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2. McKeage K. Tobramycin inhalation powder: a review of its use in the treatment of chronic pseudomonas aeruginosa infection in patients with cystic fibrosis. Drugs 2013; [Epub ahead of print] Available from: http://link.springer.com/article/10.1007%2 Fs40265-013-0141-0

Book

3. Mueller HJ, Freeman D. FT-IR spectrometry in materiolography. 2nd Ed., Ohio: American Society for Metal 1994, p.51-56.

Chapter in a book

4. Alexander RG. Considerations in creating a beautiful smile. In: Romano R, editor. The art of the smile. London: Quintessence Publishing, 2005, p.187-210.
5. Hudson FB, Hawcroft J. Duration of treatment in phenylketonuria. In: Seakins J, Saunders R, editors. Treatment of inborn errors of metabolism. London: Churchill Livingstone, 1973, p.51-56.

Thesis

6. Maden I. Effect Of Nd:YAG Laser Treatment In Addition To Scaling And Root Planning. Doctoral Dissertation, Istanbul University Institute of Health Sciences Periodontology Department, 2009.

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Figure 1. Panoramic radiograph of the patient taken 6 months after surgery, note irregular borders of the lesion.

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Table 1. *Concise explanation of the table contents (SD: standard deviation, CTA: cartilage tissue area, NBA: new bone area).*

	Control group (Mean % ± SD %)	First group (Mean % ± SD %)	Second group (Mean % ± SD %)
CTA	21.41 ± 4.2	2.5 ± 2.4	11.42 ± 4.2
NBA	11.48 ± 0.2	21.41 ± 14.22	11.41 ± 4.2

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Original research

Development of a scale for measuring university students' attitudes toward oral health

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Purpose
Valid and reliable scales which have been developed for assessing individuals' attitudes toward oral health and are based on multiple theoretical views are limited in number in the literature. Hence, there is a need for more scale development studies for further evaluation of the psychometric properties of the oral and dental health attitudes of students. The aim of this study was to develop a scale for measuring college undergraduates' attitudes toward oral and dental health.

Materials and Methods
A sample of 770 college undergraduates (241 male, 529 female) enrolled in various academic programs of three universities in Turkey participated in this study. We collected data from two separate samples. The data obtained from sample 1 (n = 470) were used for Exploratory Factor Analysis (EFA) and the data from sample 2 (n = 300) were utilized for undertaking Confirmatory Factor Analysis (CFA). To test the construct validity, EFA, CFA, convergent validity, and measurement invariance were used, respectively.

Results
In the first stage, EFA was conducted on a 48-item scale. EFA results showed that the final version of the Oral Health Attitude Scale (OHA-S) had a six-factor structure: sensitivity, importance, avoidance of harmful elements, tendency towards products and activities, awareness, and social impact. To confirm this structure, CFA was used. CFA results showed good model fit indexes. The final version of the scale consisted of 41 items with six factors. Moreover, Cronbach's alpha and Spearman-Brown Split-Half coefficients showed a good level of reliability. Moreover, t-scores were statistically significant for 27% of the lower and upper groups.

Conclusion
The developed scale was found to be a potential tool for measuring and evaluating university students' attitudes toward oral and dental health.

Keywords: *Attitude, oral and dental health, scale development, university*

Introduction

Oral and dental problems, which are among the most common issues worldwide, are critical concerns that affect the overall health of individuals (1). These diseases which create a serious economic burden on individuals reduce the quality of their lives (2). Oral diseases can cause serious health problems. The most prominent factors associated with oral and dental diseases are hygiene, tobacco use, alcohol, nutritional status, and stress. Oral hygiene is the most important of these factors in terms of preventing oral diseases (3). The general condition and quality of life of the individuals are adversely affected by poor oral hygiene (4). As a result of this situation, the deterioration of the balance in the oral environment causes dental caries (2). Dental caries have been reported globally as one

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of the most common oral diseases affecting all age groups (2) and the quality of life of individuals (5). Therefore, dental caries are an important problem that should be taken into account because they cause time and financial losses (6).

Individuals with oral and dental fear typically have poorer oral hygiene and a higher incidence of oral diseases. As a result, they avoid visiting the dentist and tend to have longer intervals between dental visits (7). In a previous study, it was stated that individuals with dental anxiety or dental fear had more decayed and missing teeth which prevented them from going to the dentist (8). Good oral health plays an essential role in the individuals' functions such as speaking, smiling, and making creative contributions to society which are impactful in the general well-being of the individual (9). In addition to clinician-related factors, (10) cognitive, affective, and behavioral characteristics of the individuals are also effective in preventing oral and dental diseases (11). Attitude, which emphasizes the combination of these features, is directly related to positive or negative tendencies toward oral and dental health. Specifically, attitude is one of the important determinants in explaining human behavior (12). It is described as a mental posture (13) or psychological tendencies (14) toward a specific object, person, issue, event, or institution. Attitude gives information about the tendencies of individuals about how to behave in a certain situation. It can lead to positive or negative tendencies in their behavior (15). Some attitude theories, including ABC Model (16), Planned Behavior Model (14), Broaden-and-Build Theory of Positive (17), and Health Belief Model (18) provide evidence for its conceptual frameworks suggesting that attitude can result in behavioral changes. A prevalent tripartite model of attitude highlights that it consists of three components (14,15): cognitive component (values, knowledge, beliefs, and thoughts), affective component (emotional or feeling segment of attitudes), and behavioral component (person's tendencies and intentions for behavioral reaction). The formation of attitude depends on the result of the interaction, combination, and organization of these three factors.

The development of positive attitudes toward oral and dental health in individuals is crucial to improving general health at the macro level and ensuring oral health and hygiene at the micro level (19). In a previous study, an instrument was developed to evaluate the patients' oral health attitudes and their tooth brushing habits (20). Importantly, the most common instruments used to measure individuals' attitudes, beliefs, and thoughts are Likert-type scales (21). Since attitudes are tendencies that individuals can learn and acquire later, it is possible to direct their tendencies by determining their existing tendencies. Although early ages make up the critical developmental period for the development of health literacy, it is important to change and guide individuals' stereotypical tendencies toward oral and dental health at later ages. Moreover, it is a necessity for them to develop these beliefs and attitudes in a more conscious way in order to set an example for other individuals at a young age.

In light of this information, valid and reliable instruments are necessary for identifying individuals' attitudes toward oral and dental health. A previous study highlighted the scarcity of instruments for university students or adults (22). Kirtiloglu and Yavuz stated that self-preventive oral behavior among Turkish non-dental university students was at a lower level compared to industrialized countries (23). Moreover, the results of another study indicated that increased oral health education was necessary for and could be effective in improving oral health in Turkey (24). While several studies have reported on oral hygiene habits among children, adolescents, adults, and university students, there is a scarcity of research focusing on non-dental university students (23,25). Although several instruments have been developed for assessing attitudes toward oral and dental health, valid and reliable scales based on multiple theoretical views are limited in the literature. Hence, there is a need for more scale development studies for further evaluation of the psychometric properties of oral and dental health attitudes of students. Therefore, the aim of this study was to develop a scale for measuring college undergraduates' attitudes toward oral and dental health.

Material and Methods

Ethical approval

The study was approved by the institutional board of Bartin University Ethics Committee (Protocol No: 2023-SBB-0120) and informed consent was taken from each of the participants.

Study sample

A total of 770 college undergraduates (241 male and 529 female) enrolled in various academic programs at three universities in Turkey participated in the current study. All of the participants gave their voluntary consent to take part in the study. Data were collected from two samples. The first sample was composed of 470 students (346 females, 124 males) between 18 and 27 years (M = 20.18, SD = 1.18) while the second sample was comprised of 300 students (183 females, 117 males) between 18 and 28 years (M = 20.86, SD = 1.5). Convenience sampling was used to select the participants. It is preferable and feasible for implementation when there are limitations in time, cost, and labor (26). These students were enrolled in various academic programs (such as education, engineering, humanities, sports, and medicine) at Usak University (n = 302), Bolu Abant Izzet Baysal University (n = 255), and Bartin University (n = 213) located in Turkey.

Sample size estimation

To estimate the sample size, G*Power software (version 3.1) was used by taking into consideration the assumption of detecting large effect size and setting up a two-tailed hypothesis. The analysis results showed that a minimum of 565 participants are required for this study ($\alpha = 0.05$; $\beta = 0.95$).

Developing the scale

We adopted Boateng *et al.*'s (27) three phases and nine steps as a guide in scale development and reporting: (i) item development, (ii) scale development, and (iii) scale evaluation. Although the first phase, item development, includes (1) the identification of the domain(s) and item generation, and (2) the consideration of content validity, the second

phase consists of (3) pre-testing questions, (4) sampling and survey administration, (5) item reduction, and (6) extraction of latent factors. The last phase, scale evaluation, requires (7) the tests of dimensionality, (8) the tests of reliability, and (9) the tests of validity. Firstly, to determine the conceptual structure, literature review was conducted to determine the existing attitude scales about oral and dental health (18, 20, 28). Then, these scales were analyzed to identify their factor structures.

Initially, the first version of the scale included 52 items consisting of general factors. To provide the face and content validity, 12 experts with Ph.D. degrees from restorative dentistry (n = 3), pediatric dentistry (n = 2), oral and maxillofacial radiology (n = 1), periodontology (n = 1), orthodontics (n = 1), measurement and assessment (n = 2), and psychology (n = 2) evaluated the scale items. They were sent an expert evaluation form in a 4-point Likert-type format about the scale items involving the statements ranging from "irrelevant" (1) to "highly relevant" (4). There was also a section in the form where experts could write their suggestions and evaluations on the items.

Based on their feedback, some items were revised or excluded from the scale (e.g., the item including the statement "A natural appearance of my teeth is important to me" was revised as "The natural and esthetic appearance of my teeth is important to me"). Specifically, the content validity ratio (CVR) was found to be 0.83. In accordance with Ayre and Scally's criteria, (29) CVR was not less than 0.66 for 12 experts. Factor analysis and item statistics were conducted for the construct validity of the measurement instrument. A pilot application was employed for the extraction of discriminability levels and the identification of items that represent the construct effectively. Moreover, the item-content validity index (I-CVI) was calculated for each item. The number of experts who rated each item as 3 or 4 regarding their relevancy was divided by the total number of experts to calculate the I-CVI. According to a previous study, (30) I-CVI should not be less than 0.78. The content validity index (S-CVI) was calculated to prove the content validity of the entire scale. Shrotryia and Dhanda (31) recommended that the S-CVI should not be less than 0.80. In this study, it was found to be 0.85. Irrelevant and redundant items were excluded from the draft scale resulting in an initial scale consisting of 48 items. While preparing the scale items, we paid attention to make the items simple and understandable. Items did not contain more than one thought, judgment, or feeling. Moreover, a language expert evaluated the items for spelling and grammar; 8 university students read the scale items for comprehensibility. The initial version of the scale had a 5-point Likert type response format ranging from strongly disagree (1) to strongly agree (5). The middle option (3) was "somewhat agree" instead of "neutral". It is a commonly used type of Likert scale in the measurement of psychometric constructs (32). The age of the participants has an important place in determining the rating on Likert scales. As the age groups of the participants decrease, there are difficulties in distinguishing multi-level scales (33). For this reason, we preferred this Likert type for university students. Higher scores indicated more positive attitudes toward oral and dental health. Since all items were positive, there were no reverse-scored items in the scale. Figure 1 shows the developmental process of OHA-S.

Procedure

Data were collected between March 15, 2023 and April 15, 2023. The scales were administered to 304 students in a paper-pencil format and 466 students in an online format via Microsoft Forms link by using a QR code. There was no missing data in the completed forms. All the non-dental university students willing to participate in the study were considered in the inclusion criteria. The exclusion criteria includ-

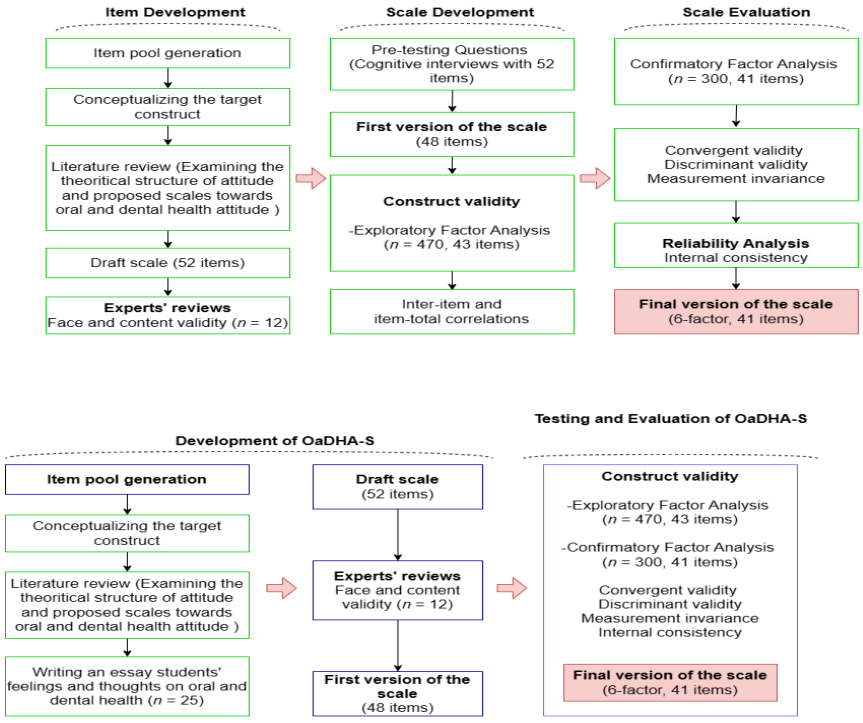

Figure 1. *Flowchart of the OHA-S development process.*

ed students who did not give consent to participate in the study. Firstly, the participants read the consent form for their voluntary and anonymous participation in the study. Then, they were informed about the purpose of the questionnaire. Completing the scale lasted approximately 10 minutes.

Data analysis

Construct validity of OHA-S was analyzed with EFA on SPSS V25.0 (IBM, Armonk, NY, USA). Kaiser-Meyer-Olkin (KMO) test for the sample adequacy and Bartlett sphericity statistics for the suitability of the data were run. Although principal component analysis was used as the extraction method, varimax was used as the rotation technique. Structural validity was subsequently tested using the CFA. It was performed using LISREL (v.8.80) with the Robust Maximum Likelihood (R-ML) method by assessing the fit indices which should be lower than 3 for Chi-square/ degree of freedom (*χ2/df*) (34), higher than 0.90 for Comparative Fit Index (*CFI*), Non-Normed Fit Index (*NNFI*) and Tucker Lewis Index (*TLI*) (35), and lower than 0.08 for Root-Mean-Square Error of Approximation (*RMSEA*) and Standardized Root Mean Square Residual (*SRMR*) (36). Cronbach's alpha (*α*) and split-half coefficients (corrected by Spearman-Brown) were calculated for displaying the internal consistency for the overall scale and its each factor. The discrimination of the items of the scale was analyzed based on the lower and upper (27%) groups by using independent samples *t*-test. To check whether the convergent validity was supported, Average Variance Extracted (*AVE*) ≥ 0.50 and Composite Reliability (*CR*) ≥ 0.70 for each factorial structure were computed (37, 38). As a requirement of convergent validity, factor loads for each item must be greater than 0.50 and the level of significance must be less than 0.05 (36). The absence of bias in the responses provided by both female and male participants and the establishment of the validity of the instrument's structure based on gender were examined in terms of measurement invariance. For testing the measurement invariance, multigroup CFA was used. Moreover, *ΔCFI, ΔRMSEA,* and *ΔSRMR* were used to investigate whether the measurement invariance was supported across the two groups of gender. Expected difference less than 0.01 in the *ΔCFI* value supports the less parameterized model (39).

Results

Factor structure of OHA-S (EFA Results)

To ensure construct validity, EFA was conducted on the 48 items. Kaiser-Meyer-Olkin (KMO) measure conducted prior to EFA was found to be 0.944 indicating sampling adequacy for this data set and Bartlett's test was statistically significant ($\chi 2 = 17520.615$ $df = 1128$, $p < 0.001$). Six-factor structure of the scale with Eigenvalues above 1 accounted for 51.6% of the total variance. For multifactorial structures, 40% or more of the total variance explained is considered sufficient (40). Items with factor loadings of 0.40 or above were retained in the scale, (41) while 5 items indicating poor factor loadings were removed from it. Factor loadings ranged from 0.43 to 0.75. Subsequently, the factors were named in accordance with the items represented by each structure. The factors were identified as sensitivity for OH (12 items), importance of OH (6 items), avoidance of harmful elements for OH (7 items), tendency towards products and activities on OH (7 items), awareness of OH (6 items), and social impact (5 items). The item-total correlations were investigated to reveal the consistency of the items in each factor. Results indicated that all item-factor correlations ranging from 0.56 to 0.85 were above the threshold of 0.30 (42). Table 1 shows EFA results including the factor loadings for each factor.

Confirmative factor analysis (CFA) results

To confirm the proposed six-factor structure of the OHA-S with 43 items, CFA was carried out using a separate sample ($n = 300$). Since the relative multivariate kurtosis value was found to be greater than 1 when the assumption of multivariate normality was examined for the first-order six-factor OHA-S, the R-ML method was used. As the factor loadings of items 7 and 28 were calculated as 0.22 and 0.14 respectively, they were excluded from the analysis. The model fit indexes were checked with 41 items. Since the proposed six sub-factors had high correlations between each other, they were moved up to the second-order level and the model was re-tested. Second-order model represented a good model fit ($\chi 2/df = 10237.35/813$; $p < 0.001$; $RMSEA = 0.080$; $GFI = 0.90$; $AGFI = 0.90$; $CFI = 0.91$; $NNFI = 0.90$). Therefore, the structural model of the scale was accepted and found to be significant ($p < 0.001$) (Figure 2). Although the chi-square value was influenced by the sample size and did not support the model fit, *RMSEA, GFI, AGFI, CFI,* and *NNFI* values supported the model fit. Finally, the calculated factor loadings varied between 0.30 and 0.80.

Internal Consistency Reliability of the OHA-S

We used the overall sample to examine the internal consistency of OHA-S. It had good internal consistency ($\alpha = 0.92$). Each of its six sub-factors also had high or acceptable internal consistency as follows: factor1 ($\alpha = 0.89$), factor2 ($\alpha = 0.85$), factor3 ($\alpha = 0.84$), factor4 ($\alpha = 0.83$), factor5 ($\alpha = 0.80$), and factor6 ($\alpha = 0.77$). The coefficient of internal consistency (split-half, corrected by Spearman-Brown) of the overall scale was calculated as 0.91. These values of the factors were computed as 0.86, 0.90, 0.81, 0.82, 0.81, and 0.75, respectively. The findings showed that the split-half coefficients were high or acceptable level. Moreover, the item discrimination was conducted by using the independent samples *t*-test (comparison of 27% lower-upper groups). Analysis results were found to be statistically significant ($p < 0.001$). Hence, *t* values ranged between 23.78 and 34.79 for factor1, 23.41 and 30.96 for factor2, 45.55 and 63.26 for factor3, 49.91 and 68.37 for factor4, 20.62 and 61.224 for factor5, and 50.19 and 69.99 for factor6. These results showed that the items in the scale had acceptable internal consistency and high discrimination in terms of attitudes toward OH.

Convergent and discriminant validity

AVE and CR values were calculated by examining all factor loading values calculated in the EFA. The findings indicated that the desired limit value was not exceeded ($AVE_1 = 0.36$; $AVE_2 =$

Table 1: *Factor loadings for each factor*

Item	Factor					
	I	II	III	IV	V	VI
#Item29. I want my teeth to be white.	0.71					
#Item39. I am worried about losing even one of my teeth.	0.70					
#Item34. When I brush my teeth, I feel relaxed.	0.70					
#Item31. I feel good when my teeth or gums are healthy.	0.66					
#Item30. My oral and dental health problems worry me.	0.64					
#Item43. I take care of my toothbrush and change it at least once a year.	0.61					
#Item40. When others smile, I notice whether their teeth have an esthetic appearance or not.	0.59					
#Item21. I take care of my oral and dental health so that there is no bad breath.	0.57					
#Item37. I am worried about discoloration of my teeth.	0.56					
#Item22. I take care to brush my teeth at least twice a day.	0.45					
#Item24. When choosing my toothbrush, I take care of several features such as hardness, softness, and shape of its bristles.	0.43					
#Item20. When my dental and oral treatment is completed, I feel happy.	0.43					
#Item3. I care about my teeth as much as my other organs in my body.		0.74				
#Item4. Oral and dental health is important for a good smile.		0.71				
#Item2. I want to have straight teeth in terms of esthetic.		0.68				
#Item5. I pay attention to my oral and dental care in order not to experience oral and dental problems.		0.66				
#Item1. Oral and dental care is important for general body health.		0.65				
#Item9. The natural and esthetic appearance of my teeth is important to me.		0.62				
#Item36. I avoid damaging foods or beverages causing tooth erosion.			0.75			
#Item14. I avoid excessive consumption of foods or beverages that cause discoloration on my teeth.			0.72			
#Item33. I avoid sugary foods due to their damage to my teeth.			0.71			
#Item23. I avoid extremely hot/cold foods or beverages due to their damage to my teeth.			0.71			
#Item8. I try to avoid foods or beverages that cause dental caries.			0.64			
#Item27. I take care to consume foods that strengthen my teeth.			0.60			
#Item26. I take care not to smoke to protect my oral and dental health.			0.46			
#Item6. New products related to oral and dental health attract my attention.				0.69		
#Item10. I am interested in the promotion or advertisement of products on oral and dental health.				0.68		
#Item44. Programs, news, and events related to oral and dental health in the media attract my attention.				0.60		
#Item11. I purchase my toothpaste by checking its ingredients.				0.57		
#Item35. I am willing to participate in seminars and trainings on oral and dental health.				0.55		
#Item7*. I try to be an example to those around me by paying attention to my oral and dental health.				0.50		
#Item18. I periodically use mouthwash for my oral and dental health.				0.44		
#Item16. Dental floss use helps me maintain my oral and dental health.					0.66	
#Item17. Teeth scaling is periodically necessary for gingival health.					0.61	
#Item25. Besides brushing my teeth, I am willing to use dental floss regularly.					0.60	
#Item45. I am willing to go to the dentist regularly for oral and dental health check-up.					0.52	
#Item46. I avoid breaking hard-shelled foods with my teeth.					0.49	

#Item47. I am willing to learn the necessary information to protect my oral and dental health.	0.43
#Item42. I feel jealous when someone's teeth are prettier than my teeth.	0.64
#Item19. I care about what other people think of my teeth.	0.59
#Item48. Even if it's a joke, it makes me very sad when my teeth made fun of my teeth.	0.55
#Item41. I am pleased to show my teeth to other people when I smile.	0.54
#Item28*. Good oral and dental health strengthens communication and socialization with people.	0.47

*Note: * After CFA, these items were removed from the scale*
Factor I="Sensitivity for OH"; Factor II="Importance of OH"; Factor III="Avoidance of Harmful Elements for OH"; Factor IV="Tendency towards Products and Activities on OH"; Factor V="Awareness of OH"; Factor VI="Social Impact".

