their apartments, as well as from children in the same age group who lived in smoke-free apartments. The results showed that the deciduous teeth of children exposed to tobacco smoke in their apartments exhibited higher levels of Pb and Cd, accompanied by elevated Pb:Ca and Cd:Ca ratios, indicative of significant accumulation of these metals when compared to the teeth of children residing in smoke-free apartments.[77]

In several biological samples, there is an alteration in the coexistence pattern of elements (regardless of whether they are antagonistic or synergistic) when in the presence of toxic elements at harmful levels. The study conducted by Malara et al. aimed to determine whether there is a variation in the amount of Cd and Zn present in the hard tissues of retained wisdom teeth in smokers and non-smokers, and whether the exposure to cigarette smoke has an effect on the coexistence of these metals within the tissues.[78] The materials used for this study were retained wisdom teeth from both smokers and non-smokers, and the Cd and Zn levels were determined using AAS. The results showed that the retained wisdom teeth of smokers exhibited higher levels of Cd and Zn compared to the non-smokers' teeth. Furthermore, the coexistence pattern of Cd and Zn in teeth was influenced by exposure to heavy metals, exhibiting a strong synergistic relationship in smokers.[78]

The study conducted by Alhasmi et al. employed laser-induced breakdown spectroscopy to detect the presence of toxic elements in the teeth of smokers and non-smokers.[79] The research investigated periodontal parameters associated with differences in exposure to these toxic elements. The aim of the study was to ascertain the impact of smoking on the accumulation of toxic elements in teeth and the associated changes in periodontal health. The study revealed that the concentrations of Pb, Cd and As were significantly higher in the teeth of smokers compared to non-smokers and the control group. Specifically, smokers exhibited elevated levels of these toxic elements compared to non-smokers, who also demonstrated higher concentrations than the control group. Overall, the control group exhibited the lowest levels of these elements.[79]

One aspect investigated by Olovčić et al. was the impact of smoking on the accumulation of toxic metals.[80] The researchers analyzed the presence of 12 different metals in dental samples collected from 2 cities in Bosnia and Herzegovina, namely Bihać and Sarajevo. The research revealed statistically significant differences in the levels of Zn between the dentin samples of smokers and non-smokers.[80]

The study conducted by Fischer et al. evaluated the significance of passive smoking on the levels of Pb and Cr found in deciduous teeth.[81] The study presented the changes in Pb and Cr content in deciduous teeth of children exposed to environmental tobacco smoke. The control group consisted of children whose deciduous teeth were not exposed to tobacco smoke at home. The analysis revealed that the Pb content was higher in the non-exposed population (13.81 μg/g) than in the passive smoking population (12.28 μg/g). Passive smoking resulted in a reduction in Cr content in deciduous teeth. The quotient of Pb and Cr contents was higher in passive-smoking boys and girls and in different types of deciduous teeth.[81] Fischer et al. also evaluated the effect of passive smoking on the concentration of other metals in deciduous teeth.[82] The increased concentration of toxic elements may disrupt the balance of trace elements which are crucial for the body's physiology. The study involved the examination of deciduous teeth that were collected during the process of being replaced by permanent dentition. The content of various elements, including Mn, Fe, Cu, Ca, K, Na, Mg, Zn, Cr, Cd, and Pb, was determined through the use of AAS. The deciduous teeth of children who were not exposed to tobacco smoke exhibited higher levels of elements that are essential for the body's physiological functions, such as Fe, Zn, K, Na, and Ca. In contrast, the deciduous teeth of children exposed to passive smoking exhibited lower levels of these essential elements. Furthermore, the study demonstrated higher levels of toxic metals in the hard tissues of the teeth of children who were exposed to passive smoking at home.[82] It is possible that these toxic metals originate from tobacco smoke.

Toxic metals are a major contaminant in cigarette smoke, leading to the accumulation of these elements in calcified tissue of the teeth after entering the human body. This creates new conditions for the coexistence and occurrence of metals, which can be described using Czarnowski's model of the equilibrium between biological and chemical cations.[83] In their study, Malara et al. investigated the impact of smoking on the cationic equilibrium present in the teeth of men.[83] The study aimed to determine whether the consumption of cigarettes had any effect on the levels of Cd, Cr, Cu, Fe, Mn, Pb, Zn, K, Na, Ca, and Mg present in the hard tissues of men's teeth, as well as on the constant value of the cationic equilibrium model. The research material consisted of extracted permanent teeth from both smokers and non-smokers. The results indicated that smokers exhibited higher levels of Cd, Cr, Cu, Pb, and Zn in their teeth, whereas the concentrations of K, Na, Ca, Mg, Fe, and Mn were lower in smokers' teeth than those found in non-smokers. Additionally, the constant value of cationic equilibrium in the teeth of smokers was observed to be lower than that of non-smokers.[83] The abovementioned studies are summarized in Table 1.