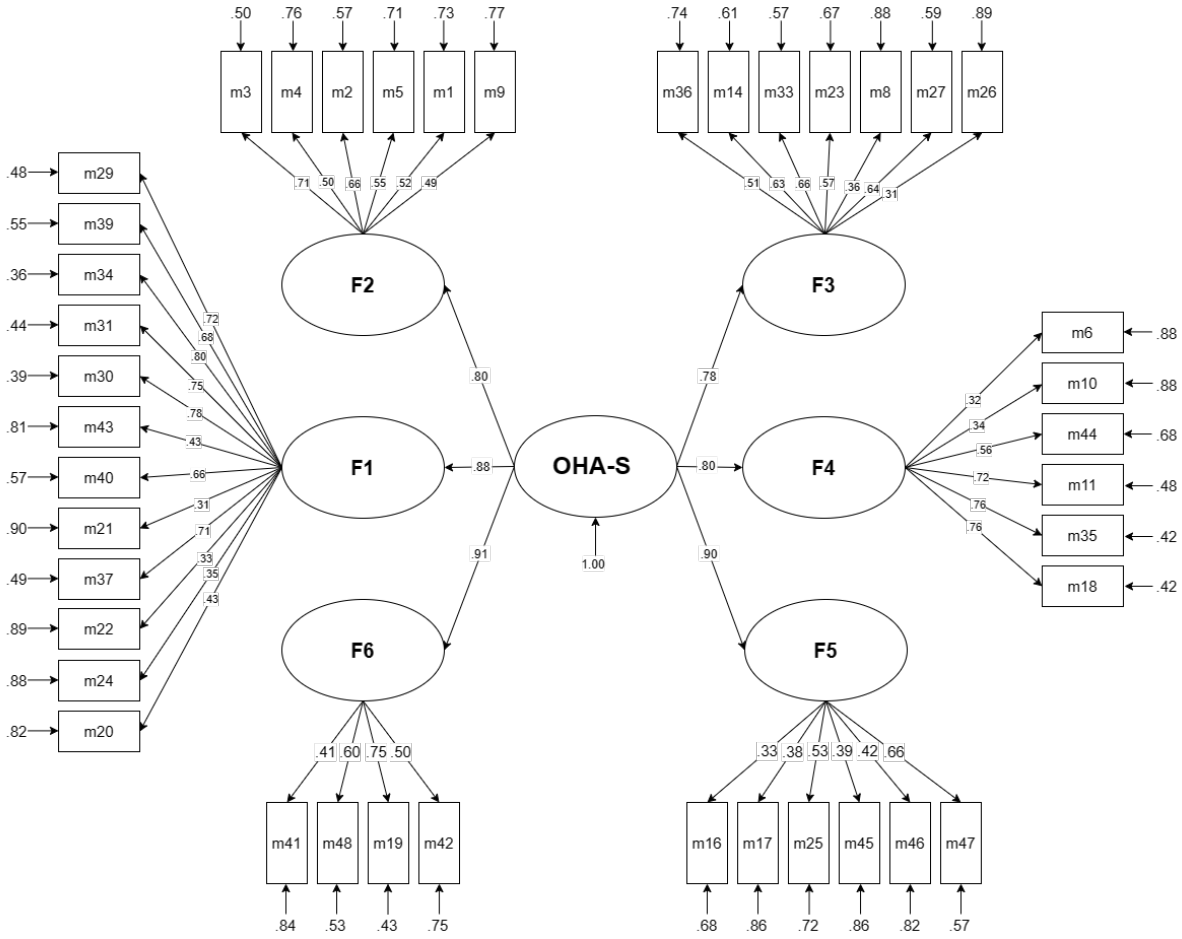

Figure 2. *Path diagram for second-order CFA.*

0.47; AVE_3 = 0.44; AVE_4 = 0.34; AVE_5 = 0.32; AVE_6 = 0.32). Moreover, CR values for all sub-factors exceeded the benchmark value and the composite reliability appeared to be high (CR_1 = 0.87; CR_2 = 0.84; CR_3 = 0.85; CR_4 = 0.78; CR_5 = 0.73; CR_6 = 0.70). When examined as a whole (CR-AVE), it was determined that the convergent validity value was mainly provided by CR. To carry out the discriminant analysis, we examined the correlations between all sub-factors and calculated partial AVE square root values. By using these partial correlation coefficients, we compared the square root values of the partial AVE. All partial coefficients were not bigger than the square root values of AVE.

Measurement invariance

As the evidence of construct validity, measurement invariance was discussed according to different demographic characteristics of the developed measurement tool and the meaning/response status of the items. Measurement invariance was examined with multigroup DFA to determine whether the fit of the model differed in terms of the gender variable (241 male, 529 female). For the model, the model fit results for configural invariance, metric invariance, and scalar invariance were examined. It was determined that figural and metric invariances were provided for the established model, but scalar invariance for the model was not achieved. It was observed that the difference values of the fit indices examined in the multigroup CFA could not be obtained with scaler invariance *(ΔGFI* (<) 0.01, *ΔSRMR* (>) 0.01, *ΔCFI* (>) 0.01, (Table 2).

After examining the measurement invariance, cut-off scores were determined according to the scale levels to ensure the usability of the scale and to make sense of the scores. Cut-off scores were examined with cluster analysis because a score

Table 2: *Examination of measurement invariance with Multigroup-CFA results*

Measurement Invariance	χ^2	df	RMSEA	GFI	CFI	SRMR
Configural invariance	10123.35*	1575	0.08	0.91	0.90	0.041
Metric invariance	10220.28*	1629	0.08	0.91	0.90	0.045
Scaler invariance	10231.14*	1625	0.08	0.90	0.85	0.056
Metric - Configural	96.93	54	0.08	0	0	0.004
Scaler - Configural	107.79	50	0.08	-0.01	0.05	0.015
Scaler - Metric	10.86	4	0.08	-0.01	0.05	0.011

*$p < .001$, *Acceptable fit indices: RMSEA ≤ 0.08; GFI ≥ 0.90; CFI ≥ 0.90; SRMR ≤ 0.08* (34–36)

Table 3: *Levels and score ranges of OHA-S*

Levels	Score ranges
Very low	41-101
Low	102-144
Improvable	145-160
High	161-184
Very high	185-205

range was not previously specified as a criterion. The score ranges of the five levels were determined. The lowest score of the scale is 41 and the highest score is 205. There are no reverse items. After the cluster analysis, the levels were named as "very low", "low", "improvable", "high" and "very high". Table 3 shows these levels and score ranges. When Table 3 is examined, the scores between 41-101 points are at a very low level for OHA-S, scores between 102-144 points are at a low level, scores between 145-160 points are at an improvable level, scores between 161-184 points are at a high level, and scores between 185-205 points are at a very high level. It was converted into a standard score at a high level.

Discussion

The current study sought to develop a scale for measuring college undergraduates' attitudes toward OH. It provided more evidence for the validity and reliability of the OHA-S and indicated acceptable psychometric features including OH knowledge. The items were initially created based on the review of the literature and the essays about students' feelings and thoughts. The draft scale consisted of 52 items, all scored on a 5-point Likert-type scale ranging from "strongly disagree-1" to "strongly agree-5". Based on the experts' feedback, the initial scale consisted of 48 items.

Good oral health is considered an important factor contributing to an individual's overall health status (43). There is a growing worldwide awareness of the fact that comprehensive health services should also include oral health (44). Oral health is not only about an individual's smile and esthetics but it has also been noted for its social impact on the individuals by resulting in low self-confidence and negatively affecting their quality of life in various ways (45). Dental caries and complications can significantly reduce human life quality and create a major economic burden by triggering systemic diseases (46). Adequate oral hygiene helps to increase individuals' self-esteem, making their quality of life better. Individuals' oral and dental health concern depends on their awareness of it and strongly influences their oral health status (47). A previous study indicated that a good oral health knowledge level helps to develop a positive attitude towards oral health (48). Several studies focused on oral health knowledge, attitude, and behavior (28) how much individuals pay attention to oral health care and how much individuals invest in oral health care (oral health values) (49), and, lastly, patients' oral and dental health attitudes and their tooth brushing behaviors (20). Although dental students were included in some studies (50, 51), students participating in our study were from different academic programs, not from the dentisstry program. Unlike other studies, this study focused on attitude and sub-factors of attitude towards OH. In the first stage, OHA-S had 48 items. To ensure the structure validity of the scale, EFA was used. As a result of the EFA, it was grouped into six factors with 43 items.

The first factor, sensitivity for OH, included susceptible reactions and feelings such as getting worried, conscientiousness, and feeling good about OH. The second factor, the importance of OH, was related to paying attention to OH and considering it important for general health and physical appearance. The third factor was named avoidance of harmful elements for OH. Essentially, avoidance occurs when individuals develop an attitude that leads them not to exhibit a certain behavior as a result of associating it with an unpleasant situation. In this sense, individuals may avoid certain behaviors for their OH. The fourth factor, tendency toward products and activities on OH, is related to factors such as directing attention to oral and dental health products, their contents, and information about the subject in the media, and selective perception. With the dynamic and directive effect of the attitude, the individual focuses on certain ones among multiple stimuli. The fifth factor, awareness of OH, was related to being aware of the basics of OH such as toothbrushing, literacy, and check-up. The last factor, social impact, refers to the reactions of others about the teeth of the individual or the formation of trends toward oral and dental health by comparison with others. According to Bandura's Social Cognitive Learning Theory (52), individuals develop new reactive tendencies by observing the reactions of others cognitively. This indicates that the beliefs underlying attitudes are socially constructed. The results provided evidence for the widely accepted contents of cognitive, affective, and behavioral components (53, 54). Cut-off scores for standard score ranges were created. The cluster analysis results showed that the attitude level was divided into five intervals as "very low", "low", "improvable", "high", and "very high".

To confirm this structure, CFA was used. CFA results indicated that the scale had an acceptable model fit and the factor loadings of two items were also calculated lower than 0.30. After these items were removed from the scale, we proposed a scale with 41 items (Appendix-A) (Turkish version; Appendix-B) (English version; Appendix-C) grouped under the aforementioned six factors (sensitivity, importance, avoidance of harmful elements, tendency towards products and activities, awareness, and social impact) (Appendix-B and Appendix-C show a rearranged version of the item rankings in the scale). While the lowest score from the overall scale was 41, the highest score was 205. OHA-S had high internal consistency. Moreover, the overall scale and its sub-factors had good internal consistency, convergent validity, and discriminant validity. In this study, the development process of OHA-S, though not being the first scale developed (22), was comprehensive and, from a multi-view perspective of attitude, it is one of the few scales developed to assess the attitudes toward OH and to understand underlying behavior and intentions in this direction.

There are several limitations of the current study. First, the sample was comprised of college undergraduates and the obtained data was specific to one country. Cultural differences could limit the generalizability of the results. With further validation and use, further studies should be performed on larger samples in different countries or for cross-culture comparisons. Second, predictive validity and test-retest reliability verifications were not performed in this study. Third, further research should be conducted on specific populations such as adults and the elderly. While further studies are needed, the development of a more contemporary assessment tool for OHA may provide opportunities for comprehensive epidemiological research and future intervention.

Conclusion

The OHA-S had good indexes of content validity, construct validity, and internal consistency. Psychometric characteristics of the OHA-S that were reported in the current study indicated that it is a potential tool for measuring and assessing college undergraduates' attitudes toward OH. There is a need for research using tools such as OHA-S to determine the oral and dental health needs of individuals and the effects of dental problems on their attitudes. Further research should be performed to strengthen the scale characteristics.

Türkçe özet: *Üniversite Öğrencilerinin Ağız Sağlığına Yönelik Tutumlarının Belirlenmesi için Ölçek Geliştirilmesi. Amaç: İlgili literatürde ağız sağlığına yönelik tutumu değerlendirmek için geliştirilen, çoklu teorik görüşlere dayanan geçerli ve güvenilir ölçekler sınırlı sayıdadır. Bu nedenle, öğrencilerin ağız ve diş sağlığına yönelik tutumların psikometrik özelliklerinin incelenmesine yönelik daha çok ölçek geliştirme çalışmasına ihtiyaç vardır. Mevcut araştırmanın amacı, üniversite öğrencilerinin ağız ve diş sağlığına yönelik tutumlarını ölçmek için geçerli ve güvenilir bir ölçme aracı geliştirmektir. Gereç ve Yöntem: Araştırmaya Türkiye'deki üç üniversitenin çeşitli akademik programlarına kayıtlı 770 üniversite öğrencisi (241 erkek, 529 kadın) katılmıştır. Araştırmanın verileri iki ayrı örneklemden toplanmıştır. İlk örneklemden (n = 470) elde edilen veriler Açıklayıcı Faktör Analizi (AFA) için, ikinci örneklemden (n = 300) elde edilen veriler ise Doğrulayıcı Faktör Analizi (DFA) için kullanıldı. Ölçeğin yapı geçerliliğini test etmek için sırasıyla AFA, DFA, yakınsak geçerlilik ve ölçme değişmezliği kullanılmıştır. Bulgular: İlk aşamada, 48 maddelik ölçek üzerinde AFA yapılmıştır. AFA sonuçları, Ağız Sağlığı Tutum Ölçeği›nin (AST-Ö) altı faktörlü bir yapıya sahip olduğunu göstermiştir: duyarlılık, önem, zararlı unsurlardan kaçınma, ürün ve faaliyetlere eğilim, farkındalık ve sosyal etki. Ölçeğin AFA sonucunda ortaya çıkan yapısını doğrulamak için DFA gerçekleştirilmiştir. DFA sonucunda elde edilen uyum indekslerinin kabul edilebilir olduğu görülmüştür. Ölçeğin son hali, altı faktörlü 41 maddeden oluşmaktadır. Ayrıca Cronbach's alpha ve Spearman-Brown Split Half (yarıya bölme) değerleri iyi düzeyde güvenilirliğe sahip olduğunu göstermiştir. Ayrıca %27 alt-üst gruplar için t puanları istatistiksel olarak anlamlıdır. Sonuç: Geliştirilen ölçeğin üniversite öğrencilerinin ağız ve diş sağlığına yönelik tutumlarını ölçmek ve değerlendirmek için potansiyel bir araç olduğu görülmüştür. Anahtar Kelimeler: Tutum, ağız ve diş sağlığı, ölçek geliştirme, üniversite*

Ethics Committee Approval: The study was approved by the institutional board of Bartin University Ethics Committee (Protocol No: 2023-SBB-0120) and informed consent was taken from each of the participants.

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: MF, CM, MF participated in designing the study. MF, CM, MF participated in generating the data for the study. MF, CM, MF participated in gathering the data for the study. CM, MF participated in the analysis of the data. MF, MF wrote the majority of the original draft of the paper. MF, CM, MF participated in writing the paper. MF, CM, MF has had access to all of the raw data of the study. MF, CM, MF has reviewed the pertinent raw data on which the results and conclusions of this study are based. MF, CM, MF have approved the final version of this paper. MF, CM, MF guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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APPENDIX

Appendix A. *Turkish version of Oral Health Attitude Scale*

Factor-1: Sensitivity/Duyarlılık (12 items)

- Dişlerimin beyaz olmasını arzu ederim.
- Dişlerimden birini bile kaybetmek beni endişelendirir.
- Dişlerimi fırçalamak beni rahatlatır.
- Dişlerimin/dişetlerimin sağlıklı olması beni mutlu hissettirir.
- Ağız ve diş sağlığı problemlerim beni endişelendirir.
- Diş fırçamı yılda en az bir kez değiştirmeye özen gösteririm.
- Başkaları gülümsediğinde onların dişlerinin estetik görünümü dikkatimi çeker.
- Ağız kokusu olmaması için ağız ve diş sağlığıma özen gösteririm.
- Dişlerimde renklenme olması beni endişelendirir.
- Dişlerimi günde en az iki kez fırçalamaya dikkat ederim.
- Diş fırçamın seçiminde fırça kıllarının sertlik, yumuşaklık ve şekil gibi özelliklerine dikkat ederim.
- Ağız ve diş tedavim tamamlandığında mutlu hissederim.

Factor-2: Importance/Önem (6 items)

- Dişlerimi vücudumdaki diğer organlarım kadar önemserim.
- İyi bir gülümseme için ağız ve diş sağlığı önemlidir.
- Estetik açıdan dişlerimin düzgün dizilimde olmasını arzu ederim.
- Ağız ve diş sorunları yaşamamak için ağız ve diş bakımıma dikkat ederim.
- Ağız ve diş bakımı genel vücut sağlığım için önemlidir.
- Dişlerimin doğal ve estetik görünümü benim için önemlidir.

Factor-3: Avoidance of harmful elements/Zararlı unsurlardan kaçınma (7 items)

- Dişlerimi aşındıran yiyecek veya içeceklerden uzak dururum.
- Dişlerimde renklenmeye neden olan yiyecek veya içecekleri aşırı tüketmekten kaçınırım.
- Şekerli gıdalardan uzak dururum, çünkü dişlerime zarar vereceğini düşünürüm.
- Aşırı sıcak/soğuk yiyecek veya içeceklerden uzak dururum, çünkü dişlerime zarar vereceğini düşünürüm.
- Diş çürümesine neden olan yiyecek veya içeceklerden uzak durmaya çalışırım.
- Dişlerimi güçlendiren besinleri tüketmeye dikkat ederim.
- Ağız ve diş sağlığımı korumak için sigara içmemeye özen gösteririm.

Factor-4: Tendency towards products and activities/Ürün ve faaliyetlere eğilim (6 items)

- Ağız ve diş sağlığı ile ilgili yeni çıkan ürünler dikkatimi çeker.
- Ağız ve diş sağlığı ile ilgili ürünlerin tanıtım veya reklamları ilgimi çeker.
- Medyada ağız ve diş sağlığı ile ilgili program, haber, etkinlik vs. ilgimi çeker.
- Diş macunumun içeriğini kontrol ederek seçerim.
- Ağız ve diş sağlığına yönelik eğitim ve seminerlere katılmada istekliyim.
- Ağız ve diş sağlığım için belirli zamanlarda ağız gargarası kullanırım.

Factor-5: Awareness/Farkındalık (6 items)

- Diş ipi kullanmak ağız ve diş sağlığımı korumaya yardımcı olur.
- Dişeti sağlığı içinbelirli zamanlarda diş taşı temizliği yaptırmak gereklidir.
- Dişlerimi fırçalamanın yanında diş ipini de düzenli kullanmada istekliyim.
- Ağız ve diş sağlığı kontrolü için düzenli periyotlarda diş hekimine gitmede istekliyim.
- Sert kabuklu yiyecekleri dişlerimle kırmaktan kaçınırım.
- Ağız ve diş sağlığımı korumak için gerekli bilgileri öğrenmede istekliyim.

Factor-6: Social impact/Sosyal etki (4 items)

- Başkalarının dişlerinin benim dişlerimden daha güzel olmasını kıskanırım.
- Başkalarının dişlerim hakkında ne düşündüğünü önemserim.
- Şaka da olsa, dişlerimle dalga geçilmesi beni üzer.
- Gülümsediğimde dişlerimi başkalarına göstermek hoşuma gider.