Table 1. Summary of the studies included in the literature review

Study	Study design	Aim of the study/study results
Rayad et al. 2023[1]	in vitro pilot study	The study evaluates the concentration of toxic metals in third molars obtained from residents of the Legnica–Głogów Copper District and identifies risk factors for metal accumulation.
Rayad et al. 2023[2]	in vitro pilot study	The study investigates the mercury content in impacted wisdom teeth of patients from the Legnica–Głogów Copper District and evaluates environmental exposure.
Bryła et al. 2021[3]	research article	The study measures the concentration of toxic metals in impacted third molars and adjacent bone tissue across different patient groups, revealing significant variations.
Fukunaka and Fujitani 2018[4]	review	The study investigates the role of zinc homeostasis in the pathogenesis of diabetes and obesity.
Taniguchi et al. 2013[5]	experimental research	The zinc transporter ZIP9/SLC39A9 is a critical regulator of B-cell receptor signaling pathways.
Ciosek et al. 2023[6]	review	The study describes the interactions of iron, zinc, copper, cadmium, and mercury with bone tissue and discusses their potential health effects.
Brodziak-Dopierala et al. 2009[7]	research article	The study explores the interactions of copper and iron with other elements in the osseous tissue of the femoral head.
Kabata-Pendias and Mukherjee 2007[8]	book	The book traces the pathway of trace elements from soil to humans, emphasizing the environmental and health implications.
Otten et al. 2006[9]	book	The guide provides dietary reference intakes for essential nutrients, including trace elements.
World Health Organization 2007[10]	guidelines	The report offers guidelines for the assessment of the iron status in populations and emphasizes the implications for public health.
Wang et al. 2018[11]	experimental research	Iron-induced oxidative stress stimulates osteoclast differentiation via the NF-κB signaling pathway in a mouse model.
Angus et al. 1988[12]	research article	The study examines the relationship between dietary intake and bone mineral density.
Nriagu 1989[13]	research article	The study provides a global assessment of the natural sources of atmospheric trace metals.
Collins et al. 2010[14]	review	The article explores the metabolic crossroads of iron and copper, highlighting their interactions.
Wang and Shi 2001[15]	review	The study investigates the molecular mechanisms underlying metal toxicity and carcinogenesis.
Dudek-Adamska et al. 2018[16]	research article	The study analyzes chromium levels in postmortem material to assess environmental exposure.
Pietrzak et al. 2021[17]	research article	The study investigates the influence of arsenic, cadmium, mercury, and lead levels on the overall survival of patients with lung cancer.
Tvinnereim et al. 2000[18]	research article	The study identifies factors that influence the concentration of heavy metals in human primary teeth.
Alomary et al. 2013[19]	research article	The study measures lead, cadmium, copper, iron, and zinc levels in deciduous teeth of children in Jordan, identifying factors affecting their concentrations.
Amadi et al. 2019[20]	review	The review explores natural antidotes and management strategies for metal toxicity.
Andersen 2004[21]	review	The review discusses the chemical and biological aspects of treating metal intoxications with chelating agents, emphasizing their mechanisms of action and effectiveness.
Fischer and Wiechuła 2016[22]	research article	The study reveals age-dependent changes in lead concentration in human teeth, indicating an increased accumulation with advancing age.
Fischer et al. 2013[23]	research article	The concentrations of elements in deciduous teeth vary with age, reflecting alterations in metal exposure and mineral metabolism as individuals grow older.
Malara et al. 2005[24]	research article	The study identifies factors influencing the presence of chromium in teeth, including environmental and dietary influences.
Fischer and Wiechuła 2016[25]	research article	The study explores the potential relationship between a child's body weight and the concentration of metals in their deciduous teeth, revealing significant correlations.
Orzechowska-Wylęgała et al. 2011[26]	research article	The accumulation of cadmium and lead in deciduous teeth is higher in children with celiac disease or food allergies.
Yalçin et al. 2021[27]	research article	The study compares the element profiles in the blood and teeth of children with congenital heart disease to those of healthy children, demonstrating significant differences.
Sitarik et al. 2020[28]	research article	The exposure to lead in utero and in the early postnatal period is associated with changes in the infant gut microbiota.

Study	Study design	Aim of the study/study results
Abdullah et al. 2012[29]	research article	There is a potential correlation between the presence of heavy metals in children's tooth enamel and the occurrence of autism spectrum disorder and disruptive behaviors.
Dickerson et al. 2017[30]	review	There are potential sex differences in the relationship between metal exposure and autism spectrum disorder. Males and females may respond differently to the effects of metal toxicity.
Adams et al. 2007[31]	research article	The study compares the levels of mercury, lead and zinc in baby teeth between children with autism spectrum disorder and a control group, indicating higher levels in the former.
Figueroa-Romero et al. 2020[32]	research article	Early life metal dysregulation is correlated with the development of amyotrophic lateral sclerosis.
Needleman et al. 1990[33]	research article	The study examines the long-term effects of low-dose lead exposure during childhood, showing its lasting impact on cognitive function.
Haavikko et al. 1985[34]	research article	The concentrations of zinc and copper in the deciduous teeth of Finnish children and adolescents are associated with early indicators of atherosclerosis.
Bauer et al. 2017[35]	research article	The correlation between manganese levels in teeth and neurobehavioral outcomes highlights the existence of sex-specific windows of susceptibility.
Gunier et al. 2015[36]	research article	Manganese levels in teeth are associated with neurodevelopmental outcomes in young Mexican-American children.
Horton et al. 2018[37]	observational study	Dentin biomarkers of prenatal and early childhood exposure to manganese, zinc and lead are associated with variations in childhood behavior.
Mora et al. 2015[38]	research article	The prenatal and postnatal manganese levels in teeth are linked to neurodevelopmental performance in children aged 7–10.5 years.
Henn et al. 2018[39]	research article	Using dentin microspatial analyses, researchers identify critical windows of susceptibility to manganese exposure that affect neurodevelopment.
Rísová 2019[40]	review	The study identifies the pathway of lead transfer from the mother's body to the child, highlighting the risks associated with prenatal exposure.
Friedman et al. 2022[41]	research article	The study analyzes the presence of multiple metals in children's deciduous teeth, revealing significant environmental exposure.
Anttila and Anttila 1987[42]	cross-sectional study	The trace element content in the enamel of deciduous incisors varies between children from rural and urban Finnish areas, as evidenced by proton-induced X-ray emission analysis.
Anttila 1987[43]	cross-sectional study	The enamel of deciduous teeth from high-radon areas exhibits a significantly higher lead content in comparison to enamel from areas with lower radon levels.
Järvinen et al. 1984[44]	analytical study	The concentrations of lead in deciduous molar enamel in Finland vary, with elevated levels detected in certain regions, suggesting the potential for environmental or occupational exposure.
Fosse and Justesen 1978[45]	environmental study	The concentration of lead in the deciduous teeth of Norwegian children is elevated, indicating a significant environmental or occupational exposure to lead.
Arora et al. 2006[46]	research article	The study uses the spatial distribution of lead in human primary teeth as a biomarker for pre- and neonatal lead exposure.
Asaduzzaman et al. 2017[47]	analytical study	Dentin from human teeth can serve as a bioindicator for heavy metal exposure and environmental pollution.
Wychowanski and Malkiewicz 2017[48]	research article	The study evaluates the concentration of metal ions in the hard tissues of teeth among residents in central Poland, revealing patterns of environmental exposure.
Nowak 1995[49]	research article	The study evaluates the prevalence of heavy metals and essential elements, including sodium, potassium and calcium, in human teeth, demonstrating regional variations.
Nowak and Chmielnicka 2000[50]	research article	The study examines the relationship between lead and cadmium and essential elements in the hair, teeth and nails of individuals who have been exposed to environmental factors.
Fischer et al. 2004[51]	research article	The study analyzes the content of lead in the teeth of individuals residing in the Silesian region, revealing elevated levels of contamination.
Malara et al. 2016[52]	research article	The study analyzes the presence of selected toxic and essential heavy metals in impacted teeth and mandibular bones of individuals exposed to environmental heavy metals.
Fischer et al. 2008[53]	analytical study	The study analyzes the co-occurrence of chromium with selected elements in impacted wisdom teeth, revealing significant interrelationships and potential sources of exposure.
Rzepnicka et al. 2002[54]	observational study	Microintoxication with lead, copper and zinc causes disturbances in the number of teeth among the youth from Legnica, Poland.
Malara et al. 2005[55]	environmental exposure assessment study	Environmental exposure affects the coexistence of cadmium and zinc in teeth, reflecting the extent of environmental contamination.
Malara and Kwapuliński 2004[56]	environmental exposure assessment study	The presence of metals in teeth is influenced by environmental factors, which can be useful in assessing population exposure.

Study	Study design	Aim of the study/study results
Malara et al. 2004[57]	analytical study	Lead interferes with the occurrence of essential elements in teeth.
Fischer et al. 2014[58]	analytical study	The study evaluates the concentration of barium in deciduous teeth, impacted teeth and facial bones of Polish residents, identifying variations associated with environmental exposure.
Fischer et al. 2008[59]	analytical study	The study analyzes the content of selenium in retained wisdom teeth, demonstrating a range of values.
Piekut et al. 2018[60]	environmental exposure assessment study	Primary teeth can serve as indicators of the extent of environmental exposure of children to heavy metals.
Wiechuła et al. 2006[61]	analytical study	The study reveals significant variation in metal concentrations in the teeth of residents of the Silesian region, indicating environmental impact.
Fischer et al. 2009[62]	analytical study	The concentrations of metals in the maxilla and mandible of deciduous and permanent teeth vary significantly.
Malara et al. 2005[63]	occupational exposure assessment study	The occurrence of selected metals in the teeth of coal miners is contingent upon age and the duration of employment.
Kwapuliński et al. 2000[64]	occupational exposure assessment study	The prevalence of selected metals varies among different types of teeth in coal miners.
Poczatek et al. 2004[65]	occupational exposure assessment study	The analysis indicates an occupational risk for industrial employees based on the concentration of specific elements in their teeth and body fluids.
Johnston et al. 2019[66]	environmental exposure assessment study	The presence of lead and arsenic in the deciduous teeth of children living near a lead-acid battery smelter indicates a considerable degree of environmental exposure.
Savabieasfahani et al. 2016[67]	environmental exposure assessment study	Prenatal metal exposure in the Middle East, as evidenced by the presence of traces in the deciduous teeth of children, is likely attributable to war-related pollution.
Haavikko et al. 1984[68]	observational study	The study reveals that the concentration of lead in both the enamel and dentin of deciduous teeth varies among children from 2 Finnish towns, indicating regional differences in environmental lead exposure.
Shishniashvili et al. 2016[69]	environmental exposure assessment study	Primary teeth and hair are reliable indicators of environmental pollution.
Tsuji et al. 2001[70]	observational study	The elevated levels of lead in the dentin of deciduous teeth collected from remote First Nations communities in the western James Bay region of northern Ontario, Canada, indicate significant environmental lead exposure in these communities.
van Wyk et al. 1983[71]	cross-sectional study	The elevated levels of lead in the deciduous teeth of children from selected urban areas in the Cape Peninsula indicate a higher degree of environmental lead exposure in these regions.
Lyngbye et al. 1990[72]	epidemiological study	The levels of lead in children's teeth are significantly associated with traffic-related lead exposure.
Gomes et al. 2004[73]	in vivo study	The lead content in the superficial enamel of deciduous teeth in preschool children is measurable, indicating environmental exposure to lead.
Fernandes and França 2023[74]	review	Dental materials can contribute to nanometal and metal ion pollution in the dental environment, potentially impacting both patients and dental professionals.
Malara et al. 2004[75]	observational study	Cigarette smoking increases the presence of cadmium and lead in the hard tissues of the teeth.
Malara et al. 2006[76]	observational study	Passive smoking elevates the levels of selected metals in deciduous teeth.
Malara et al. 2004[77]	observational study	Lead and cadmium are present in the deciduous teeth of children exposed to cigarette smoke in their apartments.
Malara et al. 2005[78]	observational study	Cigarette smoking affects the coexistence of cadmium and zinc in retained wisdom teeth.
Alhasmi et al. 2015[79]	observational study	The concentration of toxic elements in the teeth of smokers is higher than in the teeth of non-smokers. These levels correlate with adverse periodontal parameters.
Olovčić et al. 2019[80]	cross-sectional study	The concentrations of metals in human enamel and dentin are influenced by sex, geographic location and smoking habits.
Fischer et al. 2009[81]	observational study	Passive smoking affects the levels of lead and chromium in deciduous teeth.
Fischer et al. 2011[82]	observational study	The study evaluates the impact of passive smoking on the metal content in deciduous teeth, showing a significant exposure risk.
Malara et al. 2004[83]	analytical study	Cigarette smoking affects the cationic equilibrium in men's teeth.