Appendix B. *Ağız Sağlığı Tutum Ölçeği (AST-Ö)*

	Kesinlikle Katılmıyorum	Katılmıyorum	Kısmen Katılıyorum	Katılıyorum	Kesinlikle Katılıyorum
1-Dişlerimin beyaz olmasını arzu ederim.					
2-Dişlerimi aşındıran yiyecek veya içeceklerden uzak dururum.					
3-Dişlerimi vücudumdaki diğer organlarım kadar önemserim.					
4-Diş ipi kullanmak ağız ve diş sağlığımı korumaya yardımcı olur.					
5-Dişlerimden birini bile kaybetmek beni endişelendirir.					
6-Ağız ve diş sağlığı ile ilgili yeni çıkan ürünler dikkatimi çeker.					
7-İyi bir gülümseme için ağız ve diş sağlığı önemlidir.					
8-Başkalarının dişlerim hakkında ne düşündüğünü önemserim.					
9-Dişlerimi fırçalamak beni rahatlatır.					
10-Şaka da olsa, dişlerimle dalga geçilmesi beni üzer.					
11-Dişlerimde renklenmeye neden olan yiyecek veya içecekleri aşırı tüketmekten kaçınırım.					
12-Gülümsediğimde dişlerimi başkalarına göstermek hoşuma gider.					
13-Dişlerimin/dişetlerimin sağlıklı olması beni mutlu hissettirir.					
14-Dişeti sağlığı için belirli zamanlarda diş taşı temizliği yaptırmak gereklidir.					
15-Diş fırçamın seçiminde fırça kıllarının sertlik, yumuşaklık ve şekil gibi özelliklerine dikkat ederim.					
16-Estetik açıdan dişlerimin düzgün dizilimde olmasını arzu ederim.					
17-Ağız ve diş sağlığı problemlerim beni endişelendirir.					
18-Şekerli gıdalardan uzak dururum, çünkü dişlerime zarar vereceğini düşünürüm.					
19-Ağız ve diş sağlığı ile ilgili ürünlerin tanıtım veya reklamları ilgimi çeker.					
20-Ağız ve diş sorunları yaşamamak için ağız ve diş bakımıma dikkat ederim.					
21-Diş fırçamı yılda en az bir kez değiştirmeye özen gösteririm.					
22-Dişlerimi fırçalamanın yanında diş ipini de düzenli kullanmada istekliyim.					
23-Aşırı sıcak/soğuk yiyecek veya içeceklerden uzak dururum, çünkü dişlerime zarar vereceğini düşünürüm.					
24-Medyada ağız ve diş sağlığı ile ilgili program, haber, etkinlik vs. ilgimi çeker.					
25-Başkaları gülümsediğinde onların dişlerinin estetik görünümü dikkatimi çeker.					
26-Başkalarının dişlerinin benim dişlerimden daha güzel olmasını kıskanırım.					
27-Diş çürümesine neden olan yiyecek veya içeceklerden uzak durmaya çalışırım.					
28-Diş macunumun içeriğini kontrol ederek seçerim.					
29-Ağız kokusu olmaması için ağız ve diş sağlığıma özen gösteririm.					
30-Ağız ve diş sağlığı kontrolü için düzenli periyotlarda diş hekimine gitmede istekliyim.					
31-Ağız ve diş bakımı genel vücut sağlığım için önemlidir.					
32-Ağız ve diş sağlığına yönelik eğitim ve seminerlere katılmada istekliyim.					
33-Dişlerimde renklenme olması beni endişelendirir.					
34-Dişlerimi güçlendiren besinleri tüketmeye dikkat ederim.					
35-Dişlerimin doğal ve estetik görünümü benim için önemlidir.					
36-Sert kabuklu yiyecekleri dişlerimle kırmaktan kaçınırım.					
37-Dişlerimi günde en az iki kez fırçalamaya dikkat ederim.					
38-Ağız ve diş sağlığımı korumak için gerekli bilgileri öğrenmede istekliyim.					
39-Ağız ve diş sağlığımı korumak için sigara içmemeye özen gösteririm.					
40-Ağız ve diş tedavim tamamlandığında mutlu hissederim.					
41-Ağız ve diş sağlığım için belirli zamanlarda ağız gargarası kullanırım.					

Duyarlılık: 1,5,9,13,15,17,21,25,29,33,37,40; ***Önem:*** 3,7,16,20,31,35; ***Zararlı unsurlardan kaçınma:*** 2,11,18,23,27,34,39; ***Ürün ve faaliyetlere eğilim:*** 6,19,24,28,32,41; ***Farkındalık:*** 4,14,22,30,36,38; ***Sosyal etki:*** 8,10,12,26

Appendix C. *Oral Health Attitude Scale (OHA-S)*

	Strongly Disagree	Disagree	Somewhat Agree	Agree	Strongly Agree
1-I want my teeth to be white.					
2-I avoid damaging foods or beverages causing tooth erosion.					
3-I care about my teeth as much as my other organs in my body.					
4-Dental floss use helps me maintain my oral and dental health.					
5-I am worried about losing even one of my teeth.					
6-New products related to oral and dental health attract my attention.					
7-Oral and dental health is important for a good smile.					
8-I care about what other people think of my teeth.					
9- When I brush my teeth, I feel relaxed.					
10-Even if it's a joke, it makes me very sad when my teeth made fun of my teeth.					
11-I avoid excessive consumption of foods or beverages that cause discoloration on my teeth.					
12-I am pleased to show my teeth to other people when I smile.					
13-I feel good when my teeth or gums are healthy.					
14-Teeth scaling is periodically necessary for gingival health.					
15-When choosing my toothbrush, I take care of several features such as hardness, softness, and shape of its bristles.					
16-I want to have straight teeth in terms of esthetic.					
17-My oral and dental health problems worry me.					
18-I avoid sugary foods due to their damage to my teeth.					
19-I am interested in the promotion or advertisement of products on oral and dental health.					
20-I pay attention to my oral and dental care in order not to experience oral and dental problems.					
21-I take care of my toothbrush and change it at least once a year.					
22-Besides brushing my teeth, I am willing to use dental floss regularly					
23-I avoid extremely hot/cold foods or beverages due to their damage for my teeth.					
24-Programs, news, and events related to oral and dental health in the media attract my attention.					
25-When others smile, I notice whether their teeth have an esthetic appearance or not.					
26-I feel jealous when someone's teeth are prettier than my teeth.					
27-I try to avoid foods or beverages that cause dental caries.					
28-I purchase my toothpaste by checking its ingredients.					
29-I take care of my oral and dental health so that there is no bad breath.					
30-I am willing to go to the dentist regularly for oral and dental health check-up.					
31-Oral and dental care is important for general body health.					
32-I am willing to participate in seminars and trainings on oral and dental health.					
33-I am worried about discoloration of my teeth.					
34- I take care to consume foods that strengthen my teeth.					
35-The natural and esthetic appearance of my teeth is important to me.					
36-I avoid breaking hard-shelled foods with my teeth.					
37-I take care to brush my teeth at least twice a day.					
38-I am willing to learn the necessary information to protect my oral and dental health.					
39-I take care not to smoke to protect my oral and dental health.					
40-When my dental and oral treatment is completed, I feel happy.					
41-I periodically use mouthwash for my oral and dental health.					

Sensitivity: 1,5,9,13,15,17,21,25,29,33,37,40; ***Importance:*** 3,7,16,20,31,35; ***Avoidance of harmful elements:*** 2,11,18,23,27,34,39; ***Tendency toward products and activities:*** 6,19,24,28,32,41; ***Awareness:*** 4,14,22,30,36,38; ***Social impact:*** 8,10,12,26

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Original research

Prevalence and predictors of molar-incisor hypomineralization among Egyptian children: a cross-sectional study

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Purpose
The primary aim of this study was twofold: first, to assess the prevalence of molar incisor hypomineralization (MIH) within a cohort of Egyptian children and, second, to investigate the potential correlation between MIH and various factors, including age, sex, birth complications, and endogamous marriage.

Materials and Methods
This cross-sectional investigation took place in Egypt's Delta region, with approximately 3000 children aged between eight and twelve years being recruited for participation. The European Academy of Pediatric Dentistry (EAPD; 2003) criteria served as the diagnostic tool for identifying MIH cases. Upon detection of clinical signs or symptoms indicative of MIH, parents were queried regarding any potential birth complications or endogamous marriages. Lesion severity levels were diagnosed using Mathu-Muju and Wright criteria.

Results
The prevalence rate for MIH was found to be 7.2%. Molars exhibited higher susceptibility rates than incisors (64.8% vs 35.2%), with approximately 37% of participants displaying severe scores, followed by mild (33.8%) and moderate (29.2%). Male subjects had significantly more occurrences than females, with positive correlations identified between MIH incidence rates alongside each gender category as well as both birth complications and endogamous marriages.

Conclusion
Children born from complicated pregnancies or whose parents are related should receive frequent check-ups from pediatric dentists during their first permanent molar eruption period. so that early detection of MIH can be facilitated allowing timely intervention.

Keywords: *Incisors, molars, hypomineralization, children, Egyptians*

Introduction

Molar incisor hypomineralization (*MIH*) is characterized as a "qualitative defect of enamel, with systemic origin, that presents as demarcated anomalies in one to four first permanent molars (*FPMs*), which are frequently accompanied by affected incisors (1). The translucency of the tissue is compromised due to hypomineralization, resulting in color changes in the enamel; areas that are white or yellowish/brownish can be observed without any alteration in thickness (2). This condition causes tooth sensitivity, posteruptive dental tissue breakdown and predisposition to dental caries. Additionally, it creates opacity on anterior teeth, leading to cosmetic and psychosocial issues. Furthermore, *MIH* affects both the quantity and quality of tooth tissues, making the selection of appropriate restorative materials and techniques more challenging (3,4).

The ideal moment to diagnose *MIH* is when it is present clinically, regardless

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of whether it affects primary or permanent teeth, 8 or 9 years of life is the best time for diagnosing this condition (2). The examination should be carried out on clean and moist teeth. The clinical signs of *MIH* vary depending on its severity and can manifest as white-creamy opacities, yellow–brown opacities, posteruptive enamel breakdown, or atypical caries located on at least one *FPM* with or without incisor involvement. To qualify as *MIH*, the lesions must measure more than 1 mm in size (5,6). For diagnosing *MIH*, the widely used criteria are those established by the European Academy of Pediatric Dentistry (7).

The frequency of *MIH* varies widely, ranging from 2.8% to 44%, depending on the population and country under investigation. Nonetheless, recent meta-analyses suggest that approximately 13%-14% of children globally are affected by *MIH* (8,9). In Egypt, multiple research studies have been conducted to determine the prevalence of *MIH* among children aged between eight and twelve years old using different measurement criteria across various regions. The results generated by these studies were inconsistent, as Saber *et al*. (10) demonstrated a prevalence rate of 2.3% at Cairo and Future Universities; similarly, Abd El Ghaffar *et al*. (11) exhibited 2.7% at Cairo University, whereas Senosy *et al*. (12) showed an occurrence rate of 4.9% at Fayoum Governorate. However, Osman *et al*. (13) determined a significantly higher percentage of approximately 14.2% in Giza Governorate alone.

The etiology of *MIH* remains obscure, with two theories postulated: environmental insults during the prenatal, perinatal, and postnatal periods or a genetic origin (14,15). The genetic basis for *MIH* was highlighted by Vieira and Kup, who believed that variations in genes involved in enamel formation can be confirmed, thus necessitating consideration of genetic etiology (15). Perinatal complications such as labor difficulties, cesarean section delivery, premature birth and low birth weight have also been associated with *MIH* (1). Although some studies suggest correlations between several potential factors and *MIH*, most provide insufficient evidence to identify causal factors (14).

Managing *MIH* can be a complex process for both dental professionals and patients alike. Children suffering from this condition often require more extensive dental treatments, with repeated visits being necessary due to the high incidence of failed restorations because of weakened tooth structure (16). Furthermore, children with severe *MIH* defects tend to experience a lower quality of life regarding oral health compared to their unaffected peers - an issue that may be compounded by the presence of caries (17,18). As such, it is crucial that we gain an understanding of the prevalence and underlying causes of *MIH* to prevent its negative impact on affected individuals. Despite this pressing need, no research has been conducted on the prevalence of *MIH* in Egypt's Delta region. Therefore, our study aims not only to determine how widespread this condition is in this area but also to explore any potential associations between birth complications or endogamy and *MIH* development.

Materials and Methods

Study design and settings

This cross-sectional investigation was carried out at the Pediatric Dentistry Outpatient Dental Clinics situated in two prestigious university hospitals, Mansoura, and Delta Universities, during the period between September 2022 and October 2023. These two clinics offer dental services to a diverse range of patients hailing from several governorates in the Delta Region, including Dakahlia, Damietta, and Kafr El-Sheikh. A convenient sample size of three thousand children was selected from these outpatient clinics for our study.

Subjects and ethical considerations

The ethical committee at Mansoura and Delta University's dental colleges approved the protocol of this study (#FODM-RC-2023-00100/12 February,2022). Prior to the commencement of the study, the purpose and methodology were discussed and clarified with the parents of the children, and their consent was obtained. It was assured that their participation would not affect the provision of their recommended services, and they had the option to decline participation in the study at any time. Furthermore, they were informed that their data would be kept confidential.

Inclusion criteria

This study recruited physically fit and socially adept children of both sexes, aged 8 to 12 years, who possessed at least one fully or partially erupted first permanent molar and/or incisor.

Exclusion criteria

The study did not encompass children exhibiting generalized developmental defects such as amelogenesis and dentinogenesis imperfecta, dental erosion, fluorosis, hypoplasia, diffuse opacities, tetracycline stains, white spot lesions or Turner's hypoplasia. Additionally, children who wore fixed orthodontic appliances were also excluded from the study.

Data collection

Investigators' training and calibration

The examination was conducted by a pediatric dentist and a dental public health demonstrator. The two examiners underwent theoretical training, which involved identifying 25 photographs of patients with *MIH* and 40 photographs depicting other enamel defects. They were then calibrated through the identification of 30 photographs each of *MIH* and other enamel defects. Intraexaminer and interexaminer reliability were assessed using Cohen's kappa coefficient, which yielded values of 0.95 and 0.90, respectively, for *MIH* examination.

Clinical examination

The participating children underwent a clinical examination using artificial light, and infection control guidelines were strictly adhered to. To obtain an accurate diagnosis, the teeth were meticulously cleaned utilizing gauze pieces and explorer tools. *MIH* was determined if the child displayed a clinical picture of at least one first permanent molar (*FPM*) with the involvement of one or more permanent incisors

(5). Lesions that exceeded 1 mm in size were classified as *MIH* (6). The *EAPD* (2003) criteria (19) were employed for scoring *MIH*, which included demarcated opacity (scored 1), posteruptive enamel breakdown (scored 2), atypical restorations (scored 3), extracted molar due to *MIH* (scored 4), and unerupted molar due to *MIH* (scored 5). Whenever there were observable clinical signs present, parents were asked about any birth complications and endogamous marriages. Finally, lesion severity levels ranging from mild to severe cases were diagnosed based on the Mathu-Muju and Wright criteria (20). Mild MIH manifests as demarcated opacities situated in nonstress bearing regions, unaccompanied by caries in the affected enamel and devoid of hypersensitivity. In cases where incisor involvement is present, it typically presents as a mild form. Moderate MIH is characterized by delineated opacities on molars and incisors, posteruptive enamel breakdown that affects only one or two surfaces without involving the cusps, and a need for atypical restorations. Additionally, patients with this condition may experience normal dental sensitivity. Severe MIH is characterized by posteruptive enamel breakdown, crown destruction, caries associated with affected enamel, and a history of dental sensitivity and aesthetic concerns.

Statistical analysis

The data were gathered, structured, and scrutinized by means of *SPSS* version 20.0 (*IBM* Corp, Armonk, NY, USA). Standard descriptive statistics such as frequencies were computed to ascertain the features of the sample. The chi-square test was employed to compare two or more frequencies. Linear regression analysis was performed to identify the influence of significant predictors on dependent variables. The confidence interval was established at 95%, and a p value less than 0.05 was deemed statistically significant.

Results

The average age of the study participants was 9.55±1.31 years, with males comprising 48% and females comprising 52%. The prevalence of *MIH* in the studied population was found to be 7.2%, equivalent to a total of 216 cases out of a sample size of 3000 children (Figure 1). Among these affected children, severe *MIH* occurred in approximately in 37% (80) of cases, while mild and moderate scores were reported in approximately 33.8% (73) and 29.2% (63), respectively (Figure 2). About the *EAPD* score distribution, Figure (3) indicates that teeth with demarcated opacity accounted for 53.9% (297), whereas those with enamel breakdown or atypical restorations made up 38.3% (211) and 7.8% (43), respectively. There were no extracted or unrecorded molars due to *MIH*. Of the 216 affected children, a total of 30.1% (65) had experienced birth complications. Moreover, endogamous parents represented 26.4% (57) of the affected children (Figure 4).

Most of the impacted teeth were molars, accounting for 64.8% of cases. In terms of incisors, a moderate rating on the *MIH* scale was predominant at 12.3%, with severe and mild scores following at 9.4% and 7.3%, respectively. Interestingly, among affected molars, severe *MIH* had the highest prevalence rate at 29.4%, while mild and moderate scores accounted for 28.9% and 15.8%, respectively (as per Table 1).

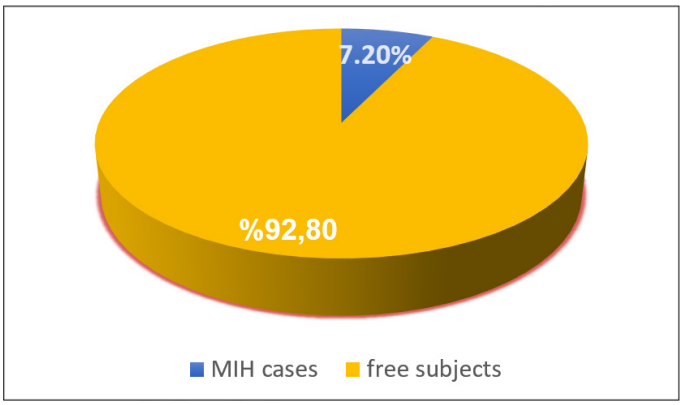

Figure 1. *Distribution of MIH among studied children.*

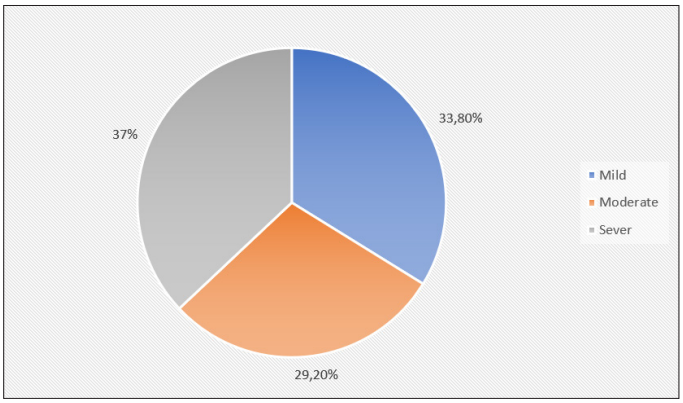

Figure 2. *Mild, moderate, and sever MIH scores among studied children.*

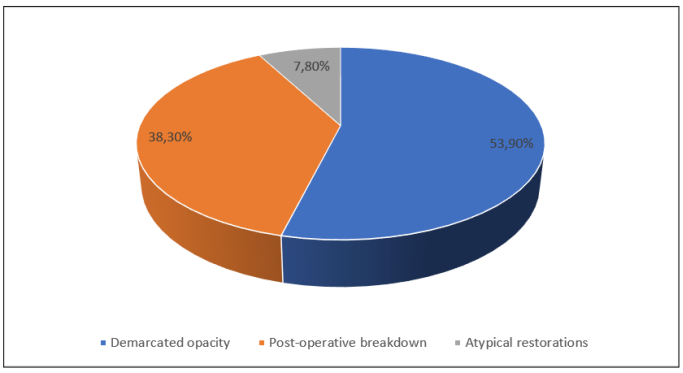

Figure 3. *Distribution of EAPD scores among affected teeth.*

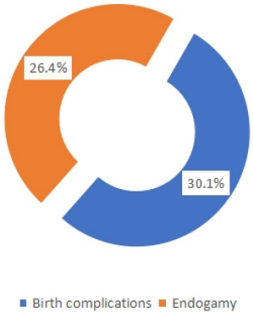

Figure 4. *Distribution of birth complications and endogamy among affected children.*

As evidenced in Table 2, the male cohort exhibited a higher *MIH* score than their female counterparts (145 vs. 71). Notably, females with mild *MIH* scores exceeded those with severe scores (34 vs. 17), while for males, the opposite held true: children with severe *MIH* scores were more prevalent than those with mild ones (63 vs. 39). A statistically significant difference was detected between genders (p<0.0001). Table 3 illustrates the correlation between *MIH* scores and age. Among the children examined, 122 cases were identified in those under ten years of age, whereas 94 cases were found in those over the age of ten. In younger children, mild *MIH* was predominant, with a total of 45 cases compared to severe *MIH*, which had only 36 cases reported. Conversely, for older children, there was an increased incidence of severe MIH, with a total of 44 reports as opposed to mild *MIH*, which had only been reported in 28 instances. The linear regression model incorporating all four predictors yielded an adjusted R^2 of 0.319, with F (352.76) = 64.194 and P <0.0001. The results indicate a significant positive correlation between *MIH* and each gender, birth complications, and endogamous marriage, with the most substantial impact observed for birth complications (Beta=0.366, P <0.001), followed by endogamous marriage (Beta =0.231, P=0.001), and gender (Beta=0.084, P <0.001). Please refer to Table 4 for further details of these findings.

Table 1. *Distribution of MIH score across affected molars and incisors (N=551)*

Affected teeth	Mild	Moderate	Severe	Total
	N (%)	N (%)	N (%)	N (%)
Incisors	40 (7.3)	68 (12.3)	52 (9.4)	194 (35.2)
Molars	159 (28.9)	87 (15.8)	162 (29.4)	357 (64.8)
Total	199 (36.1)	155 (28.1)	214 (38.8)	551 (100)
Chi-square	12.6	0.333	10.53	8.49
P value	0.0004*	0.564	0.001*	0.004*

**: Statistically significant difference by Chi-square test at p<0.05.*

Table 2. *Association between gender and MIH scores.*

Gender	MIH status	Mild	Moderate	Sever	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
Females	Present	34 (2.2)	20 (1.3)	17 (1.1)	71 (4.6)
1559	Absent	1525 (97.8)	1539 (98.7)	1542 (98.9)	1488 (95.4)
Males	Present	39 (2.7)	43 (3)	63 (4.4)	145 (10.1)
1441	Absent	1402 (97.3)	1398 (97)	1378 (95.6)	1296 (89.9)
Test of significance	Chi-square	0.871	10.541	31.068	34.005
	P value	0.351	0.001*	0.0001*	0.0001*

**: Statistically significant difference by Chi-square test at p<0.05.*

Discussion

Enamel hypomineralization arises from an imbalance in the activity of ameloblasts responsible for forming enamel during its maturation phase (21). It affects one or more permanent molars and may include incisors (22). Given that a lack of knowledge regarding prevalence rates and risk predictors may lead to rapid caries progression, dental tissue loss, and sensitivity issues associated with MIH, we conducted a study exploring these factors among Egyptian children.