Limitations

It is important to consider a number of potential biases in this review. One potential source of bias is the use of language. The review included only articles written in English and Polish. Another potential source of bias is publication bias, which can occur when only studies with significant findings are published. It is important to note that this narrative review is limited to studies published between 1978 and 2023. Consequently, research published outside of the specified time frame may have been excluded.

Conclusions

The accumulation of toxic metals in the human body is an important issue due to its potential to result in a variety of adverse health outcomes. A visible sign of exposure to such substances is the development of oral complications. Human teeth can be negatively impacted by environmental chemicals, drugs, or physical agents during both embryonic development and after they emerge in the oral cavity. According to the presented publications, the concentration of heavy metals in human teeth is influenced by both environmental and non-environmental factors. The findings of these studies suggest that teeth can serve as a valuable tool for monitoring the presence of pollutants in the environment. It is crucial to emphasize the urgent need to minimize exposure to hazardous metals. The accumulation of toxic metals, such as Pb, Cd and Hg, can lead to structural damage to the teeth and gums. The presence of these metals can result in the weakening of tooth enamel, thereby increasing the susceptibility of the teeth to the development of cavities and mechanical damage. The presence of toxic metals in the body can disrupt the balance of the oral microbiota, fostering the growth of cavity-causing bacteria and increasing the risk of tooth decay. Toxins can affect tooth mineralization, resulting in weakened teeth that are more susceptible to erosion and fractures. In children, toxic metals can impede the normal development of the teeth, resulting in complications with tooth eruption and deformities. Additionally, heavy metals can affect inflammation and gum health, potentially leading to chronic periodontal diseases, such as gingivitis and periodontitis. Toxins present in the body can affect the effectiveness of dental treatments and interactions with medications used for oral health issues.[1–3] The presence of toxic metals in the body can lead to significant adverse consequences for overall health. Metals such as Pb and Hg are recognized for their detrimental effects on the nervous system. The exposure to Pb may result in cognitive impairments, developmental delays in children and various neurological disorders. On the other hand, the exposure to Hg can lead to tremors, memory loss and additional neurological complications. Heavy metals, including Cd and Hg, can accumulate in the kidneys, resulting in a gradual impairment of their functionality. This accumulation may cause kidney damage or disease, thereby compromising the body's capacity to filter waste and regulate fluid balance. Additionally, toxic metals can affect cardiovascular health. For example, Cd has been associated with hypertension and atherosclerosis, while Pb exposure has been shown to increase the risk of heart disease. Prolonged exposure to specific metals can weaken the immune system, increasing susceptibility to infections and illnesses. This decline in immune function can adversely affect the body's overall ability to combat diseases and sustain health. Certain toxic metals have the potential to interfere with endocrine function, impacting hormone equilibrium and resulting in complications such as thyroid dysfunction, reproductive issues and metabolic irregularities. Prolonged exposure to specific heavy metals has been associated with an increased risk of cancer development. Notably, As and Cd are recognized as carcinogens that play a role in the onset of different cancer types.[1–3,6] Reducing exposure to hazardous metals requires a multifaceted approach involving strict regulations, increased public awareness and enhanced monitoring systems. Governments and environmental agencies must implement and enforce rigorous guidelines to limit the release of toxic metals into the environment. Public education campaigns should raise awareness about the sources and risks of metal exposure, encouraging individuals to take preventive measures. Additionally, technological advances can improve the detection and analysis of metal concentrations in teeth, providing valuable data for environmental health studies. Furthermore, interdisciplinary collaboration among scientists, healthcare professionals and policymakers is crucial for the development of effective strategies to mitigate the impact of toxic metals on human health. By integrating findings from dental research with broader environmental health studies, we can gain a more comprehensive understanding of the impact of these metals on various aspects of health and well-being. This holistic approach will enable the formulation of targeted interventions to reduce metal exposure and the associated health risks.