The present study's findings unveiled a *MIH* prevalence of 7.2% among participants. In Egypt, Saber *et al*. (10) and Abd El Ghafar *et al*. (11) reported a lower prevalence than our study at 2.3% and 2.7%, respectively. Conversely, Osman *et al*. (13) demonstrated a higher MIH prevalence of 14.2%. Moreover, in certain Arabic countries such as Saudi Arabia, the *MIH* prevalence was recorded at 15.2% in Riyadh (23)

Table 3. *Association between age and different MIH scores.*

Age	MIH status	Mild	Moderate	Sever	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
8 to less than 10 (n=1799)	Present	45 (2.5)	41 (2.3)	36 (2)	122 (6.8)
	Absent	1754 (97.5)	1758 (97.7)	1763 (98)	1677 (93.2)
10 to 12 (n=1201)	Present	28 (2.3)	22 (1.8)	44 (3.7)	94 (7.8)
	Absent	1173 (97.7)	1179 (98.2)	1157 (96.3)	1107 (92.2)
Test of significance	Chi-square	0.088	0.701	7.669	1.178
	P value	0.77	0.40	0.006*	0.278

**: Statistically significant difference by Chi-square test at p<0.05.*

Table 4. *Association between age, gender, birth complication, endogamy, and MIH.*

Predictors	Unstandardized Coefficients	Standardized Coefficients	T	P value	95.0% Confidence Interval for B	
	B	Beta			Lower Bound	Upper Bound
Age	0.002	0.004	0.283	0.777	-0.013	0.018
Gender	0.043	0.084	5.493	0.0001*	0.028	0.059
Birth complications	0.650	0.366	16.868	0.0001*	0.575	0.726
Endogamy	0.438	0.231	10.664	0.0001*	0.358	0.519

**: Statistically significant difference at p<0.05. Dependent variable: Total MIH*

and 8.6% in Jeddah (24). In Syria (25), the prevalence was noted to be as high as 39.9%, while Jordan recorded rates of 13.17% (26) and 17.6% (27). At Khartoum State in Sudan (28), it was found to be 20.1%. Globally, a Meta-analysis conducted 2021 showed global *MIH* prevalence of 13.5% (29). Moreover, the systematic review conducted at the year-end of 2022 revealed that the rate of *MIH* prevalence stood at 15.05% (30), whereas at Chennai, it was 12.9% in 2018 (19). At Rome in Italy the prevalence was 18.2% in 2022 (31). Israel marked 10.3% (32) during their research conducted in 2023. The variation observed across these studies could be attributed to differences with respect to sample size, whereby some used larger samples compared to others. Additionally, sampling techniques varied, with some being randomized, while others were not. Furthermore, different diagnostic criteria were employed by different researchers for assessing *MIH*, which also played a part in the disparity observed among study outcomes.

The prevalence of severe *MIH* scores (37%) was higher among the children in our study compared to mild or moderate scores. Similarly, Lopes *et al*. (29) demonstrated that 36.3% of the *MIH* cases were moderate to severe. This differs from findings reported by Zawaideh *et al*. (27), where 44% of participants showed a mild score. Conversely, Hamdan *et al*. (26) found that most of their sample had severe scores. They attributed this outcome to the breakdown of enamel with age, as well as an inherent weakness in enamel structure, which increases susceptibility to *MIH*. Our results supported this notion, as almost half of affected children aged ten and above had severe *MIH*, while those younger than ten years old exhibited only mild symptoms.

The current investigation revealed a higher incidence of affected teeth displaying demarcated opacity compared to those exhibiting posteruptive enamel breakdown. This finding was congruent with Allazzam *et al*. (24), who demonstrated a 56.5% occurrence of demarcated opacity versus a 26.1% prevalence of postoperative breakdown. Furthermore, Saber *et al*. (10) reported that out of 148 teeth analyzed, only 11 exhibited enamel breakdowns, while the rest displayed demarcated opacity. Additionally, Almuallem *et al*. (23) found that in *FPMs* and permanent incisors, there was a respective occurrence rate of 68.6% and 96.5% for demarcated opacity cases.

With regard to the affected incisors and molars, this study indicates that a greater number of teeth were impacted in molars compared to incisors. Moderate *MIH* scores were observed in most affected incisors (12.3%), while severe scores were more widely distributed among the affected molars (29.4%). These findings differ from those reported by Abd El Ghafar *et al*. (11), who found that only 30% of the affected teeth were molars. Conversely, Padavala and Sukumaran (19) demonstrated that 13 children had affected molars versus 9 with affected incisors. Moreover, Abdalla *et al*. (28) reported that 65.8% versus 34.2% of the impacted teeth were molars and incisors, respectively. Lopes *et al*. (29) revealed that affected incisors were seen in 36.6% of the study participants. Almuallem *et al*. (23) and Al-Nerabieah *et al*. (25) supported our results by highlighting *FPMs* as being more severely impacted than permanent incisors, an observation also made by Weerheijm *et al*. (22). They noted that hypomineralization severity is generally less pronounced in impacted incisors when compared to their molar counterparts. Additionally, Nisii *et al*. (31) 71.4% of the affected teeth were molars.

In terms of gender, males exhibited a higher *MIH* score than females. However, this result was not corroborated by Saber *et al*. (10), Osman *et al*. (13), and Almuallem *et al*. (23), who found no significant differences between sexes. Furthermore, Abdalla *et al*. (28) discovered that the prevalence of *MIH* was higher among females than males at 53% versus 47%, which aligned with the findings of Allazzam *et al*. (24) and Padavala and Sukumaran's study in 2018 (19). These studies demonstrated that there was a higher incidence rate of *MIH* among males than females.

Regarding the association between gender and *MIH* prevalence, it should be noted that there is a significant relationship between them. Nevertheless, this finding did not concur with Allazzam *et al*. (24), Mishra and Pandey's research in 2016 (32), or Alhowaish *et al*'s study in 2021 (33); they failed to show any meaningful correlation between sex or age groups' prevalence rates of *MIH*. Bahrololoomi *et al*'s research conducted in 2020 (34), however, showed a substantial correlation between the extent, severity and size of lesions caused by *MIH* based on gender criteria. Moreover, Nisii et *al*'s examination performed in 2022 (31) indicated that patient gender stood out as an essential variable within the optimal model since it significantly influenced both probability levels associated with suffering from *MIH* symptoms.

Our study demonstrated a significant association between birth complications and *MIH*, which is consistent with the findings of Bukhari *et al*. (30) and Pitiphat *et al* (35). Furthermore, Juárez-López *et al*. (36) and Berenstein *et al*. (37) did not find an association between *MIH* and prematurity, cesarean birth, or other birth complications. Our findings may be attributed to the fact that complications during birth can cause suffering in the child and deficiencies in oxygenation that affect amelogenesis (24). Additionally, a meta-analysis of 45 studies indicated that perinatal factors such as cesarean section, prematurity, and birth complications can lead to hypoxia, which is a greater risk factor for *MIH* (38).

The current study observed an endogamous marriage to be correlated with *MIH*. This finding is consistent with Jeremias *et al*. (39), who reported evidence of the genetic influence on *MIH*. This result supports the multifactorial nature of *MIH* etiology, which was explained by genetic variation in the *AMELX* gene, which is associated with amelogenesis imperfecta and *MIH*. The *AMELX* gene plays a crucial role in amelogenesis, as it codes for amelogenin, the primary protein of dental enamel produced by ameloblasts during the secretion stage of amelogenesis (40).

The patient sample utilized in this study may not accurately represent the overall population, as they were primarily sourced from pediatric outpatient dental clinics. The distinction between *MIH* and other developmental defects, or early caries, can be challenging, which may result in an underestimation or overestimation of the prevalence of *MIH*.

Conclusion

Based on the findings of this study, a moderate prevalence of *MIH* (7.2%) was reported in the study population of children in comparison to other study results. Molars were found to be more commonly affected by *MIH* than incisors.

The severe score was more prevalent than other scores. Demarcated opacities were widely distributed among affected teeth. Males were found to be more affected by *MIH* than females. Significant correlations were observed between *MIH* and sex, birth complications, and endogamy. It is imperative that children who have experienced birth complications or have relatives as parents or those who are currently undergoing the process of erupting their first permanent molars receive regular monitoring by a pediatric dentist to detect any instances of molar incisor hypomineralization at the earliest possible stage.

Türkçe öz: *Mısır'lı çocuklarda büyük azı-kesici diş hipomineralizasyonunun prevalansı ve belirleyicileri: kesitsel bir çalışma Amaç: Bu çalışmanın birincil amacı iki yönlüydü: birincisi, bir grup Mısırlı çocuk arasında büyük azı-kesici diş hipomineralizasyonu (MIH) prevalansını değerlendirmek ve ikincisi, MIH ile yaş, cinsiyet, doğum komplikasyonları ve akraba evliliği gibi çeşitli faktörler arasındaki olası korelasyonu araştırmak. Hastalar ve Yöntemler: Bu kesitsel araştırma, Mısır'ın Delta bölgesinde gerçekleştirildi ve sekiz ile on iki yaşları arasında yaklaşık 3000 çocuk katılım için seçildi. MIH vakalarını belirlemek için Avrupa Pediatrik Diş Hekimliği Akademisi (EAPD; 2003) kriterleri tanı aracı olarak kullanıldı. MIH'ı gösteren klinik belirti veya semptomlar tespit edildiğinde, ebeveynlere olası doğum komplikasyonları veya akraba evlilikleri hakkında sorular soruldu. Lezyon şiddet seviyeleri Mathu-Muju ve Wright kriterlerine göre teşhis edildi. Sonuçlar: MIH prevalans oranı %7,2 olarak bulundu. Büyük azı dişlerinde kesici dişlere göre daha yüksek duyarlılık oranları görüldü (%64,8'e karşı %35,2), katılımcıların yaklaşık %37'si ciddi skorlar gösterirken, bunu hafif (%33,8) ve orta (%29,2) dereceler izledi. Erkek denekler, kadınlara göre anlamlı derecede daha fazla vaka gösterdi ve MIH insidans oranları ile her iki cinsiyet kategorisinin yanı sıra hem doğum komplikasyonları hem de akraba evlilikleri arasında pozitif korelasyonlar tespit edildi. Sonuç: Komplike gebeliklerden doğan veya ebeveynleri akraba olan çocuklar, ilk daimi büyük azı dişlerinin sürme döneminde pediatrik diş hekimleri tarafından sık sık kontrol edilmelidir. Bu şekilde MIH'ın erken tespiti sağlanarak zamanında müdahale yapılabilir. Anahtar kelimeler: kesici dişler, büyük azı dişleri, hipomineralizasyon, çocuklar, Mısırlılar*

Ethics Committee Approval: The ethical committee at Mansoura and Delta University's dental colleges approved the protocol of this study (#FODMRC-2023-00100/12 February,2022).

Informed Consent: The informed contents were provided by the parents or legal guardians of the participants.

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Author contributions: AME, RMA participated in designing the study AME, RMA participated in generating the data for the study. AME, RMA participated in gathering the data for the study. AME participated in the analysis of the data. AME wrote the majority of the original draft of the paper. AME, RMA participated in writing the paper. AME, RMA has had access to all of the raw data of the study. AME, RMA, DA has reviewed the pertinent raw data on which the results and conclusions of this study are based. AME, RMA, DA have approved the final version of this paper. AME, RMA, DA guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

The effect of ozone water disinfection on color stability of nanoparticles reinforced maxillofacial silicones

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Purpose
The aim of this study is to evaluate and compare the color stability of nanoparticles reinforced maxillofacial silicone after disinfection with neutral soap, 4% chlorhexidine, and ozone water.

Materials and Methods
According to ISO 4823, a metal die was fabricated, and 96 samples were created using Room Temperature Vulcanizing silicone (RTV), Heat Temperature Vulcanizing silicone (HTV), and 3% silicone dioxide nanoparticle-reinforced RTV and HTV silicones. The samples were disinfected using neutral soap, 4% chlorhexidine, and ozone water for 10 minutes, three times a day, for 60 days. The samples were divided into four groups: Group 1 (RTV), Group 2 (3% SiO_2 nanoparticle-reinforced RTV), Group 3 (HTV), and Group 4 (3% SiO2 nanoparticle-reinforced HTV). The color stability of the maxillofacial silicones was evaluated before and after disinfection using a UV spectrophotometer. The obtained color stability values were statistically analyzed using two-way ANOVA and Tukey's HSD test. Values were considered significant when $p<0.05$.

Results
The 3% SiO_2 nanoparticle-reinforced HTV silicone showed better color stability compared to HTV and RTV silicones, with the least difference observed in the 3% SiO_2 nanoparticle-reinforced RTV.

Conclusion
Ozone water caused the least change in the color of maxillofacial silicone compared to other disinfectant solutions.

Keywords: *Color stability, disinfection, maxillofacial silicone, nanoparticles, ozone*

Introduction

The aim of each maxillofacial prosthodontist is to restore the patient's esthetics and phonetics, which will improve their self-esteem and help them lead a near-normal life (1). The field of maxillofacial prosthetics primarily deals with the replacement of maxillofacial structures by artificial substitutes, which may be fixed or removable (2).

Color is the prime factor recognized by many patients who wear facial prostheses (3-8). The main reasons for the discoloration of maxillofacial silicone material are microbial in-growth, rupture, and aging. The prosthesis is exposed to various environmental factors such as personal hygiene products, environmental pollution, UV rays, temperature, humidity, skin secretions, and disinfection solutions (9-15). Disinfection is essential for the protection of the prosthesis as well as the surrounding tissues (16).

Hygiene is important for the health of the soft tissues underneath the prostheses. Disinfection is a process used to eliminate microorganisms from the maxillofacial silicone prosthesis without affecting its color and

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mechanical properties. The chemical solution used for disinfection should not elicit any reaction in human tissues and should not affect the properties of the silicone. The choice of disinfectant is based on antimicrobial effect, biocompatibility, and preservation of the material's properties (17). Disinfectants such as effervescent tablets, neutral soap, plant extract, sodium hypochlorite solution, and 4% chlorhexidine are available to disinfect maxillofacial silicone materials (18).

Nanomaterials play a significant role in basic scientific innovation and clinical dentistry by changing the properties of materials (19). Nanomaterials such as titanium dioxide, barium sulfate, ceramic powder makeup, and zinc oxide coloring agents have been used as reinforcement materials in maxillofacial silicones. Materials with small particle sizes have large surface areas and strong interactions between the organic polymer and inorganic nanoparticles (20, 21). Silicon dioxide (SiO_2) nanoparticles (NPs) have more biomedical applications due to their biocompatibility (22-24). SiO_2 NPs are small in size; hence they have strong interactions with the organic polymer (25). Therefore, this study was conducted to evaluate and compare the color stability of maxillofacial silicone reinforced with SiO_2 nanoparticles after disinfection with 4% chlorhexidine, neutral soap, and ozone water. A hypothesis was formulated that there would be no differences in color stability among the maxillofacial silicone materials after disinfection.

Materials and Methods

Experimental design

According to ISO-4823, a master die in the shape of a ring, measuring 3 mm in height and 30 mm in diameter, was created (26) (Figure 1). A total of 96 disc-shaped samples were fabricated using room temperature vulcanizing silicone (RTV silicone, M511, Technovent P&O International, Delhi, India) and heat temperature vulcanizing silicone (HTV silicone, Copsil T-30, TN Resin, P&O International, Delhi, India). Then, 3% SiO_2 nanoparticles (30 to 50 nm) were incorporated into both the RTV silicone and HTV silicone, resulting in 6 samples for each group. The study commenced following the approval of the institutional review board (SRMU/M&HS/SRMDC/2017/F/003).

Sample distribution

The samples made from room temperature vulcanizing (RTV) silicone comprised Group 1. Room temperature vulcanizing (RTV) silicone reinforced with 3% SiO_2 nanoparticles comprised Group 2. Heat temperature vulcanizing (HTV) silicone comprised Group 3. Heat temperature vulcanizing (HTV) silicone reinforced with 3% SiO_2 nanoparticles (NPs) comprised Group 4. Each group was further subdivided based on the disinfection treatment. Samples that were not disinfected were considered as the control (Group A). The samples subjected to disinfection with neutral soap were considered as Group B. Samples disinfected with 4% chlorhexidine were considered as Group C. Samples disinfected with ozone water were considered as Group D.

Figure 1. *The representative photograph of the master die.*

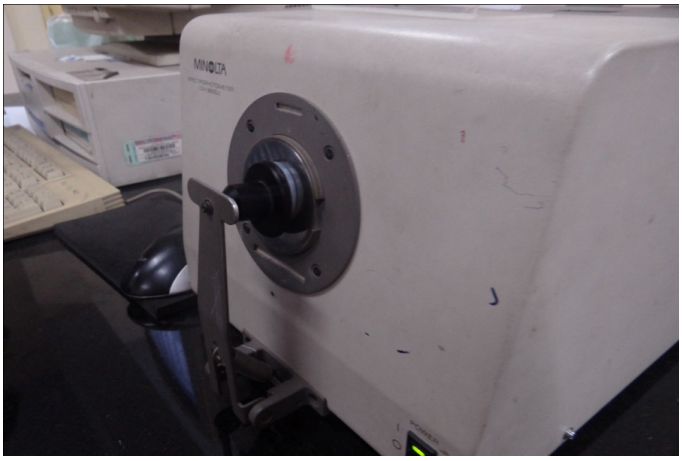

Figure 2. *The spectrophotometer device used in this study.*

Sample fabrication

Room temperature vulcanizing (RTV) maxillofacial silicone's base (10 gm) is mixed with 1 gm catalyst (10:1 ratio, totaling 11 gm) on a glass plate for 30 minutes using a stainless-steel spatula to achieve a homogeneous mixture. This mixture is then placed in a vacuum chamber for 20 minutes to remove air bubbles. The mixture is poured into a stainless-steel split mold, which is coated with a special separating medium and allowed to dry for 30 minutes. The samples are left to cure at room temperature (23 ± 2°C) for 24 hours. After curing, the samples are retrieved, and any excess material (flash) is trimmed off with a sharp scalpel. Similarly, heat temperature vulcanizing (HTV) silicone is mixed in a 1:1 ratio and poured into the stainless-steel split mold. It is then allowed to polymerize at 90°C for 1 hour in a hot air oven (Servo Enterprises, Chennai, India).

Experimental sample fabrication

RTV maxillofacial silicone (Technovent M511 RTV silicone, PO Internationals, Haryana, India) base (10 gm) and catalyst (1 gm) were weighed using a digital analytical balance. Initially, SiO_2 nanoparticles (30 to 50 nm) (Aerosil, Pharm, Mumbai, India) were added to the pre-weighed catalyst of the maxillofacial silicone and mixed for 10 minutes. Then,

the pre-weighed base was added and mixed for 30 minutes on a clean glass plate using a stainless-steel spatula to achieve a homogeneous mix. This mixture was placed in a vacuum chamber for 20 minutes to obtain an air bubble-free sample. Next, the mixture was poured into a stainless-steel split mold coated with a separating medium and allowed to dry for 30 minutes, followed by polymerization at room temperature (23 ± 2°C) for 24 hours. After curing, the excess material was trimmed off with a scalpel.

The HTV silicone (Copsil T-30 TN resin, PO Internationals, Haryana, India) catalyst and base were taken in a 1:1 ratio. Initially, the SiO_2 nanoparticles were added to the pre-weighed HTV silicone elastomer base and mixed for 30 minutes. Then, the catalyst was added and mixed for 20 minutes on a clean glass plate using a stainless-steel spatula to obtain a homogeneous mixture. This mixture was placed in a vacuum chamber for 30 minutes to acquire an air bubble-free sample. The mixture was then poured into a stainless-steel split mold and allowed to polymerize at 90°C for 1 hour in a hot air oven. Finally, the excess material was trimmed off with a scalpel.

Disinfection procedure

The samples were disinfected using neutral soap (Johnson, Chennai, India), 4% chlorhexidine (Microshield, Chennai, India), and ozone water for 10 minutes. After disinfection, the samples were rinsed with water for 10 seconds. Ozone water was produced by passing a high-voltage electrical discharge at a constant flow rate into the apparatus and injecting it into the diffuser through the output tube at a concentration of 10 ppm. The ozone water was generated in the apparatus for 20 minutes. Ozone is an unstable compound that deteriorates very quickly, with a half-life of 40 minutes at 20°C. The disinfection procedure was repeated three times a day for 60 days. After each disinfection procedure, all samples were stored in a light-proof black box at a controlled temperature of 23 ± 2°C and a relative humidity of 50 ± 10%.

Evaluation of color stability

The color stability of the silicone was evaluated using a UV light reflection spectrophotometer (MINOLTA spectrophotometer CM-3600d, Chennai, India). The color changes were calculated using the CIE Lab* system. In this system, the "L" axis represents brightness, ranging from 0 (black) to 100 (perfect white). The coordinate "a" indicates the amount of red (positive values) and green (negative values), while the coordinate "b" represents the amount of yellow (positive values) and blue (negative values). The system calculates the value of ΔE (color change) between two readings using the following formula (25) : $\Delta E = [\,(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\,]^{1/2}$

Statistical analysis

The values were statistically analyzed using SPSS version 17 (IBM, Armonk, NY, United States). The sample size was established using power analysis at a 95% confidence interval ($\alpha = 0.05$). The results were analyzed using two-way ANOVA and Tukey's HSD post hoc test. A significance value of $p<0.05$ was considered statistically significant.