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.

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References

1. Rayad S, Dobrzyński M, Kuźniarski A, et al. An in-vitro evaluation of toxic metals concentration in the third molars from residents of the Legnica–Głogów Copper Area and risk factors determining the accumulation of those metals: A pilot study. *Appl Sci*. 2023;13(5):2904. doi:10.3390/app13052904
2. Rayad S, Dobrzyński M, Kuźniarski A, et al. Mercury content in impacted wisdom teeth from patients of the Legnica–Głogów Copper Area – an in vitro pilot study. *J Xenobiot*. 2023;13(3):463–478. doi:10.3390/jox13030029
3. Bryła E, Dobrzyński M, Konkol D, Kuropka P, Styczyńska M, Korczyński M. Toxic metals content in impacted third molars and adjacent bone tissue in different groups of patients. *Materials (Basel)*. 2021;14(4):793. doi:10.3390/ma14040793
4. Fukunaka A, Fujitani Y. Role of zinc homeostasis in the pathogenesis of diabetes and obesity. *Int J Mol Sci*. 2018;19(2):476. doi:10.3390/ijms19020476
5. Taniguchi M, Fukunaka A, Hagihara M, et al. Essential role of the zinc transporter ZIP9/SLC39A9 in regulating the activations of Akt and Erk in B-cell receptor signaling pathway in DT40 cells. *PLoS One*. 2013;8(3):e58022. doi:10.1371/journal.pone.0058022
6. Ciosek Ż, Kot K, Rotter I. Iron, zinc, copper, cadmium, mercury, and bone tissue. *Int J Environ Res Public Health*. 2023;20(3):2197. doi:10.3390/ijerph20032197
7. Brodziak-Dopierala B, Kwapuliński J, Paukszto A, Kowol J, Bogunia M, Ahnert B. Interactions of copper and iron with other elements in the osseous tissue of the femur head. *Fresenius Environ Bull*. 2009;18(10a):1963–1966. https://www.researchgate.net/publication/286570065_Interactions_of_copper_and_iron_with_other_elements_in_the_osseous_tissue_of_the_femur_head. Accessed September 11, 2024.
8. Kabata-Pendias A, Mukherjee AB. *Trace Elements from Soil to Human*. Berlin/Heidelberg, Germany: Springer; 2007. doi:10.1007/978-3-540-32714-1
9. Otten JJ, Hellwig JP, Meyers LD, eds. *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. Washington, DC: National Academies Press; 2006:328–339.
10. World Health Organization. *Assessing the Iron Status of Populations*. 2nd ed. Geneva, Switzerland: WHO Press; 2007.
11. Wang X, Chen B, Sun J, et al. Iron-induced oxidative stress stimulates osteoclast differentiation via NF-κB signaling pathway in mouse model. *Metabolism*. 2018;83:167–176. doi:10.1016/j.metabol.2018.01.005
12. Angus RM, Sambrook PN, Pocock NA, Eisman JA. Dietary intake and bone mineral density. *Bone Miner*. 1988;4(3):265–277. PMID:3191284.
13. Nriagu JO. A global assessment of natural sources of atmospheric trace metals. *Nature*. 1989;338:47–49. doi:10.1038/338047a0
14. Collins JF, Prohaska JR, Knutson MD. Metabolic crossroads of iron and copper. *Nutr Rev*. 2010;68(3):133–147. doi:10.1111/j.1753-4887.2010.00271.x
15. Wang S, Shi X. Molecular mechanisms of metal toxicity and carcinogenesis. *Mol Cell Biochem*. 2001;222(1–2):3–9. PMID:11678608.
16. Dudek-Adamska D, Lech T, Konopka T, Kościelniak P. Chromium in postmortem material. *Biol Trace Elem Res*. 2018;186(2):370–378. doi:10.1007/s12011-018-1328-8
17. Pietrzak S, Wójcik J, Baszuk P, et al. Influence of the levels of arsenic, cadmium, mercury and lead on overall survival in lung cancer. *Biomolecules*. 2021;11(8):1160. doi:10.3390/biom11081160
18. Tvinnereim HM, Eide R, Riise T. Heavy metals in human primary teeth: Some factors influencing the metal concentrations. *Sci Total Environ*. 2000;255(1–3):21–27. doi:10.1016/s0048-9697(00)00436-8
19. Alomary A, Al-Momani IF, Obeidat SM, Massadeh AM. Levels of lead, cadmium, copper, iron, and zinc in deciduous teeth of children living in Irbid, Jordan by ICP-OES: Some factors affecting their concentrations. *Environ Monit Assess*. 2013;185(4):3283–3295. doi:10.1007/s10661-012-2790-y
20. Amadi CN, Offor SJ, Frazzoli C, Orisakwe OE. Natural antidotes and management of metal toxicity. *Environ Sci Pollut Res Int*. 2019;26(18):18032–18052. doi:10.1007/s11356-019-05104-2
21. Andersen O. Chemical and biological considerations in the treatment of metal intoxications by chelating agents. *Mini Rev Med Chem*. 2004;4(1):11–21. doi:10.2174/1389557043487583
22. Fischer A, Wiechuła D. Age-dependent changes in Pb concentration in human teeth. *Biol Trace Elem Res*. 2016;173(1):47–54. doi:10.1007/s12011-016-0643-1
23. Fischer A, Wiechuła D, Przybyła-Misztela C. Changes of concentrations of elements in deciduous teeth with age. *Biol Trace Elem Res*. 2013;154(3):427–432. doi:10.1007/s12011-013-9744-2
24. Malara P, Kwapuliński J, Drugacz J, Orzechowska-Wylęgała B. Factors influencing the presence of chromium in teeth. *J Elem*. 2005;10(4):951–957.
25. Fischer A, Wiechuła D. Is there a dependence between children's body weight and the concentration of metals in deciduous teeth? *Homo*. 2016;67(6):508–514. doi:10.1016/j.jchb.2016.09.002
26. Orzechowska-Wylęgała B, Obuchowicz A, Malara P, Fischer A, Kalita B. Cadmium and lead accumulate in the deciduous teeth of children with celiac disease or food allergies. *Int J Stomatol Occlusion Med*. 2011;4(1):28–31. doi:10.1007/s12548-011-0005-8
27. Yalçin SS, Dönmez Y, Aypar E, Yalçin S. Element profiles in blood and teeth samples of children with congenital heart diseases in comparison with healthy ones. *J Trace Elem Med Biol*. 2021;63:126662. doi:10.1016/j.jtemb.2020.126662
28. Sitarik AR, Arora M, Austin C, et al. Fetal and early postnatal lead exposure measured in teeth associates with infant gut microbiota. *Environ Int*. 2020;144:106062. doi:10.1016/j.envint.2020.106062
29. Abdullah MM, Ly AR, Goldberg WA, et al. Heavy metal in children's tooth enamel: Related to autism and disruptive behaviors? *J Autism Dev Disord*. 2012;42(6):929–936. doi:10.1007/s10803-011-1318-6
30. Dickerson AS, Rotem RS, Christian MA, Nguyen VT, Specht AJ. Potential sex differences relative to autism spectrum disorder and metals. *Curr Environ Health Rep*. 2017;4(4):405–414. doi:10.1007/s40572-017-0164-x
31. Adams JB, Romdalvik J, Ramanujam VMS, Legator MS. Mercury, lead, and zinc in baby teeth of children with autism versus controls. *J Toxicol Environ Health A*. 2007;70(12):1046–1051. doi:10.1080/15287390601172080
32. Figueroa-Romero C, Mikhail KA, Gennings C, et al. Early life metal dysregulation in amyotrophic lateral sclerosis. *Ann Clin Transl Neurol*. 2020;7(6):872–882. doi:10.1002/acn3.51006
33. Needleman HL, Schell A, Bellinger D, Leviton A, Allred EN. The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. *N Engl J Med*. 1990;322(2):83–88. doi:10.1056/NEJM199001113220203
34. Haavikko K, Anttila A, Helle A, Pesonen E. Atherosclerosis precursors in Finnish children and adolescents. XIV. Zinc and copper concentrations in deciduous teeth. *Acta Paediatr Scand Suppl*. 1985;318:213–219. doi:10.1111/j.1651-2227.1985.tb10096.x
35. Bauer JA, Henn BC, Austin C, et al. Manganese in teeth and neurobehavior: Sex-specific windows of susceptibility. *Environ Int*. 2017;108:299–308. doi:10.1016/j.envint.2017.08.013
36. Gunier RB, Arora M, Jerrett M, et al. Manganese in teeth and neurodevelopment in young Mexican-American children. *Environ Res*. 2015;142:688–695. doi:10.1016/j.envres.2015.09.003
37. Horton MK, Hsu L, Henn BC, et al. Dentine biomarkers of prenatal and early childhood exposure to manganese, zinc and lead and childhood behavior. *Environ Int*. 2018;121(Pt 1):148–158. doi:10.1016/j.envint.2018.08.045
38. Mora AM, Arora M, Harley KG, et al. Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort. *Environ Int*. 2015;84:39–54. doi:10.1016/j.envint.2015.07.009
39. Henn BC, Austin C, Coull BA, et al. Uncovering neurodevelopmental windows of susceptibility to manganese exposure using dentine microspatial analyses. *Environ Res*. 2018;161:588–598. doi:10.1016/j.envres.2017.12.003