Results

The mean and standard deviation of color change in the maxillofacial silicones after disinfection are listed in Table 1. The within-group comparisons were performed using two-way ANOVA and are presented in Table 2. Multiple group comparisons among the disinfection solutions were conducted using Tukey's HSD test. A significance value of $p<0.05$ indicated significant changes in color among the disinfection solutions for all the maxillofacial silicone materials (Table 3). The samples disinfected with ozone water exhibited the least color change compared to those disinfected with neutral soap and 4% chlorhexidine. Multiple group comparisons among the silicone materials were also conducted using Tukey's HSD test (Table 4). The results showed a significant difference in color change among RTV, HTV, and 3% SiO_2-reinforced RTV and HTV silicones.

Discussion

Maxillofacial silicone is a popular material used for the fabrication of maxillofacial prostheses due to its physical and mechanical properties. Typically, silicone pigmentation is achieved by adding opacifiers and nanoparticles to the base (15, 27). These additions block ultraviolet rays, thereby reducing color instability (18, 28).

Soap consists of water, alkali, and cassia. Evidence of the use of materials like soap dates to 2800 BC. Previous studies have shown that neutral soap has antibacterial and antimicrobial effects (29). Four percent chlorhexidine has a rapid antimicrobial action, making it useful as a topical antibiotic

Table 1. *Mean and standard deviation of color stability.*

Materials	Disinfection Solutions	Mean	Std. Deviation
RTV	Neutral soap	6.6533	1.19709
	4%chlrohexidine	7.9900	1.05451
	Ozone water	4.4000	1.41944
	Total	6.3478	1.91440
3%RTV	Neutral soap	4.0833	.58674
	4%chlrohexidine	6.5700	1.45737
	Ozone water	2.9900	.53826
	Total	4.5478	1.78524
HTV	Neutral soap	7.3533	.97885
	4%chlrohexidine	8.1033	1.04086
	Ozone water	4.1067	.69044
	Total	6.5211	1.98145
3%HTV	Neutral soap	4.2400	.94986
	4%chlrohexidine	5.8417	1.28672
	Ozone water	2.8617	.81969
	Total	4.3144	1.58745
Total	Neutral soap	5.5825	1.72176
	4%chlrohexidine	7.1263	1.50126
	Ozone water	3.5896	1.10624
	Total	5.4328	2.05237

***Table 2**. Two-way ANOVA results for color stability (R Squared = .782, Adjusted R Squared = .742).*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	233.740a	11	21.249	19.516	.000
Intercept	2125.085	1	2125.085	1951.743	.000
Materials	73.001	3	24.334	22.349	.000
Disinfection solutions	150.903	2	75.452	69.297	.000
Materials * Disinfection solutions	9.836	6	1.639	1.506	.192
Error	65.329	60	1.089		
Total	2424.154	72			
Corrected Total	299.069	71			

***Table 3.** Tukey HSD test for pairwise group comparisons for disinfection solution.*

(I) Disinfection Solutions	(J) Disinfection Solutions	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Neutral soap	4%chlrohexidine	-1.5437*	.30122	.000	-2.2677	-.8198
	Ozone water	1.9929*	.30122	.000	1.2690	2.7168
4%chlrohexidine	Neutral soap	1.5437*	.30122	.000	.8198	2.2677
	Ozone water	3.5367*	.30122	.000	2.8128	4.2606
Ozone water	Neutral soap	-1.9929*	.30122	.000	-2.7168	-1.2690
	4%chlrohexidine	-3.5367*	.30122	.000	-4.2606	-2.8128

***Table 4.** Tukey HSD test for multiple group comparison for maxillofacial silicone The error term is Mean Square(Error) = 1.089.*

(I) Materials	(J) Materials	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RTV	3%RTV	1.8000*	.34782	.000	.8809	2.7191
	HTV	-.1733	.34782	.959	-1.0925	.7458
	3%HTV	2.0333*	.34782	.000	1.1142	2.9525
3%RTV	RTV	-1.8000*	.34782	.000	-2.7191	-.8809
	HTV	-1.9733*	.34782	.000	-2.8925	-1.0542
	3%HTV	.2333	.34782	.908	-.6858	1.1525
HTV	RTV	.1733	.34782	.959	-.7458	1.0925
	3%RTV	1.9733*	.34782	.000	1.0542	2.8925
	3%HTV	2.2067*	.34782	.000	1.2875	3.1258
3%HTV	RTV	-2.0333*	.34782	.000	-2.9525	-1.1142
	3%RTV	-.2333	.34782	.908	-1.1525	.6858
	HTV	-2.2067*	.34782	.000	-3.1258	-1.2875

solution for the skin and as a wound cleanser. It is effective against all kinds of microbial agents and yeast (30, 31). Ozone water has antimicrobial, disinfectant, biocompatibility, and healing properties, which is why it has been proposed for various treatments in dentistry. Thus, neutral soap, 4% chlorhexidine, and ozone water were selected for the disinfection of maxillofacial silicone materials (32).

Polyzois et al. (15) mentioned in their study that a Delta E value greater than 2 units indicates a perceptible color change. All the ΔE values obtained in this research were higher than the threshold value for human eye perception. Therefore, the results indicate that significant color change occurred during the polymerization of the silicone after disinfection with neutral soap, 4% chlorhexidine, and ozone water (33). Nanoparticles act as UV shields; their electrons vibrate when exposed to UV radiation, scattering light among themselves. Thus, the smaller the nanoparticles, the better the protection against solar radiation (34). Nano-sized SiO_2, TiO_2, and ZnO have strong interfacial reactions with the organic polymer. This improves the physical and optical properties of the organic polymer and increases its resistance to environmental aging. Additionally, nanoparticles block UV rays, which enhances the color stability of silicone elastomer (35).

In this research, the samples were disinfected with neutral soap, 4% chlorhexidine, and ozone water for 10 minutes, three times a day, for 60 days, and significant differences in the color of RTV and HTV with 3% SiO_2 NP reinforced RTV and HTV silicones were observed. Group 4, containing 3% SiO_2 NPs reinforced HTV silicone, showed the least change in color after disinfection compared to Groups 1, 2, and 3 maxillofacial silicone materials. Ozone water also had the least effect on color compared to neutral soap and 4% chlorhexidine, thereby rejecting the null hypothesis.

Previous study results showed that titanium dioxide, barium sulfate, ceramic powder makeup, and zinc oxide nanoparticles reinforced maxillofacial silicone exhibited greater color stability (36). The results of the present study confirmed these findings, as SiO_2 NPs have strong interfacial reactions with the organic polymer. Aimee Maria Guiotti et al. (37) found the mean color stability of maxillofacial silicone for neutral soap to be 6.92 (1.56) and for 4% chlorhexidine to be 7.65 (1.35). In the present study, the color stability obtained for room temperature vulcanizing (RTV) silicone was 6.65 (1.19) for neutral soap, 7.99 (1.05) for 4% chlorhexidine, and 4.40 (1.42) for ozone water, which validated the results of the previous study. The results showed statistically significant changes in the color stability of maxillofacial silicone disinfected with neutral soap, 4% chlorhexidine, and ozone water ($P<0.05$). Ozone water showed the least significant difference in color stability compared to neutral soap and chlorhexidine disinfection because ozone water cannot penetrate as deeply as other disinfection solutions (38).

The ΔE value of room temperature vulcanizing (RTV) silicone, with 3% SiO_2 NPs in RTV, exhibited a lower ΔE value than heat temperature vulcanizing (HTV) silicone and 3% SiO_2 NPs in HTV silicone. Therefore, HTV silicone and 3% SiO_2 NP-reinforced HTV silicones can be recommended for the fabrication of maxillofacial prostheses due to their better color stability. A limitation of the study is that the manipulation of maxillofacial silicone material with NPs was done manually, and uniform mixing cannot be ensured. Ozone water caused the least change, but it might still be unacceptable regarding color change. Therefore, clinical correlation of the parameters is required to validate the study's results. Ozone water showed the least change in the color stability of maxillofacial silicone, so it can be used as a disinfection solution for maxillofacial silicones.

Conclusion

The 3% SiO_2 NPs-reinforced HTV silicone showed better color stability compared to HTV and RTV silicones, but the least difference was observed with 3% SiO_2 NP-reinforced RTV. Neutral soap, 4% chlorhexidine, and ozone water showed statistically significant changes in the color stability of maxillofacial silicone. Ozone water caused the least change in the color of maxillofacial silicone compared to other disinfectant solutions; hence, it can be recommended for the disinfection of maxillofacial silicones.

Türkçe öz: *Ozon suyu ile dezenfeksiyonun nanopartiküllerle güçlendirilmiş maksillofasiyal silikonların renk stabilitesi üzerindeki etkisi. Amaç: Bu çalışmanın amacı, nötr sabun, %4 klorheksidin ve ozon suyu ile dezenfeksiyon sonrasında nanopartiküllerle güçlendirilmiş maksillofasiyal silikonun renk stabilitesini değerlendirmek ve karşılaştırmaktır. Gereç ve Yöntem: ISO 4823'e göre bir metal kalıp üretildi ve oda sıcaklığında vulkanize edilen silikon (RTV), yüksek sıcaklıkta vulkanize edilen silikon (HTV) ve %3 silikon dioksit nanopartikül ile güçlendirilmiş RTV ve HTV silikonları kullanılarak 96 numune oluşturuldu. Numuneler, 60 gün boyunca günde üç kez, 10 dakika süreyle nötr sabun, %4 klorheksidin ve ozon suyu kullanılarak dezenfekte edildi. Numuneler şu dört gruba ayrıldı: Grup 1 (RTV), Grup 2 (%3 SiO_2 nanopartikül ile güçlendirilmiş RTV), Grup 3 (HTV) ve Grup 4 (%3 SiO_2 nanopartikül ile güçlendirilmiş HTV). Maksillofasiyal silikonların renk stabilitesi, dezenfeksiyon öncesi ve sonrası UV spektrofotometre kullanılarak değerlendirildi. Elde edilen renk stabilitesi değerleri, iki yönlü ANOVA ve Tukey'nin HSD testi kullanılarak istatistiksel olarak analiz edildi. Değerler, $p < 0.05$ olduğunda anlamlı kabul edildi. Bulgular: %3 SiO_2 nanopartikül ile güçlendirilmiş HTV silikon, HTV ve RTV silikonlarına kıyasla daha iyi renk stabilitesi gösterdi. En az fark, %3 SiO_2 nanopartikül ile güçlendirilmiş RTV'de gözlemlendi. Sonuç: Maksillofasiyal silikonun renginde en az değişikliğe neden olan dezenfektan çözeltisi ozon suyu oldu. Anahtar Kelimeler: renk stabilitesi; dezenfeksiyon; maksillofasiyal silikon; nanopartiküller; ozon*

Ethics Committee Approval: This study has been reviewed and approved by the local ethics committee (SRMU/M&HS/SRMD-C/2017/F/003).

Informed Consent: Not required.

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Author contributions: ASC, MB participated in designing the study. SD, ASC, MB participated in generating the data for the study. SD, ASC, MB participated in gathering the data for the study. SD, ASC participated in the analysis of the data. SD, ASC participated in writing the paper. SD has had access to all raw data of the study. ASC has reviewed the pertinent raw data on which the results and conclusions of this study are based. SD, ASC, MB have approved the final version of this paper. ASC guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

The effect of preheating on microhardness and flexural strength of bulk-fill resin composites: an *in-vitro* study

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Purpose
The objective of this study was to assess the impact of preheating on the microhardness and flexural strength of bulk-fill resin composites.

Materials and Methods
In this in vitro study, forty-two specimens were prepared of each composite, X-tra fil and Opus Bulk Fill, resulting in 84 disk-shaped specimens for microhardness testing and 84 bar-shaped specimens for flexural strength analysis. The specimens were divided into four groups as follows: Group 1: X-tra fil composite with preheating (at 68°C for 15 minutes), group 2: X-tra fil composite without preheating (at room temperature), group 3: Opus Bulk Fill composite with the same preheating method, group 4: Opus Bulk Fill composite without preheating. Microhardness was assessed using the Vickers test with a diamond indenter, and flexural strength was measured using a 3-point flexural test. Statistical comparisons were performed on the calculated results.

Results
In the preheated groups, both X-tra fil and Opus Bulk Fill composites exhibited significantly higher mean flexural strength compared to the non-preheated groups ($p<0.001$). However, there was no significant difference in the mean microhardness between the two groups for either type of composite ($p=0.719$). Additionally, the mean flexural strength and microhardness of X-tra fil composite, in both preheated and non-preheated conditions, were higher than those of the Opus Bulk Fill composite ($p<0.001$).

Conclusion
Preheating bulk-fill composites to 68°C has no detrimental effect on their microhardness and increases the flexural strength of these materials. Furthermore, the degree of microhardness and flexural strength in bulk-fill composites varies between brands and is influenced by their chemical compositions.

Keywords: *Composite resins, heating, x-tra fil composite resin, vickers test, microhardness*

Introduction

One of the recently introduced restorative materials is bulk-fill composite resins, in which the rate of polymerization shrinkage is reduced, and the depth of cure is increased up to 4 mm (1,2). Reduction in restoration time and an increase in the depth of cure have led to the widespread use of bulk-fill composites (3). Also, by increasing the bonding ability of bulk-fill composites to dentin, the marginal compatibility of the restoration has also improved (4). On the other hand, placing a block of these materials prevents voids formation and results in a dense restoration (5).

The high viscosity and stickiness of composite systems can cause problems during placement and adaptation (6). One of the proposed methods to solve this problem is preheating the composite, which leads

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to a reduction in viscosity, improved adaptation of the composite, reduced film thickness, and better handling (7,8). Composite preheating is recommended for all types of composite restorations, especially in deep cavities of posterior teeth where polymerization and adaptation in the deep layers of the material are of concern (9). Temperature has a significant effect on polymerization efficiency. Higher mobility of monomers due to increased temperature facilitates the connection between polymer chains and leads to improvement of mechanical and physical properties of composites, such as increased flexural strength and surface hardness (10).

Preheating of composites may be done by placing compoules or syringes of composite resin material in a composite heater or a water bath (11). Several studies have shown that preheating has no negative effect on the mechanical properties of nanohybrid and microhybrid resin composites (12,13). Also, preheating resin composites may increase polymerization, decrease shrinkage forces, and improve surface microhardness (14). Mechanical properties of restorative materials are of paramount importance as they directly impact their durability. Surface hardness, one of the key characteristics, is positively correlated with compressive strength, resistance to intraoral stresses, and the degree of conversion. When a material has low surface hardness, it is more susceptible to wear, which can result in restoration failure (15). Previous studies have demonstrated that preheating does not affect the flexural strength of bulk-fill and conventional composites. Additionally, the microhardness of bulk-fill composites remains unaffected by preheating. However, one study indicated that preheating does not impact the polymerization of bulk-fill composite resins, but it does enhance the microhardness of these composites (10,16,17).

Given the limited information available on bulk-fill composites and the influence of preheating on their properties, this in vitro study aims to investigate the effect of preheating on the microhardness and flexural strength of X-tra fil and Opus Bulk Fill composites. The null hypotheses tested in this study were that there are no differences in the microhardness and flexural strengths of the pre-heated and untreated composite samples.

Materials and Methods

Ethical approval

This project received Ethical approval with code (IR.TBZMED.VCR.REC.1400.316).

Sample size determination

To determine the sample size, the microhardness values were obtained from the study of Lucey *et al.* (18) and the flexural strength values were obtained from the study of Abdulmajeed *et al.* (16) Considering 95% confidence, 80% test power, two-tailed test, using G-Power version 3.1.9.6, the minimum sample size in each group was calculated as seventeen. To increase the study power, the sample size increased to 21 samples in each group (20% increase). And a total of 168 samples were used in this study.

Composite materials ans study groups

Two types of bulk fill composites, X-tra Fil (VOCO, Cuxhaven, Germany) Universal color and Opus Bulk Fill APS (FGM, Joinville-SC, Brazil) A1 color were selected for microhardness study. The characteristics of the composites are presented in Table 1. 84 microhardness disk-shaped samples were prepared based on the type of composite (X-tra fil or Opus Bulk Fill) and the preparation temperature (24°C or 68°C) in 4 groups (Figure 1), each group containing 21 samples, with a diameter of 4 mm and a thickness of 2 mm. Group 1: X-tra fil composite with preheating (the composites were placed for 15 minutes in a thermostatically controlled water bath set to 68 °C) Group 2: X-tra fil composite at room temperature Group 3: Opus Bulk Fill composite with preheating (similar to group 1) Group 4: Opus Bulk Fill composite without preheating (similar to group 2).

Polymerization

The composites were packed in an aluminum mold with 4 mm diameter and 2 mm thickness. A plastic strip and a glass slide were placed on them to remove the excess material. Then they were cured with a LED light cure device (Demetron A2, Kerr, Orange, CA, USA) at an intensity of 1000 mw/

Table 1: *Specifications of composite resin materials used in the study*

Material	X-tra fil	Opus Bulk Fill
Type	High viscosity bulk-fill	High viscosity bulk-fill
Color	Universal	A1
Filler size	2-3 micrometer	0.7-10 micrometer
Filler loading	86%w/70%v	76%w/58%v
Organic matrix	Bis-GMA, UDMA, TEGDMA	Urethanedimethacrylic monomers, Co- initiator, Stabilizers
Inorganic filler	*	Silanized silicon dioxide
Manufacturer	VOCO, Cuxhavan, Germany	FGM, Joinville, Brazil
Lot Number	2016174	240120

*The type of fillers has not been provided by the manufacturer.

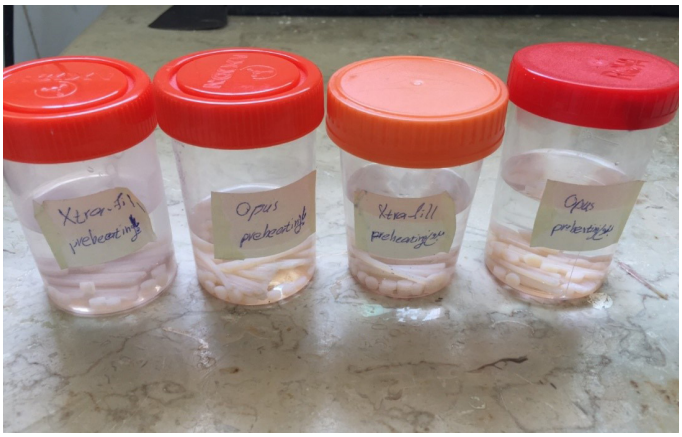

Figure 1. *Insertion The samples in 4 groups from left to right: X-tra fil composite with preheating, Opus Bulk Fill composite with preheating, X-tra fil composite without preheating, Opus Bulk Fill composite without preheating.*

cm2 for 40 seconds. After polymerization, the samples were separated from the mold and 600-grit silicon carbide abrasive was used to trim the excess material, and the dimensions of all samples were evaluated using a digital caliper (Super Caliper; Mitutoyo Corporation, Kanagawa, Japan).

Microhardness and flexural strength measurements

After fixing the samples in a holder, the surface was set perpendicular to the pyramidal intender with a square base. The surface microhardness of the samples was measured by Vickers test using a diamond intender (Innovatest, Micro Vickers tester, Micro-Met II, Buehler, IL, USA) with a load of 50 g for 15 seconds. An indented microscope with an eyepiece lens of 40 magnification was used to measure the indentations. Three depressions were created for each test sample and their mean was calculated (19).

To measure the flexural strength,84 rod-shaped samples were divided in four group based on the type of composite (X-tra fil or Opus Bulk Fill) and preparation temperature (24°C or 68°C), each group containing 21 square sectioned samples 25 mm long. They were prepared with dimensions of 2 x 2 mm. Flexural strength was determined by the ISO 4049 three-point flexural test. This test was performed using universal testing machine (H5K-S; Hounsfield Test Equipment, Redhill, UK) at a crosshead speed of 0.5 mm/min. The following formula was used to calculate the flexural strength: $\sigma = 3FL/2wt$; where F = maximum force applied; L = distance between the support beams; w = width of the specimen; and t = thickness of the specimen (16)

Statistical analysis

The results were reported by descriptive statistics mean and standard deviation. The Shapiro-Wilk test was used to test the distribution of quantitative variables. In order to compare microhardness and flexural strength between preheated and control groups, due to the normality of the dependent variable, analysis of covariance (ANCOVA) was used. A probability value of less than 0.05 was considered significant. Statistical Package for Social Sciences (SPSS) for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA) was utilized for statistical analysis.

Results

The mean flexural strength in X-tra fil composite group was higher than the mean flexural strength in Opus Bulk Fill composite in both preheated and non-preheated modes ($p<0.001$) (Table 2). Also, in the preheated group the mean flexural strength was higher than the group without preheating ($p<0.001$) in both types of X-tra fil composite and Opus Bulk Fill. The interaction effect of Composite/Preheating was not significant in the mean flexural strength ($p=0.860$). Figure 2 shows the amount of flexural strength based on the type of composite and the intervention performed. The mean microhardness in X-tra-fil composite group in both modes was higher than the mean microhardness in Opus Bulk Fill composite group ($p<0.001$) (Table 3). There wasn't any significant difference in the microhardness of two groups with and without preheating ($p=0.719$). The interaction effect of Composite/ Preheating was not significant in the mean microhardness ($p=0.532$). Figure 3 shows the microhardness based on the type of composite and the intervention performed.