40. Rísová V. The pathway of lead through the mother's body to the child. *Interdiscip Toxicol*. 2019;12(1):1–6. doi:10.2478/intox-2019-0001
41. Friedman A, Bauer JA, Austin C, et al. Multiple metals in children's deciduous teeth: Results from a community-initiated pilot study. *J Expo Sci Environ Epidemiol*. 2022;32(3):408–417. doi:10.1038/s41370-021-00400-x
42. Anttila A, Anttila A. Trace-element content in the enamel surface and in whole enamel of deciduous incisors by proton-induced X-ray emission of children from rural and urban Finnish areas. *Arch Oral Biol*. 1987;32(10):713–717. doi:10.1016/0003-9969(87)90114-2
43. Anttila A. Lead content of deciduous tooth enamel from a high-radon area. *Acta Odontol Scand*. 1987;45(4):283–288. doi:10.3109/00016358709097540
44. Järvinen V, Anttila A, Lappalainen R, Rytömaa I. Lead content of deciduous molar enamel in Finland, as measured by proton-induced X-ray emission. *Scand J Work Environ Health*. 1984;10(2):103–107. doi:10.5271/sjweh.2360
45. Fosse G, Justesen NP. Lead in deciduous teeth of Norwegian children. *Arch Environ Health*. 1978;33(4):166–175. doi:10.1080/00039896.1978.10667329
46. Arora M, Kennedy BJ, Elhlou S, et al. Spatial distribution of lead in human primary teeth as a biomarker of pre- and neonatal lead exposure. *Sci Total Environ*. 2006;371(1–3):55–62. doi:10.1016/j.scitotenv.2006.07.035
47. Asaduzzaman K, Khandaker MU, Binti Baharudin NA, et al. Heavy metals in human teeth dentine: A bio-indicator of metals exposure and environmental pollution. *Chemosphere*. 2017;176:221–230. doi:10.1016/j.chemosphere.2017.02.114
48. Wychowanski P, Malkiewicz K. Evaluation of metal ion concentration in hard tissues of teeth in residents of Central Poland. *Biomed Res Int*. 2017;2017:6419709. doi:10.1155/2017/6419709
49. Nowak B. Occurrence of heavy metals and sodium, potassium and calcium in human teeth. *Analyst*. 1995;120(3):747–750. doi:10.1039/an9952000747
50. Nowak B, Chmielnicka J. Relationship of lead and cadmium to essential elements in hair, teeth, and nails of environmentally exposed people. *Ecotoxicol Environ Saf*. 2000;46(3):265–274. doi:10.1006/eesa.2000.1921
51. Fischer A, Wiechuła D, Kwapuliński J. The lead content of the teeth in inhabitants of the Silesian region. *Czas Stomatol*. 2004;57(3):177–182.
52. Malara P, Fischer A, Malara B. Selected toxic and essential heavy metals in impacted teeth and the surrounding mandibular bones of people exposed to heavy metals in the environment. *J Occup Med Toxicol*. 2016;11:56. doi:10.1186/s12995-016-0146-1
53. Fischer A, Wiechuła D, Malara P, et al. The study of co-occurrence of chromium with selected elements in impacted wisdom teeth. *Czas Stomatol*. 2008;61(1):48–54.
54. Rzepnicka M, Wicher K, Szymaniak M. Disturbances in the number of teeth in youth from Legnica, in the context of microintoxication with Pb, Cu, Zn. *Stomatol Współcz*. 2002;9(4):50–54.
55. Malara P, Kwapuliński J, Drugacz J, Malara B. Impact of environmental exposure on coexistence of cadmium and zinc in teeth. *J Elem*. 2005;10(4):959–965.
56. Malara P, Kwapuliński J. Environmental conditioning of metals occurrence in teeth. *Med Śr*. 2004;7(2):113–119.
57. Malara P, Kwapuliński J, Malara B. The influence of lead on occurrence of some essential elements in teeth. *Acta Toxicol*. 2004;12(1):47–53. https://www.researchgate.net/publication/286551127_The_influence_of_lead_on_occurrence_of_some_essential_elements_in_teeth,%20access. Accessed September 10, 2024.
58. Fischer A, Malara P, Wiechuła D. The study of barium concentration in deciduous teeth, impacted teeth, and facial bones of Polish residents. *Biol Trace Elem Res*. 2014;161(1):32–37. doi:10.1007/s12011-014-0061-1
59. Fischer A, Wiechuła D, Kwapuliński J, Malara P, Łazarczyk-Henke J. Content of selenium in retained wisdom teeth [in Polish]. *Bromatol Chem Toksykol*. 2008;41(4):981–986. https://ptfarm.pl/pub/File/wydawnictwa/bromatologia/4_08/Bromat%204%20s.%200981-0986.pdf. Accessed September 10, 2024.
60. Piekut A, Moskalenko O, Gut K. Can primary teeth be an indicator of the environmental exposure of children to heavy metals? [in Polish] *Med Srod*. 2018;21(4):18–23. https://www.environmed.pl/pdf-113108-42887?filename=Can%20primary%20teeth%20be%20an.pdf. Accessed September 10, 2024.
61. Wiechuła D, Fischer A, Kwapuliński J, Loska K, Fischer T, Kurpas P. Multivariate statistical analysis of metal concentrations in teeth of residents of Silesian region, southern Poland. *Arch Environ Contam Toxicol*. 2006;51(2):314–320. doi:10.1007/s00244-004-0202-1