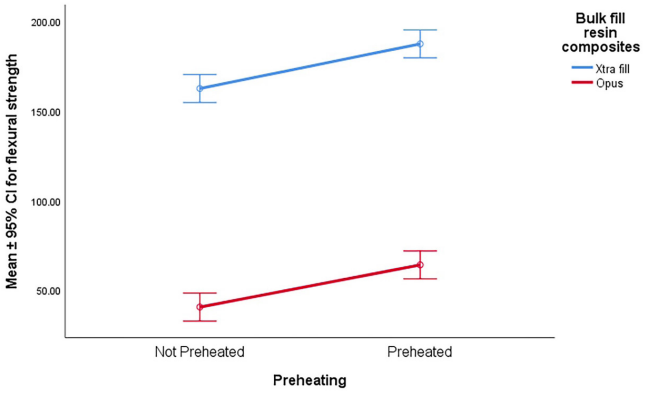

Figure 2. *Flexural strength based on the type of composite and the intervention.*

Table 2: *Flexural strength of X-tra fil and Opus Bulk Fill resin composites with and without preheating*

Composite	Preheating	Mean	SD	P-value		
				Composite	Preheating	Composite /Preheating
X-tra fil	Not Preheated	162.50	26.24	<0.001	<0.001	0.860
	Preheated	187.41	15.77			
Opus Bulk Fill	Not Preheated	40.59	11.70			
	Preheated	64.11	14.69			

SD: Standard Deviation

Table 3: *Microhardness of resin of X-tra fil and Opus Bulk Fill composites with and without preheating. SD: Standard Deviation)*

Composite	Preheating	Mean	SD	P-value		
				Composite	Preheating	Composite/ Preheating
X-tra fil	Not Preheated	73.63	6.34	<0.001	0.719	0.532
	Preheated	73.32	5.67			
Opus Bulk Fill	Not Preheated	35.08	4.17			
	Preheated	36.25	5.38			

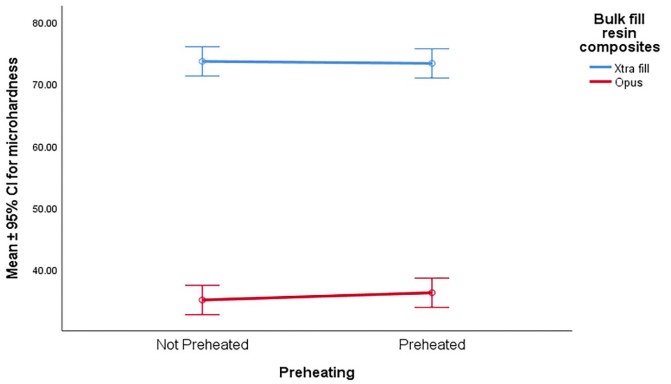

Figure 3. *Microhardness based on the type of composite and the intervention.*

Discussion

In the present study, preheating did not have any significant effect on the microhardness of X-tra fil and Opus Bulk Fill composites. In the study conducted by Degirmenci and Can (10), microhardness increased in Bulk Fill composites due to preheating, while microhardness in micro-hybrid composites decreased. Another study by Theobaldo *et al.* (17) found that preheating had no impact on the microhardness of Surefil SDR bulk composite. Interestingly, even though the conventional flow composite Filtek Z350 (used as a control group) had a higher volume percentage of filler (55%), its lower monomer-to-polymer conversion degree compared to the preheated bulk fill flow composite resulted in similar microhardness values. Therefore, it's evident that various factors influence the microhardness of composite resin materials.

In Lucey *et al.*'s study (18), preheating increased the microhardness of the hybrid (conventional) Spectrum TPH composite, which contrasts with the findings of the present study. This discrepancy can be attributed to the differences in the studied composites. Additionally, Nada and El-Mowafy (20) reported in their study that the effect of preheating on the surface microhardness of composites depends on the composite brand, its chemical composition, and particularly the type of monomer used. In the current study, preheating increased the flexural strength of both X-tra fil and Opus Bulk Fill composites. A study by Deb *et al.* (13) observed a significant increase in the flexural strength of the hybrid composite Spectrum TPH and Flow composite SDI after preheating, which was attributed to increased molecular activity in the polymer system and enhanced crosslinking in polymer chains. Kramer *et al.* (21) found that preheating increased the flexural strength of Filtek Supreme XT (conventional) nanocomposite and Tetric-Evo Cream bulk fill composite. The higher conversion degree of preheated composite resin was thought to have a positive effect on flexural strength. Alshali also reported that preheating composites before curing can increase polymerization and degree of conversion by temporarily reducing viscosity. In Abdulmajeed et al.'s study (16), preheating did not affect the flexural strength of Filtek One BulkFill composite and Filtek Supreme Ultra conventional composite, in contrast to the results of the present study. The discrepancy may be attributed to the differences in composite brands.

In the current study, both microhardness and flexural strength of X-tra fil bulk composite were higher than those of Opus Bulk Fill composite both before and after preheating. Alshali *et al.* (22) and Nag *et al.* (23) showed that microhardness values have a direct relationship with the amount of inorganic filler. They also demonstrated that the morphology and distribution of filler particles, particle shape and density, the ratio of monomer, type of monomer, crosslinking of polymers, and degree of conversion (DC) can account for variations in the microhardness of different resin composites. It was mentioned in Nag *et al.*'s study (23) that the manufacturers of X-tra fil bulk-filled composite increased the size of filler particles and filler content to enhance its microhardness. As a result, the microhardness of X-tra fil composite with 86% filler content was higher than that of Opus Bulk Fill composite with 79% filler content, both before and after preheating. Furthermore, in Degirmenci and Can's study (10), Estelite Bulk Fill Flow composite (EST), which contains BisGMA monomer, exhibited higher microhardness values than G-aenial Posterior and SDR Plus composites. BisGMA is a monomer with high molecular weight, strong hydrogen bonding capacity, and low molecular mobility. It is considered the most viscous and least flexible among dental resin monomers. The strong intermolecular bonding between hydroxyl groups in BisGMA likely contributed to the higher microhardness of EST, similar to the findings in the present study, where X-tra fil bulk composite containing BisGMA exhibited higher microhardness than Opus Bulk Fill composite.

Gomes *et al.* (24) demonstrated that the mechanical properties of bulk-filled composite resin depend on their filler content, with higher filler volume corresponding to higher flexural strength. In the current study, X-tra fil composite with 86% filler by volume had higher flexural strength than Opus Bulk Fill composite with 79% filler by volume. Additionally, the study by Deb *et al.* (13) identified an inverse relationship between flexural strength and shrinkage under preheating conditions. Therefore, composites with less shrinkage, such as X-tra fil, tend to exhibit higher flexural strength.

Microhardness in resin composites can be measured using either the Vickers or Knoop tests. Both methods involve creating an indentation with a diamond tip under a predefined force for a specified duration (25). In the present study, the Vickers test was employed for surface microhardness testing. Research has indicated a very strong correlation (r=0.991) between the Knoop and Vickers hardness tests (26). The Knoop test is based on linear measurements, while the Vickers test measures values based on an area. As a result, it is challenging to determine which test is more accurate (27), and either test can be used for material comparisons. The present study utilized X-tra fil and Opus Bulk Fill composites. These two brands were selected based on their relative acceptance and popularity among clinicians.

It is important to note that the present study examined only two brands of composite and two preheating temperatures, with samples analyzed 24 hours after curing. Future studies may explore other brands of bulk-fill composites, a wider range of preheating temperatures, and different post-curing timeframes, which could yield varying results. Additionally, the non-anatomical geometry of the samples, prepared according to ISO standards, may have influenced the results. To better simulate clinical conditions, the samples were polymerized from one side only. Moreover, this in vitro study was conducted without dental tissue, and results may differ in clinical settings. Future studies could investigate the effect of preheating on dental tissue and pulp by testing composites with dental tissue involved.

Conclusion

In light of the findings from this study, it can be stated that preheating bulk-fill composites to a temperature of 68 degrees Celsius does not compromise their microhardness. In fact, it results in an increase in their flexural strength. Additionally, substantial variations in both microhardness and flexural strength are observed among different bulk-fill composites, primarily stemming from differences in their chemical compositions.

Türkçe özet: *Ön ısıtmanın yığın dolgulu reçine kompozitlerin mikrosertliği ve eğilme mukavemeti üzerindeki etkisi: in vitro bir çalışma. Amaç: Bu çalışmanın amacı, ön ısıtmanın yığın dolgulu reçine kompozitlerin mikrosertliği ve eğilme mukavemeti üzerindeki etkisini değerlendirmektir. Gereç ve Yöntem: Bu in vitro çalışmada, X-tra fil ve Opus Bulk Fill kompozitlerinin her birinden kırk iki örnek hazırlandı; sonuçta mikro sertlik testi için 84 disk şeklinde örnek ve eğilme mukavemeti analizi için 84 çubuk şeklinde örnek elde edildi. Örnekler şu şekilde dört gruba ayrıldı: Grup 1: Ön ısıtmalı X-tra fil kompozit (68°C'de 15 dakika), grup 2: Ön ısıtmasız X-tra fil kompozit (oda sıcaklığında), grup 3: Opus Aynı ön ısıtma yöntemine sahip Bulk Fill kompoziti, grup 4: Ön ısıtmasız Opus Bulk Fill kompoziti. Mikro sertlik, elmas uçlu Vickers testi kullanılarak değerlendirildi ve bükülme mukavemeti, 3 noktalı bükülme testi kullanılarak ölçüldü. Hesaplanan sonuçlar üzerinde istatistiksel karşılaştırmalar yapıldı. Bulgular: Ön ısıtmalı gruplarda hem X-tra fil hem de Opus Bulk Fill kompozitleri, ön ısıtmasız gruplarla karşılaştırıldığında önemli ölçüde daha yüksek ortalama eğilme mukavemeti sergiledi (p<0.001). Ancak her iki kompozit türü için de iki grup arasında ortalama mikrosertlik açısından anlamlı bir fark yoktu (p=0,719). Ayrıca, X-tra fil kompozitinin ortalama eğilme mukavemeti ve mikrosertliği, hem ön ısıtmalı hem de ön ısıtmasız koşullarda, Opus Bulk Fill kompozitininkinden daha yüksekti (p<0.001). Sonuç: Bulk-fill kompozitlerin 68°C'ye kadar önceden ısıtılmasının mikrosertlikleri üzerinde zararlı bir etkisi yoktur ve bu malzemelerin eğilme mukavemetini arttırır. Ayrıca, toplu dolgulu kompozitlerdeki mikro sertlik ve bükülme mukavemetinin derecesi markalar arasında farklılık gösterir ve kimyasal bileşimlerinden etkilenir. Anahtar kelimeler: kompozit reçineler, ısıtma, x-tra fil kompozit reçine, vickers testi, mikrosertlik*

Ethics Committee Approval: This project has been reviewed and approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1400.316).

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Original research

Effect of ionizing radiation on the microstructure and physical properties of endodontic gutta-percha points

Purpose
Patients undergoing radiotherapy for head or neck cancer often require root canal treatments, which can be compromised by the effects of radiation. This investigation aimed to determine whether ionizing radiation (IR), in doses similar to those used in conventional therapy, affects the surface and physicomechanical properties of various brands of endodontic gutta-percha points (EGPs).

Materials and Methods
One hundred and twenty-three EGPs from three brands (Meta-Biomed, Dentsply, and Hygenic) were divided into groups and either exposed or not exposed to IR at a total dose of 50 Gy, divided into 25 fractions. Tensile strength and microhardness tests were performed on all EGPs. Scanning electron microscopy was utilized to identify possible microstructural surface changes due to IR exposure. The proportion of organic to inorganic components in each brand was also determined.

Results
Exposure to IR resulted in significant changes only in the EGPs from the Meta-Biomed brand, including a notable decrease in tensile strength and an increase in microhardness. Furthermore, the surface microstructure of these EGPs displayed dark lines and striations over a large area, with some lines deeply embedded in the center and cavities of variable depths and extensions observed, leading to irregular and non-smooth surfaces. This brand had the highest proportion of organic components.

Conclusion
The physicomechanical properties and surface microstructure of Meta-Biomed brand EGPs were significantly affected by IR at doses used in conventional therapy for head or neck cancer, while the other brands were less affected or unaffected.

Keywords: *Radiotherapy, endodontic gutta-percha points, microstructure, head and neck cancer, root canal therapy*

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Introduction

Head and neck cancers are serious and debilitating illnesses typically treated with a combination therapy approach, including surgery, chemotherapy, and radiotherapy using ionizing radiation (IR) (1). Radiotherapy has been proven effective in controlling and curing malignant tumors (2). However, it is well known that radiotherapy can cause oral complications as side effects on healthy soft and hard tissues, significantly affecting quality of life (3). These complications can be temporary or permanent, requiring ongoing dental care due to their long-lasting effects, sometimes persisting for months or years. The most common complications include mucositis, xerostomia, dysesthesia, bacterial and fungal infections, trismus, osteoradionecrosis, periodontitis, and radiation-induced dental caries (4–7).

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A thorough oral examination should be performed before initiating radiotherapy, and necessary treatments should be administered, with efforts made to avoid tooth extraction whenever possible to reduce the risk of osteoradionecrosis during or after IR exposure. Consequently, root canal treatment is frequently performed on many patients (1). Despite the high success rate of current root canal treatments, the prognosis may be compromised in immunosuppressed patients with xerostomia and alterations in oral microbiota; thus, these factors must be considered during treatment (8,9). Furthermore, it is crucial to consider the materials used in root canal treatments, such as those for filling root canals and for provisional or definitive tooth restoration, as these materials will also be exposed to IR. Few studies have explored the impact of IR on the adhesive properties of restorative materials (10) and different types of endodontic sealers (11,12). However, the effects of IR on endodontic gutta-percha points (EGPs), which are the core of root canal fillings and considered the "gold standard" among endodontic filling materials, have not been thoroughly investigated (13). EGPs are available in various brands, primarily composed of zinc oxide, gutta-percha polymer, waxes, resins, and barium sulfate (14), with compositions varying by brand (15). These differences in composition can lead to variations in physicomechanical properties, such as brittleness, stiffness, tensile strength, and radiopacity, largely depending on the ratios of organic (gutta-percha polymer and waxes/resins) to inorganic (zinc oxide and metal sulfates) components (16).

Given their composition, it is logical to hypothesize that IR could affect the properties of EGPs similarly to its effects o n enamel and dentin tissues (17,18). This study aims to determine whether IR, in doses akin to those used in conventional therapy for head or neck cancer, impacts the surface microstructure and physicomechanical properties of different brands of EGPs. There would be no significant difference in the surface microstructure and physicomechanical properties of different brands of EGPs after exposure to IR in doses similar to those used in conventional therapy for head or neck cancer.

Materials and methods

Gutta-percha points and study groups

One hundred and twenty-three #45, 0.2 taper EGPs from three brands, as listed in Table 1, were utilized. These were divided into four groups. Group 1 included 60 EGPs, with 20 from each brand; half of these were subjected to IR and all were tested for tensile strength. Group 2 comprised 45 EGPs, 15 from each brand, to assess surface microhardness before and after IR exposure. Group 3 consisted of 18 EGPs, six from each brand, with half receiving IR; all were examined using a scanning electron microscope (SEM). Group 4 involved using 1g of EGPs from each brand to determine the organic/inorganic content ratio. Given that the physicomechanical tests conducted on groups 1 and 2 are unconventional for these materials, and no standards exist regarding the methodology or the required sample size, the approach was first standardized through pilot tests. These preliminary tests identified optimal experimental conditions, and the sample sizes for each group were determined based on methodologies from other studies that examined similar physicomechanical properties in different dental materials.

Table 1: *Endodontic gutta-percha points selected for this study*

Brand	Manufacturer	Lot number
Meta-Biomed	Meta Biomed Co, Ltd, Chungbuk, Korea	GE19030068
Dentsply	Dentsply Maillefer, Ballaigues, Switzerland	031217
Hygienic	Hygienic, Coltene/ Whaledent, Inc., USA	K17196

A crucial step in the pretest procedure involved converting the conical shape of each EGP into a flat form in a standardized manner. This was accomplished by pressing each EGP at 400 Newtons between two metallic plates using a computer-controlled universal testing machine (UTM) (CMS Metrology, Model WDW-5Y, Querétaro, Mexico), thereby facilitating all subsequent tests.

Ionizing radiation exposure

All EGPs were wrapped in gauze and submerged in sterile distilled water. Each group was then placed inside plastic bags and categorized based on whether they were designated to receive IR exposure. The bags not intended for IR exposure were stored at room temperature, shielded from light, heat, and any potential sources of IR. Conversely, the bags designated for IR exposure were processed by the Oncologic Center of Querétaro S.A. de C.V. This was done using a medical linear accelerator machine (Trilogy Linear Accelerator; Varian Medical Systems), which delivered IR using 6 MV X-rays from a distance of 100 cm. A total dose of 50 Gy, divided into 25 fractions (2 Gy per fraction), was administered over five consecutive days per week for six weeks, mirroring the standard radiotherapy protocol for head and neck cancer.

Tensile strength test

The tensile strength of each EGP in Group 1 was measured using the same UTM previously mentioned, but this time equipped with rubber grips. Paper tape was applied to both ends of each EGP, leaving a 10 mm section exposed. The rubber grips secured the ends of the EGP during the test. A crosshead speed of 1.0 mm/min was applied until the EGP was pulled apart, and the maximum load was recorded in Newtons. The room temperature was maintained at 29 ± 1°C.

Microhardness test

Microhardness for Group 2 EGPs was assessed before and after IR exposure using a microhardness tester (CMS Metrology, Model CHV-1, Querétaro, Mexico). A force of 0.98N (0.1kgf) was applied with a diamond indenter for 10 seconds. Measurements were recorded in Vickers hardness number (VHN), calculated with the equation: VHN = 1.854 (L/d^2), where L is the applied load (kgf) and d is the mean diagonal length (mm). The final value was derived from three indentations on different areas of one side of each EGP.

Surface microstructural observations (SEM)

EGPs from Group 3 were examined for potential microstructural surface changes due to IR exposure. Both IR-exposed and non-exposed EGPs were mounted on a holder and scanned with an SEM (Hitachi TM1000, Mito City, Japan) operating at 15 kV. Images were captured from at least three different locations at various magnifications using a backscattering electron detector.

Organic-inorganic proportion

To differentiate and quantify the organic and inorganic components of each brand, a recognized method was employed (19). Briefly, 1 g of EGPs from each brand was dissolved in 20 ml of chloroform for 24 hours. The solution was then centrifuged for 15 minutes at 10,000 rpm. The inorganic components solidified and were separated from the organic supernatant; solids were collected by filtration and the mass of both phases was determined after evaporating the solvent.

Statistical analysis

The results were statistically analyzed using the Student's t-test and the paired t-test, as appropriate, and two-way ANOVA with post hoc Tukey-Kramer multiple comparison test, following the normal distribution confirmation by the Smirnov-Kolmogorov test. All analyses were conducted using GraphPad Instat, version 3.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

Results

The tensile strength analysis of the EGPs not subjected to IR revealed that the Hygienic and Dentsply brands had similar tensile strengths, yet both were significantly different ($p<0.0001$) from the Meta-Biomed brand, which exhibited the lowest tensile strength. Furthermore, after IR exposure, the Meta-Biomed brand was the only one to experience a significant decrease in tensile strength ($p<0.0001$). Conversely, the Dentsply and Hygienic brands did not show any significant change in tensile strength following IR exposure (Table 2). A similar pattern was observed in the microhardness test; the Meta-Biomed EGPs underwent a significant increase in microhardness ($p<0.0001$) after IR exposure, indicating they were the most affected among the brands tested (Table 3).

The surface microstructure of the three EGP brands, when not exposed to IR, displayed smooth, homogeneous surfaces with uniform contrast at lower magnifications (x250, x500). However, at higher magnifications (x2000, x10000), several particles with higher contrast, all smaller than one micron, were observed, immersed in a matrix of organic elements with lower contrast. After IR exposure, the Hygienic brand exhibited no noticeable changes on its surface. Meanwhile, the Dentsply EGPs displayed a few dark lines and striations across their entire surface. These features were even more abundant in the Meta-Biomed brand, covering a large surface area. Additionally, some of these lines were deep, and cavities of varying depths and extents were noted in the center of some, resulting in irregular and non-smooth surfaces (Figure 1). With respect to the organic/inorganic content in each group, very similar proportions in the Dentsply and Hygienic EGPs were observed. In contrast, a high proportion of organic components was found in Meta-Biomed EGP (Table 4).

Table 2: *Tensile strength (Newtons) of different brands of EGPs exposed or not to IR*

	Hygenic (n=20)	Dentsply (n=20)	Meta-Biomed (n=20)	P value[b]
		X ± SD (Range)		
No IR (n=10)	5.11 ± 0.57 (4.15 - 6.15)	4.97 ± 0.64 (3.85 - 5.80)	3.73 ± 0.60 (2.75 - 4.50)	≤ 0.0001
After IR (n=10)	4.67 ± 0.63 (3.80 - 6.10)	4.58 ± 0.39 (4.10 - 5.20)	1.57 ± 0.35 (1.05 - 2.40)	≤ 0.0001
P value[a]	0.0636	0.1115	≤ 0.0001	

X: Mean; SD: Standard deviation; EGPs: Endodontic gutta-percha points; IR: Ionizing radiation; [a]: Student t-test; [b]: ANOVA; Post hoc Tukey-Kramer test in the no radiated and radiated groups comparisons resulted in no statistical significance when comparing Hygenic Vs. Dentsply, while Hygenic Vs. Meta-Biomed and Dentsply Vs. Meta-Biomed resulted significative ($p<0.0001$).

Table 3. *Microhardness (HV0.1) of different brands of EGPs before and after being exposed to IR*

	Hygenic (n=15)	Dentsply (n=15)	Meta-Biomed (n=15)	P value[b]
		X ± SD (Range)		
Before IR	49.01 ± 5.00 (42.2 - 55.2)	47.83 ± 4.92 (41.1 - 58.7)	35.36 ± 3.04 (31.6 - 40.8)	≤ 0.0001
After IR	49.36 ± 4.76 (42.8 - 56.0)	49.74 ± 6.59 (41.7 - 60.5)	43.60 ± 2.66 (39.8 - 47.6)	0.0161
P value[a]	0.1222	0.1146	≤ 0.0001	

X: Mean; SD: Standard deviation; EGPs: Endodontic gutta-percha points; IR: Ionizing radiation; [a]: Paired t-test; [b]: ANOVA; Post hoc Tukey-Kramer test in pre-Ionizing Radiation comparisons resulted in no statistical significance ($p>0.05$) when comparing Hygenic Vs. Dentsply. Hygenic Vs. Meta-Biomed, and Dentsply Vs. Meta-Biomed were different ($p<0.0001$). Comparing post-Ionizing Radiation groups: Hygenic Vs. Dentsply were not statistically significant, while Hygenic Vs. Meta-Biomed and Dentsply Vs. Meta-Biomed were ($p<0.01$).

Discussion

Patients undergoing therapy for head or neck cancer typically receive a cumulative dosage ranging from 30 to 70 Gy over five to seven weeks (18), which is sufficient to cause numerous undesirable changes in oral tissues, including mucosal, muscular, vascular, osseous, and dental tissues (20). Before therapy, patients subjected to IR should be orally evaluated and receive periodontal, dental, and endodontic treatments to eliminate all oral diseases and prevent or minimize complications in the post-IR period, thus providing better oral health conditions (21). Since the risk of developing osteoradionecrosis persists throughout a patient's life,

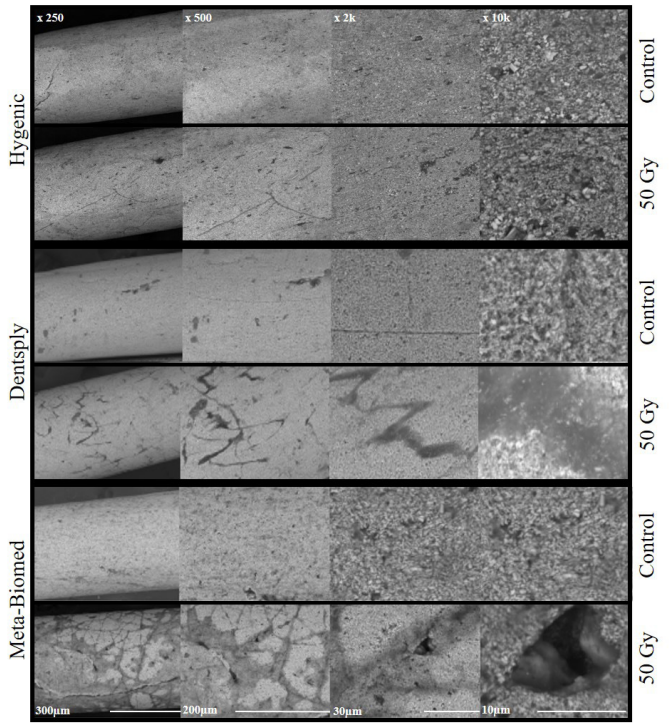

Figure 1. *Scanning electron microscope photographs at different magnifications (250, 500, 2000, 10,000 x) representatives of the surface microstructure of each EGP brand without IR and after IR exposure (50Gy). The scale bars indicated at the bottom apply to all images in the same column. Note the erosion of the Meta-Biomed brand after IR exposure.*

Table 4: *Percentage of organic and inorganic content in each EGP brand*

	Hygenic	Dentsply	Meta-Biomed
Organic	13.55 %	13.70 %	15.62 %
Inorganic	86.45 %	86.30 %	84.38 %

all efforts must be directed towards preventing extractions. Therefore, root canal treatment, both before and after IR therapy, emerges as an essential alternative for these patients (22), also offering the opportunity to rehabilitate teeth and improve the quality of life. Achieving a successful long-term root canal treatment hinges on several factors, including obtaining an hermetic seal through root canal filling—alongside cleaning and shaping the canal—as one of the key aspects to prevent bacterial passage and recontamination (23).

Despite EGPs being the primary material for root canal filling, achieving a hermetic seal is impossible without endodontic sealers, which come in various compositions (24). An ideal endodontic sealer must adhere firmly to both dentin and EGPs, among other properties. The interaction with dentin or EGPs might vary depending on their composition, leading to expected differences in adhesive properties. There is existing information on the effects of IR on different endodontic sealers and significant data on IR's impact on dentin and enamel (11, 25–27). However, the effects of IR on EGPs remain unclear, although logically, IR could affect them due to their organic and inorganic composition.

Historically, the composition of EGPs has varied over time and by manufacturer. The primary component, zinc oxide, constitutes a wide range of 36.6–75%, imparting antibacterial properties and serving as a vulcanizing agent; gutta-percha polymers account for 18–22%, and barium sulfate, added for radiopacity, ranges from 1.1–31.2% (19, 28, 29). The variance in components and their proportions directly influences the physicomechanical properties of EGPs. This study tested two such properties, providing a reference for the physical effects of IR on EGPs. Tensile strength, significantly correlated with the percentage of gutta-percha polymer (19), and the rigidity of EGPs are affected by the concentration of inorganic components and gutta-percha polymer, with small amounts of plasticizers enhancing flexibility and compactness (30, 31).

The study revealed distinct differences between Hygienic and Dentsply brands compared to Meta-Biomed EGPs, with and without IR exposure, suggesting variations in component composition and proportions. These differences are consistent with surface microstructure changes, potentially linked to their composition (19, 28). However, without detailed information on the exact formulas, establishing clear explanations remains challenging. Although this study began to quantify the organic and inorganic phases present in each brand of EGP, the lack of detailed component analysis is a significant limitation; still, it was observed that Meta-Biomed EGPs had a higher organic content compared to others (32, 33).

IR's harmful effects on organic components could explain the observed surface striations and cavities, resulting from the degradation of organic matter into harmful byproducts (33). This degradation impacts the EGP's internal integrity and, by extension, its inorganic structure, leading to physical changes potentially caused by the thermal effects of IR absorption. The post-IR effects on some EGPs complicate the prognosis for patients receiving IR therapy, as the damage to EGPs adds to that already known to affect endodontic sealers and dentin, compromising adhesion and the success of root canal treatments (34).

This study's in vitro design limits the direct applicability of its findings to clinical situations, as root canal filling materials in patients do not directly receive IR. Further research is necessary to fully understand the effects of IR on EGPs, particularly through the investigation of the exact components and their proportions in each brand, to elucidate which are more susceptible to IR damage.

Conclusion

Despite the study's limitations, it was found that the physicomechanical properties and surface microstructure of Meta-Biomed EGPs were significantly affected by IR at doses typical of conventional head or neck cancer therapy, while other brands showed no such effects.

Türkçe özet: *İyonlaştırıcı radyasyonun endodontik güta-perkaların mikro yapısı ve fiziksel özellikleri üzerine etkisi. Amaç: Baş veya boyun kanserinde radyoterapi gören hastalar genellikle radyasyon etkileri nedeniyle kök kanal tedavilerine ihtiyaç duyarlar. Geleneksel tedavi sırasında kullanılanlara benzer dozlardaki iyonlaştırıcı radyasyonun (IR), farklı markalardaki endodontik güta-perka noktalarının (EGP'ler) yüzeyini ve fizikomekanik özelliklerini etkileyip etkilemediğinin belirlenmesi son*

derece önemlidir ve bu çalışmanın amacını oluşturmaktadır. Gereç ve Yöntem: Üç markaya (Meta-Biomed, Dentsply ve Hygenic) ait 123 EGP, gruplara ayrılarak 25 fraksiyona bölünmüş toplam 50 Gy dozda IR'ye maruz bırakılıp bırakılmadı. Tüm EGP'lere çekme mukavemeti ve mikrosertlik testleri yapıldı. IR'ye maruz kalma nedeniyle olası mikroyapısal yüzey değişikliklerini tanımlamak için taramalı elektron mikroskobu gözlemleri kullanıldı. Her markanın organik-inorganik oranı belirlendi. Bulgular: IR'ye maruz kaldıktan sonra yalnızca Meta-Biomed markasının EGP'leri önemli değişiklikler yaşadı, çekme mukavemetinde önemli bir azalma ve mikro sertlik arttı. Ayrıca yüzey mikro yapısında geniş bir yüzey alanını etkileyen koyu çizgiler görülüyordu; bu çizgilerden bazıları merkezde derindi ve düzensiz ve pürüzsüz olmayan yüzeyler oluşturan, değişken derinlik ve uzantılara sahip boşluklar gözlemlendi. Organik bileşen oranı en yüksek markaydı. Sonuç: Test edilen markalardan biri olan Meta-Biomed'in fiziko-mekanik özellikleri ve yüzey mikro yapısı, konvansiyonel baş veya boyun kanseri tedavisi sırasında kullanılan dozlarda IR'den önemli ölçüde etkilenirken, diğer markalar daha az etkilendi veya hiç etkilenmedi. Anahtar Kelimeler: radyoterapi; endodontik güta-perka; mikro yapı; baş ve boyun kanseri, kanal tedavisi

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: MVG, CLC, LFEC, RADP participated in designing the study. MANR, RAPN, RADP participated in generating the data for the study. MANR, MVG participated in gathering the data for the study. MVG, CLC, LFEC, RADP participated in the analysis of the data. RADP wrote the majority of the original draft of the paper. MANR, MVG, RAPN, LFEC participated in writing the paper. MVG, RADP has had access to all of the raw data of the study. MANR, MVG, RAPN, CLC, LFEC, RADP has reviewed the pertinent raw data on which the results and conclusions of this study are based. MANR, MVG, RAPN, CLC, LFEC, RADP have approved the final version of this paper. RADP guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

Enamel surface roughness after orthodontic adhesive removal: an in vitro study comparing four clearance methods

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Purpose
Adhesive remnants removal is the last key step influencing orthodontic treatment outcomes. Four different clearance methods (CM) of orthodontic adhesive were evaluated to determine, which achieved the smoothest enamel surface in the shortest time.

Materials and Methods
75 intact premolars extracted for orthodontic purposes were included, sixty had an orthodontic bracket bonded and subsequently removed, and fifteen served as the control group. Four CMs were used to clear the tooth surface of 15 premolars each: carbide bur (CB), carbide bur with titanium nitride surface treatment + fine carbide bur (CBCB), glass fiber-reinforced composite instrument (GFCB), zirconia bur + glass fiber-reinforced composite bur (ZBCB). The processing time was recorded. In ten premolars from each group, the enamel surface was evaluated by atomic force microscopy estimating mean roughness (Ra), roughness profile value (Rq), and roughness depth (Rt). Enamel Damage Index (EDI) was assessed with a scanning electron microscope on 5 remaining premolars.

Results
Significant differences were observed in all evaluated parameters - Ra ($p<0.0001$), Rq ($p<0.0001$), and Rt ($p<0.0001$). GFCB exhibited the smoothest surface in all parameters. The lowest EDI exhibited teeth treated by GFCB, however, the differences were not significant. Working with GFCB took the longest time (mean 116 s), and the shortest with CBCB (mean 49 s).

Conclusion
Using CB is the fastest clearance method, but the enamel surface roughness was highest. Clearing with a set of instruments CBCB proved to be a fast method with satisfying remaining enamel roughness.

Keywords: *Enamel roughness, clearance method, orthodontics, adhesive, tooth surface*

Introduction

The goal of orthodontic treatment is to achieve treatment plan objectives efficiently while avoiding any iatrogenic damage. At the end of the active phase of the treatment, when removing fixed orthodontic appliances or attachments, the focus should be on preventing damage to hard dental tissues through the use of appropriate techniques and instruments during adhesive remnants removal. The roughness of the inadequately treated enamel surface might bring future problems, as the threshold roughness value of the enamel surface for bacterial adhesion has been determined as 0.2 µm (1). Conventional methods of adhesive removal can lead to macroscopically visible deep grooves ranging from 10 to 20 µm (1).

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In 1979, Zachrisson and Årtun evaluated the enamel surface after adhesive remnants removal using a scanning electron microscope (2). According to the degree of enamel damage, diamond tools were found to be unacceptable for adhesive remnants removal. Even fine diamond cutters caused coarse scratches. Using the fine diamond polishing discs led to an acceptable enamel surface but with deep scratches. Subsequently, a carbide bur (CB) was recommended as the best instrument for adhesive remnants removal (3-7). Further studies presented glass fiber reinforced composite instrument (GFCB) for adhesive remnants removal. It was originally designed to remove pigmentation and polish the enamel surface and established itself in orthodontics as a suitable tool for removing adhesive residues. Karan *et al.* (8) in 2010 in her study presented the effect of GFCB on an enamel surface - the achieved surface was smoother than compared with CB treatment. The same result was reached by Mohebi *et al.* (9); however, they identified the carbide CB as the tool of choice because of the shorter treatment time. In the present study two more CM methods were presented and compared to CB and GFCB, which are used in clinical practice, but were not compared to the effectivity of these two standard instruments.

The present study aimed to assess four CM used for the adhesive remnant removal in terms of the treatment duration for each CM and especially the resulting enamel surface roughness using atomic force microscopy and scanning electron microscopy. The null hypothesis of the study was that resulting enamel surface roughness and treatment time would not differ between the four CM.

Materials and Methods

Ethical approval

The survey was approved by the ethics committee of the Institutional Review Board EK/1/25/03/2021.

Experimental design

The material consisted of 75 intact premolars, all of which were extracted as part of an orthodontic extraction treatment plan. Each tooth crown was inspected for any visible cracks, scratches, or other damage on its buccal surface by visual inspection using dental light (A-dec 500 LED dental light) and a magnifying glass. Only intact and healthy teeth were included in the sample. The extracted teeth were randomly divided into five groups - 15 premolars in each.

To the buccal surface of sixty premolars metal premolar brackets Mini Master MBT (American Orthodontics, Sheboygan, WI, USA) were bonded in a standard manner. The brackets were positioned in the center of the vestibular surface of the anatomical crown according to the bracket placement rules. The prescribed bonding protocol was followed closely, every tooth surface was first etched for 30 seconds using 36% phosphoric acid (M+W Big Etch, M+W Dental, Büdingen, Germany), then rinsed with water for 30 seconds and dried with an air syringe. Subsequently, Transbond™ MIP adhesive (3M Unitek, St. Paul, MN, USA) was applied to the etched enamel surface, excesses were removed with a suction device. To ensure bond failure between the bracket and the adhesive layer during bracket removal, the bracket base was lubricated with a thin layer of petroleum jelly, allowing most of the adhesive to remain on the tooth surface (10). Then, Transbond™ PLUS Color Change Adhesive (3M Unitek, St. Paul, MN, USA) was applied to the bracket base, and the bracket was pressed onto the prepared enamel. The excess adhesive was removed with a probe, and each bracket was light-cured using a 3M ESPE Ortholux™ Luminous Curing light (3M Unitek, St. Paul, MN, USA) for 40 seconds (10 seconds each from the mesial, occlusal, distal, and gingival sides). The teeth with attached brackets were stored in water at room temperature for 24 hours. The following day, the brackets were removed by an experienced orthodontist using bracket-removing pliers (Dentaurum Premium Line 004-349, Dentaurum, Ispringen, Germany), following the "wing model" introduced by Brosh (11). The debonding pliers that were inserted under the occlusal and gingival wings of the bracket and pressed. As the bracket base was coated with petroleum jelly, minimal strength was necessary to debond the bracket. This method was proven to reduce the risk of enamel damage (12).

The sixty premolars with adhesive remnants were randomly divided into four groups of 15 premolars each (table 1). For each group, a different CM was used to remove the adhesive remnants: group 1- carbide bur (CB); group 2 - carbide bur with titanium nitride surface treatment + fine finishing with a carbide bur (CBCB); group 3 - glass fiber-reinforced composite bur (GFCB); group 4 - zirconia bur + fine finishing with a glass fiber-reinforced composite bur (ZBCB; figure 1)

The remaining 15 premolars with no adhesive served as a control group to assess the natural enamel surface. On each premolar a new instrument was used to eliminate the influence of tool wear on the results. Manufacturer's protocols were strictly followed for the use of the individual instruments, including rotation speed. Micromotor handpiece was used for all instruments, the rotation speed was 30000 revolutions per minute (rpm) for CB and CBCB, 10000 rpm for GFCB, and 15000-20000 rpm for ZBCB. Final fine enamel polishing was not included in the study design, as it cannot eliminate the grooves and pits on the surface (13-15).

On 10 premolars from each group evaluation of the enamel surface roughness was performed using an atomic force microscope (AFM; Dimension Icon, Bruker, MA, USA). Five scans were performed on each tooth in the area where the adhesive was removed, the exact location determined by

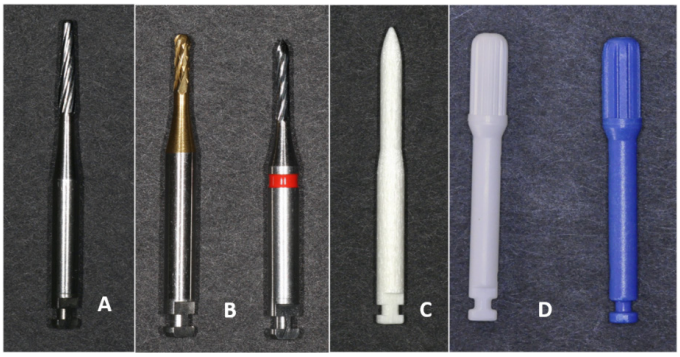

Figure 1. *Four CM instruments/set of instruments A) carbide bur, B) carbide bur with titanium nitride surface treatment + fine carbide bur, C) glass fiber-reinforced composite bur, D) zirconia bur + glass fiber-reinforced composite bur.*

Table 1. *The instruments used to remove adhesive remnants in each sample group.*

Samples groups	Adhesive remnant	Handpiece used	Rotation speed (rpm)	Instrument	Manufacturer
CB	Yes	Micromotor	30000	Carbide bur	NTI-Kahla GmbH, Kahla, Germany
CBCB	Yes	Micromotor	30000	Carbide bur with titanium nitride surface treatment + fine carbide bur	NTI-Kahla GmbH, Kahla, Germany
GFCB	Yes	Micromotor	10000	Glass fiber-reinforced composite bur	Stainbuster, Abrasive Technology Inc, Lewis Center, Ohio, USA
ZBCB	Yes	Micromotor	15000-20000	Zirconia bur + glass fiber-reinforced composite bur	DSI, Dental Solutions Israel, Ashdod, Israel
Control Group	No	None	None	None	None

Rpm – revolution per minute, CB – carbide bur; CBCB - carbide bur with titanium nitride surface treatment + fine diamond bur; GFCB - Glass fiber-reinforced composite bur; ZBCB - zirconia bur + glass fiber-reinforced composite bur

the overseeing orthodontist. For each scan, an area of 25x25 µm was probed on a flat section of the enamel surface. The scanning was performed using the PeakForce Tapping Technology and ScanAsyst probes (40 kHz, 0.4 N/m) with applied forces of about 5-7 nN, low enough to induce any surface damage. The AFM images were analyzed using the specialized Gwyddion software to obtain the values of individual parameters listed below. The evaluating parameters were adopted from the study by Karan *et al.* (8) and were as follows (all expressed in nanometers): arithmetic mean roughness parameter (Ra) - represents the arithmetic mean of all parts of the roughness profile; root mean square roughness parameter (Rq) - represents the root mean square of all values of the roughness profile; roughness depth (Rt) or total height of the R-profile - representing the sum of the highest peak of the profile and the depth of the deepest valley of the R-profile within the measured path. Overall, 50 measurements were obtained for each group and each measured parameter.

The remaining five premolars from each sample group were investigated by a scanning electron microscope (JSM-IT500HR JEOL InTouchScope™, Tokyo, Japan) at 5.0 kV with a 10 mm working distance and a 500× magnification. After obtaining the micrographs, one was randomly picked from each specimen. The presence of adhesive remnants was evaluated on each. Evaluation of the enamel surface was performed using the Enamel Damage Index (EDI) according to the exact procedure established by Schuler and van Vaese (16). The individual values were assigned by a single trained and experienced evaluator. The index has four levels: 0 - smooth enamel surface without grooves and cracks 1 - acceptable enamel surface with scattered grooves, covering only 1-10% of the enamel surface; 2 - rough surface with deep furrows or grooves covering 11-50% of the enamel surface; 3 - coarse furrows and wide grooves covering more than 50% of the enamel surface, damage visible to the naked eye. Observations using the electron microscope were performed without gold coating of the enamel surface, which can sometimes be an altering factor for the detection of lesions on the enamel surface (17).

To determine the time required to remove the adhesive residue with each instrument, the time interval in seconds needed for complete adhesive remnant removal was measured for each sample. Adhesive removal on all samples was performed by one experienced orthodontist in a standard manner, while an assistant recorded the time using stopwatches. Only the exact treatment time was measured, the timer was stopped for every replacement of the instrument in the CBCB and ZBCB groups.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA), NCSS 10 statistical software (2015, NCSS, LLC., Kaysville, UT, USA), and MS Excel 2016 (Microsoft Corp., Redmond WA, USA). The sample size was determined using G*Power 3.1.9.7. Considering the parameters obtained by examining pilot samples, effect size determined from pilot samples, 95% confidence level (1-α), and 80% test power (1- β), 8 samples for each group were deemed sufficient. The normal distribution of variables was assessed using Shapiro-Wilk tests for the quantitative variables. For the comparison of five independent samples, analysis of variance (ANOVA) was used, followed by Bonferroni post hoc tests. Qualitative data were evaluated using Fisher's exact test. All tests were conducted at a significance level of 5%. In case of comparing the times needed to remove adhesive remnants, *t*-test was used. To adjust for multiple comparisons and keep the familywise α at 0.05, the Bonferroni correction was used. The resulting α for a single comparison was 0.0167. Box plots were used to visualize the distribution of quantitative variables.