62. Fischer A, Wiechuła D, Postek-Stefańska L, Kwapuliński J. Concentrations of metals in maxilla and mandible deciduous and permanent human teeth. *Biol Trace Elem Res*. 2009;132(1–3):19–26. doi:10.1007/s12011-009-8383-0
63. Malara P, Łangowska-Adamczyk H, Kwapuliński J, Kyrcz-Florek Z. Occurrence of selected metals in teeth of coal-miners in the function of age and years of employment [in Polish]. *Stomatol Współcz*. 2005;12(5):25–32.
64. Kwapuliński J, Malara B, Malara P. Occurrence of selected metals in various teeth of coal miners. *Med Śr*. 2000;3(2):55–64.
65. Poczatek M, Machoy Z, Gutowska I, Chlubek D. A comparative analysis of occupational risk in industry employees based on concentrations of some elements in teeth and body fluids [in Polish]. *Ann Acad Med Stetin*. 2004;50(1):107–114. https://eurekamag.com/research/012/371/012371878.php?srsltid=AfmBOortr6G8T4l_q_Pba-1Tr1SJaTGe3FsuBEjq573FXpbbtRrMMEW7e. Accessed September 10, 2024.
66. Johnston JE, Franklin M, Roh H, Austin C, Arora M. Lead and arsenic in shed deciduous teeth of children living near a lead-acid battery smelter. *Environ Sci Technol*. 2019;53(10):6000–6006. doi:10.1021/acs.est.9b00429
67. Savabieasfahani M, Ali SS, Bacho R, Savabi O, Alsabbak M. Prenatal metal exposure in the Middle East: Imprint of war in deciduous teeth of children. *Environ Monit Assess*. 2016;188(9):505. doi:10.1007/s10661-016-5491-0
68. Haavikko K, Anttila A, Helle A, Vuori E. Lead concentrations of enamel and dentine of deciduous teeth of children from two Finnish towns. *Arch Environ Health*. 1984;39(2):78–84. doi:10.1080/00039896.1984.10545839
69. Shishniashvili TE, Suladze NN, Margvelashvili VV. Primary teeth and hair as indicators of environmental pollution. *J Clin Pediatr Dent*. 2016;40(2):152–155. doi:10.17796/1053-4628-40.2.152
70. Tsuji LJ, Karagatzides JD, Katapatuk B, et al. Elevated dentine-lead levels in deciduous teeth collected from remote first nation communities located in the western James Bay region of northern Ontario, Canada. *J Environ Monit*. 2001;3(6):702–705. doi:10.1039/b106163c
71. van Wyk CW, Grobler SR. Lead levels in deciduous teeth of children from selected urban areas in the Cape Peninsula. *S Afr Med J*. 1983;63(15):559–562. PMID:6845044.
72. Lyngbye T, Hansen ON, Grandjean P. Predictors of tooth-lead level with special reference to traffic. A study of lead-exposure in children. *Int Arch Occup Environ Health*. 1990;62(6):417–422. doi:10.1007/BF00379057
73. Gomes VE, da Luz Rosário de Sousa M, Barbosa F Jr, et al. In vivo studies on lead content of deciduous teeth superficial enamel of preschool children. *Sci Total Environ*. 2004;320(1):25–35. doi:10.1016/j.scitotenv.2003.08.013
74. Fernandes ACBCJ, França R. Nanometals and metal ion pollution from dental materials in dental environment. *Micro*. 2023;3(2):471–483. doi:10.3390/micro3020031
75. Malara P, Łangowska-Adamczyk H, Kwapuliński J, Malara B. The effect of cigarette smoking on the presence of cadmium and lead in the hard tissues of the teeth [in Polish]. *Czas Stomatol*. 2004;57(2):118–124.
76. Malara P, Kwapuliński J, Malara B, Drugacz J. Effect of passive smoking on the level of selected metals in deciduous teeth [in Polish]. *Przegl Lek*. 2006;63(10):1002–1006. PMID:17288202.
77. Malara P, Kwapuliński J, Drugacz J. Lead and cadmium occurrence in deciduous teeth of children exposed to cigarette smoke in apartments [in Poland]. *Przegl Lek*. 2004;61(10):1122–1125. PMID:15794269.
78. Malara P, Kwapuliński J, Drugacz J, Malara B. Effect of cigarette smoking on coexistence of cadmium and zinc in retained wisdom teeth [in Polish]. *Przegl Lek*. 2005;62(10):1072–1074. PMID:16521956.
79. Alhasmi AM, Gondal MA, Nasr MM, Shafik S, Habibullah YB. Detection of toxic elements using laser-induced breakdown spectroscopy in smokers' and nonsmokers' teeth and investigation of periodontal parameters. *Appl Opt*. 2015;54(24):7342–7349. doi:10.1364/AO.54.007342

80. Olovčić A, Ramić E, Memić M. Human enamel and dentin: Effect of gender, geographic location, and smoking upon metal concentrations. *Anal Lett*. 2019;53(2):245–261. doi:10.1080/00032719.2019.1646753
81. Fischer A, Kwapuliński J, Nogaj E, Ahnert B, Rzepka J. The important mean of passive smoking for lead and chromium content in deciduous teeth [in Polish]. *Przegl Lek*. 2009;66(10):669–672. PMID:20301907.
82. Fischer A, Wiechuła D, Kwapuliński J. Evaluation of the passive smoking effect on metal content in deciduous teeth. *J Stomatol*. 2011;64(8):620–630.
83. Malara P, Kwapuliński J, Malara B. The influence of smoking on cationic equilibrium in men's teeth. *J Elem*. 2004;9(3):413–419.

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