Results

Results of the evaluation of the enamel surface performed by AFM for intact enamel and enamel treated by different CM are listed in table 2. The lowest values of all three scores were achieved by GFCB (Ra=98.25; Rq=118.67; Rt=421.97), the roughness depth achieved by this instrument was lower than in intact enamel. The highest values of all three scores were found for enamel treated by CB (Ra=238.31; Rq=286.7; Rt=1034.22). The results for ZBCB were close to the results of intact enamel (Ra=144.71; Rq=175.33; Rt=605.95), the results of

Rt and Rq for CBCB were mediocre. The analysis of variance revealed statistically significant differences among the groups for all examined parameters - Ra ($p<0.0001$; power=1.0000), Rq ($p<0.0001$; power=1.0000), and Rt ($p<0.0001$; power=1.0000). The evaluation of the EDI index was performed on the SEM images (table 3, fig. 2A-E). No adhesive remnants were found on any teeth in the sample. The teeth treated with the GFCB instrument showed the smoothest enamel surface (1 tooth classified as 0, 3 teeth classified as 1, and 1 tooth classified as 2), while the teeth treated by CB had the worst EDI results. EDI results for ZBCB and CBCB were in between, with most of the teeth EDI 1 or 2. No statistically significant differences were found between the four CM according to Fisher's exact test ($p=0.201$). The treatment with the GFCB instrument takes significantly longer (mean = 116 s) compared to all other groups of teeth treated with other instruments (fig.3): the CB (mean = 66 s; $p<0.0001$; power=0.99689), the CBCB (mean = 49 s; $p<0.0001$; power=0.99999), and the ZBCB (mean = 61 s; $p<0.0001$; power=0.99759). No significant differences in time intervals were found among the other instruments.

Table 2. *Evaluation of the enamel surface performed by atomic force microscope.*

Parameters	Instrument	Average	SD	95% CI average		Minimum	Maximum	p
				Lover limit	Upper limit			
Ra	CB	238.31	79.16	215.81	260.81	95.28	431.20	<0.0001***
	CBCB	148.07	53.22	132.95	163.20	64.04	270.10	
	GFCB	98.25	40.69	86.68	109.81	31.79	218.10	
	ZBCB	144.71	53.97	129.38	160.05	61.25	304.10	
	Control	134.41	62.75	116.58	152.25	33.00	268.50	
Rq	CB	286.97	89.79	261.45	312.49	119.90	497.00	<0.0001***
	CBCB	178.58	60.83	161.29	195.87	79.20	304.90	
	GFCB	118.67	46.85	105.35	131.98	38.02	254.80	
	ZBCB	175.33	61.60	157.82	192.84	78.44	344.80	
	Control	158.98	69.89	139.12	178.85	42.58	305.90	
Rt	CB	1034.22	336.55	938.57	1129.87	400.06	1874.00	<0.0001***
	CBCB	630.70	202.24	573.22	688.17	324.30	1313.00	
	GFCB	421.97	161.17	376.16	467.77	146.60	880.60	
	ZBCB	605.95	206.07	547.38	664.51	278.20	1170.00	
	Control	539.69	225.86	475.50	603.88	138.60	1034.00	

****p<0,001; 95% CI – 95% confidence interval; Ra - mean roughness value - arithmetic mean value of the roughness profile; Rq - mean value - quadratic mean of all roughness profile values; Rt - depth of roughness - is the sum of the highest and lowest points of the measured area. CB – carbide bur; CBCB - carbide bur with titanium nitride surface treatment + fine diamond bur; GFCB - Glass fiber-reinforced composite bur; ZBCB - zirconia bur + glass fiber-reinforced composite bur*

Table 3. *Contingency table with EDI results.*

Instrument	Findings	EDI (surface roughness)				Total
		0	1	2	3	
CB	count	0	0	2	3	5
	%	0.0%	0.0%	40%	60%	100%
CBCB	count	0	1	2	2	5
	%	0.0%	20%	40%	40%	100%
GFCB	count	1	3	1	0	5
	%	20.0%	60.0%	20.0%	0.0%	100%
ZBCB	count	0	2	3	0	5
	%	5.0%	30.0%	40.0%	25.0%	100%
Total	count	1	6	8	5	20
	%	5.0%	30.0%	40.0%	25.0%	100%

EDI - enamel damage index; CB – carbide bur; CBCB - carbide bur with titanium nitride surface treatment + fine diamond bur; GFCB - Glass fiber-reinforced composite bur; ZBCB - zirconia bur + glass fiber-reinforced composite bur

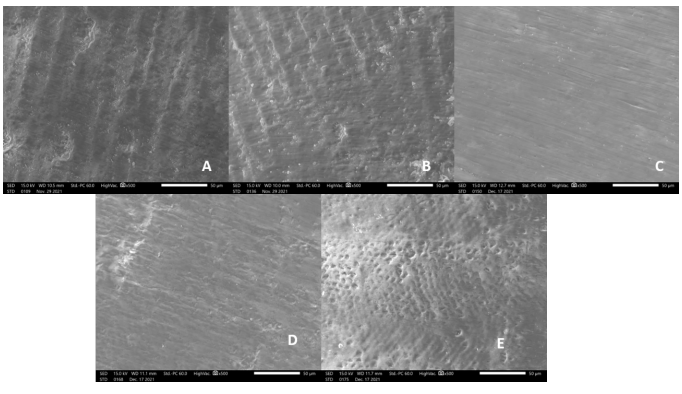

Figure 2. *Scanning electron microscope images of enamel at 500x magnification A) carbide bur, B) carbide bur with titanium nitride surface treatment + fine diamond bur, C) glass fiber-reinforced composite bur, D) zirconia bur + glass fiber-reinforced composite bur, E) intact enamel.*

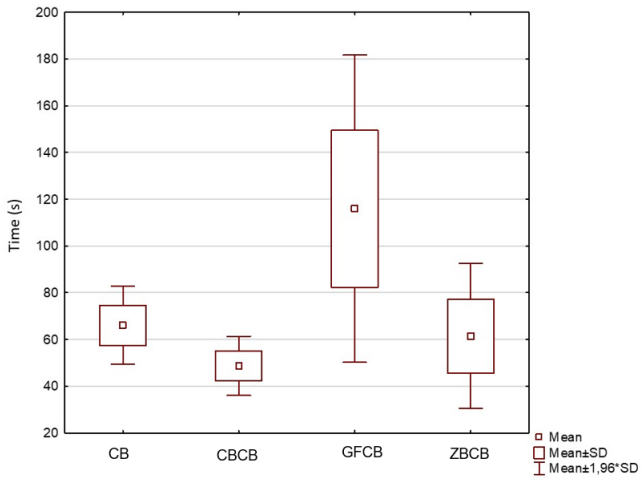

Figure 3. *The mean values and standard deviations of time needed to remove the adhesive remnants for each instrument. CB – carbide bur; CBCB - carbide bur with titanium nitride surface treatment + fine carbide bur; GFCB - glass fiber-reinforced composite bur; ZBCB - zirconia bur + glass fiber-reinforced composite bur.*

Discussion

Care must be taken when removing adhesive remnants after fixed appliance or aligner treatment as any roughness of the enamel surface may result in excessive plaque accumulation and increased pigment deposition (18,19). Even if the enamel surface appears clinically undamaged minuscule adhesive remnants or grooves and scratches from inadequately used instruments might surpass the threshold roughness value for bacterial adhesion. Bonetti *et al.* (20) observed residual adhesive in 20% of the teeth examined in vivo with a scanning electron microscope. We have not found any adhesive remnants on the teeth in the sample, probably due to easier cleaning of the adhesive remnants under in vitro conditions with optimal lighting.

Iatrogenic scratching, grooving or infractions of the enamel may occur during mechanical bracket removal due to force applied by pliers or possible direct mechanical damage (21). In the present study before bracket bonding a thin layer of petroleum jelly was applied to its base, which caused the bond to break at the bracket base and the adhesive, leaving all adhesive on the tooth (10). This procedure facilitated smooth bracket removal with low force. Therefore, any alteration to the enamel surface found was attributed solely to the instrument used for adhesive remnants removal.

To compare the effects of the instruments on the enamel surface, we selected the four most frequently used CM: carbide bur, which is considered the gold standard for removal of the adhesive remnants; as well as carbide bur with nitride treatment and composite or zirconia bur. Although CB are considered safe, when used incautiously with high pressure and higher than recommended rotation speed they can cause enamel pitting and pulpal thermal changes (22,23). In the present study, the speed was always set according to the manufacturer's recommendations to test the I/SI efficiency properly, on the other hand, Zachrisson *et al.* (2) in 1979 recommended lower speeds for safety reasons for CB.

Results from SEM showed that the enamel surface after adhesive remnants removal with the GFCB was the smoothest while the surface treated by CB presented the worst results, however, the differences between the four investigated CM methods were insignificant. Results from an electron microscope might be biased due to the subjective assessment of the EDI by the evaluator and therefore inaccurate. Shah *et al.* (10) compared the effect of the GFCB instrument on the enamel surface with other three fine polishing systems using SEM together with a surface roughness tester, and their results confirmed that the enamel surface appeared closest to natural enamel when using the GFCB instrument. Garg *et al.* (24) compared the GFCB with another composite bur and a CB using the same investigative method. Their results showed a significantly smoother enamel surface when using both composite burs compared to the standard CB. In the present study SEM results were complemented by an AFM investigation to obtain objective values of enamel roughness since AFM is more sensitive to surface topography even down to the nanoscale (25). The results of all three investigated roughness parameters indicate that the smoothest enamel surface was achieved with the GFCB instrument. It produced the least rough enamel surface compared to CB, CBCB, and ZBDB. The resulting Ra, Rq, and Rt values were even lower after using the GFCB instrument than in an intact enamel. CBCB created a satisfactory final enamel surface according to all Ra, Rq, and Rt values. Enamel treated by CB achieved the highest Ra and Rt scores, while the highest Rq score was estimated for ZBCB. Mohebi *et al.* (9) reached a similar conclusion when comparing the effect of the GFCB versus the CB by AFM in a high-speed and low-speed handpiece. Karan *et al.* (8) also confirmed that the GFCB left a smoother enamel surface than the CB. However, Sugsompian *et al.* (26) concluded in their study that all investigated clearance methods (Sof-Lex disc, sandblaster, tungsten carbide bur, and white stone bur) resulted in a clinically acceptable enamel surface roughness.

Chair time is nowadays the most expensive part of orthodontic treatment, therefore cleaning of the adhesive remnants should be as quick and effective as possible. Removing the adhesive remnants in perfect in vitro conditions by an experienced orthodontist with CBCB took less than a minute and with CB 66 seconds on average. Using the GFCB instrument it took almost twice as much time. Caution must be drawn when interpreting this result - this study was done in vitro, thus the result might not reflect the clinical situation. In the case of sets of instruments (CBCB and ZBCB) the clinical treatment time might be higher because of the need to replace the instruments, however in reality the replacement occurs just once for each dental arch, which should not affect the time interval needed for adhesive remnants removal much. For the time-consuming nature of the preparation with the composite instruments, Mohebi *et al.* (9) recommended starting with the removal of the thickest layers of adhesive using a carbide bur and completing the work with a composite bur when only a thin layer of adhesive is present, which might decrease the clearance duration.

Clinically patients' discomfort might be a problem during adhesive remnants removal. While in some studies vibrations of specified frequency and magnitude are discussed to be effec-

tive in pain relief during the active phase of orthodontic treatment, others report that vibrations of the tooth during drilling a cavity cause unpleasant feelings for patient (27,28). Clearing the adhesive remnants with burs corresponds more with the latter and might differ according to the used speed of the bur (23). Yet, there are no studies of patient discomfort during adhesive remnants removal by burs, most studies concentrate on the pain felt during mechanical removal of the brackets (29,30). The personal perception of the operator in the present study was, that while working with a zirconia bur the vibrations were higher than while using other tools. Further studies are necessary to estimate the levels of patient discomfort using different instruments for adhesive remnants removal.

Instruments for adhesive remnants removal are usually not disposable, but their durability is not much discussed or researched. It is usually up to the treating clinician to evaluate the instrument suitable for its continued use. In studies dealing with the effect of the instrument on the enamel surface, a new instrument is usually used for each tooth to avoid biased results by instrument wear, as in the case of the present study. However, it is not clearly stated how often the instrument should be changed in everyday practice. According to a study by Pines and Schulman, the greatest edge abrasion of the CB occurs when used directly on enamel and edge blunting occurs after preparation of approximately 11 enamel surfaces, *i.e.*, one dental arch (31). In addition to edge abrasion caused by the inorganic filler, the reduction in tool efficiency also results in clogging of the sawdust space between the blades. On the other hand, according to the manufacturer's leaflet, GFCB remains sharp thanks to the glass fibers throughout use, reducing its mass. There are no publications about the wear of the CBCB and ZBCB that the authors are aware of. More studies on the wear of instruments used for adhesive remnants clearance are needed as studies on brand-new instruments may not represent a standard clinical situation.

This study has some limitations that should be considered when interpreting the results. A limitation of the current study is that it does not utilize the repeated investigation method (i.e. one investigation before bracket placement and the second after its removal for AMF and SEM investigations), as using the control sample might not bring the exact results. Another limitation is the possibility of overlooking smaller grooves or scratches during the initial inspection of the enamel surface by visual inspection under dental light and magnifying glass, which could have an impact on the results. Also, although every bracket base was coated with petroleum jelly and minimal strength was necessary to debond the bracket, the possibility of enamel damage while removing the brackets with pliers cannot be excluded. Adhesive remnants were cleaned by hand by one experienced orthodontist, therefore differences in applied pressure on the individual instruments cannot be excluded.

Conclusion

The results have shown significant differences in the enamel roughness and treatment time among the different clearance methods in vitro. The Glass fiber-reinforced composite bur achieved the smoothest enamel surface, but it required the longest processing time. Using a carbide bur was the fastest clearance method, but the enamel surface roughness was the highest. Using the set of instruments - carbide bur with titanium nitride surface treatment and fine carbide bur - proved to be a fast method to remove the remaining adhesive with satisfying remaining enamel roughness.

Türkçe öz: *Ortodontik yapıştırıcı çıkarıldıktan sonra mine yüzey pürüzlülüğü. Dört temizleme yöntemini karşılaştıran bir in vitro çalışma. Amaç: Yapıştırıcı kalıntılarının çıkarılması, ortodontik tedavi sonuçlarını etkileyen son önemli adımdır. En kısa sürede en pürüzsüz mine yüzeyini hangi yöntemin elde ettiğini belirlemek amacıyla dört farklı ortodontik yapıştırıcı temizleme yöntemi (CM) değerlendirildi. Gereç ve Yöntem: Ortodontik amaçlarla çekilen 75 sağlam küçük azı dişi çalışmaya dahil edildi, bunların altmışına ortodontik braket yapıştırıldı ve daha sonra çıkarıldı, on beşi kontrol grubu olarak kullanıldı. Her bir CM, 15 küçük azı dişinin yüzeyini temizlemek için kullanıldı: karbür frez (CB), titanyum nitrür yüzey işlemi + ince karbür frez (CBCB), cam elyaf takviyeli kompozit alet (GFCB), zirkonya frez + cam elyaf takviyeli kompozit frez (ZBCB). İşlem süresi kaydedildi. Her gruptan on küçük azı dişinde, atomik kuvvet mikroskobu ile ortalama pürüzlülük (Ra), pürüzlülük profil değeri (Rq) ve pürüzlülük derinliği (Rt) tahmin edilerek mine yüzeyi değerlendirildi. Kalan beş küçük azı dişinde taramalı elektron mikroskobu ile Mine Hasar Endeksi (EDI) değerlendirildi. Bulgular: Tüm değerlendirilen parametrelerde - Ra ($p<0.0001$), Rq ($p<0.0001$) ve Rt ($p<0.0001$) - önemli farklılıklar gözlemlendi. GFCB, tüm parametrelerde en pürüzsüz yüzeyi sergiledi. En düşük EDI, GFCB ile tedavi edilen dişlerde gözlemlendi, ancak farklar önemli değildi. GFCB ile çalışmak en uzun süreyi aldı (ortalama 116s) ve en kısa süre CBCB ile çalışıldı (ortalama 49s). Sonuç: CB kullanımı en hızlı temizleme yöntemi olsa da, mine yüzey pürüzlülüğü en yüksekti. CBCB alet seti ile temizleme, kalan mine pürüzlülüğünde tatmin edici sonuçlarla hızlı bir yöntem olduğunu kanıtladı. Anahtar kelimeler: mine pürüzlülüğü, temizleme yöntemi, ortodonti, yapıştırıcı, diş yüzeyi*

Ethics Committee Approval: The survey was approved by the ethics committee of the Institutional Review Board EK/1/25/03/2021.

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: DR, MK, AB, LS participated in designing the study. DR, AB, LS participated in generating the data for the study. DR, AB, LS, AL participated in gathering the data for the study. DR, MK,PK participated in the analysis of the data. DR, MK, WU wrote the majority of the original draft of the paper. PK, AL, WU participated in writing the paper. DR, MK, AB, LS has had access to all of the raw data of the study. DR, MK, WU has reviewed the pertinent raw data on which the results and conclusions of this study are based. DR, MK, AB, LS, PK, AL, WU have approved the final version of this paper. WU guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

Management of jump space in immediate implants with and without demineralised freeze dried bone allograft: a randomised controlled trial

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Purpose
The present study aimed to evaluate and compare the management of Jump space (JS) in immediate implants with and without Demineralised freeze-dried bone allograft (DFDBA) with flapless approach.

Materials and Methods
The present study included 40 sites with immediate implant placement in the maxillary anterior region. Group 1 patients were treated without augmentation while Group 2 patients with DFDBA in the JS. Both the groups were further subdivided according to the horizontal dimensions as JS less (G1S1, G2S1) or more than 2mm (G1S2, G2S2). Plaque index (PI), Gingival Index (GI), Probing depth (PD), Testori esthetic score (TS), VAS score, Crestal Bone height (CBH), Ridge width (RW), Vertical distance (VD) and radiolucent area (RA) were evaluated radiographically with CBCT at baseline and 12 months' post therapy.

Results
Significant differences were observed in CBH in the midfacial region in G1S1-G2S1 with the mean of 0.34±0.19mm and G1S2 –G2S2 with 0.75±0.26 mm at 12 months. Significant differences in TS were observed in G1S1 and G2S1 with mean value of 0.55±0.53 while G1S2 and G2S2 exhibited value of 1.33±0.82.

Conclusion
DFDBA shows better CBH preservation in midfacial region, reduction in RA indicating greater resolution of JS thereby leading to better hard and soft tissue healing.

Keywords: *Immediate implants, jumping gap, peri implant tissues, bone graft, tissue healing*

Introduction

The advent of immediate flapless dental implants marked a significant stride in the realm of minimally invasive implantology, revolutionizing treatment protocols by streamlining procedures and maximizing patient comfort, all while boasting an impressive success rate (1). Within this innovative approach lies a pivotal challenge: effectively managing the space, known as the jump space (JS), between the implant periphery and the surrounding bone, particularly when implants are inserted into fresh extraction sockets (2). Navigating the intricacies of the JS and achieving primary socket closure present ongoing hurdles for implantologists, demanding meticulous attention, especially in the aesthetic zone where the buccal bony plate tends to be thin. The potential for soft tissue recession due to buccal bone resorption underscores the critical importance of surgical interventions to optimize outcome. However, the landscape of tech-

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niques for managing the buccal space remains marked by controversy and confusion. The quest for an ideal approach encompasses three key objectives: facilitating optimal bone fill within the space, attaining the most coronal level of bone-to-implant contact (BIC) and mitigating buccal bone resorption and soft-tissue recession to the greatest extent possible.

Various techniques and materials have been developed to reliably facilitate tissue regeneration in implant dentistry, aiming to achieve optimal peri-implant hard and soft tissue quantity and quality. Among these advancements, human decalcified freeze-dried bone allografts (DFDBA) provides a vital solution serving in periodontal regeneration and alveolar ridge maintenance ensuring adequate bone volume for endosseous implant placement (3). DFDBA operates as a multifaceted material, functioning to maintain space, promote bone growth and harnessing the potential of bone morphogenetic proteins to induce new bone formation (4).

The space between socket walls and the implant surface presents a crucial consideration, often necessitating augmentation to reliably achieve bone-to-implant contact (BIC) and mitigate the risk of soft tissue collapse (5). Nonetheless, conflicting viewpoints within the literature have stirred controversy on this matter. While certain studies have reported successful horizontal space regeneration, even in cases where the space is smaller than 2 mm and a stable blood clot is present (6,7), recent investigations have revealed a contrasting trend. These newer findings suggest that spaces exceeding 2 mm tend to exhibit superior fill rates, even in the absence of additional grafting material (8).

Numerous biomaterials have been tried with varying results to fill the JS between the implant body and the buccal cortex (9). However, there remains a notable paucity in the literature concerning the efficacy of using DFDBA specifically for filling the vertical distance and JS around immediate implants placed using a flapless approach, as well as its impact on soft tissue dimensions. Consequently, the present study was devised to examine the influence of DFDBA in immediate implants with JS measuring 2 mm or more on both hard and soft tissue dimensions. The null hypothesis was that there is no significant difference in the hard and soft tissue dimensions with the use of DFDBA in immediate implants with JS measuring 2mm or more.

Materials and Methods

Ethical approval

The study was carried out between February 2021 and March 2022. Approval for this clinical trial was obtained from the Institutional Ethics Committee of our institute, aligning with the updated principles of the Helsinki Declaration for biomedical research. The Institutional Ethics Committee (IEC) provided approval under the registration number IEC/VSPMDCRC/06/2019. Furthermore, the trial was registered with the Clinical Trials Registry of India under registration number CTRI/2021/01/030620 and adhered to the CONSORT statement and EQUATOR guidelines for reporting.

Sample size determination

The sample size was determined based on the findings of a study by Paknejad M. *et al*. (9), wherein the authors examined the impact of flapless implant placement combined with graft material on the height of buccal bone. A power analysis indicated an effect size of 1.25. To achieve this effect with 95% confidence and 80% power, the estimated number of sites per group was determined to be 12 (total: 24). Anticipating potential attrition, 20 sites per group were enrolled in the study.

Study design and patient selection

This study was designed as a parallel-group, randomized clinical trial with four arms, each with an equal allocation ratio 1:1:1:1. Patients indicated for immediate implant therapy in the maxillary anterior region up to premolars i.e from teeth number 15 to 25 (according FDI tooth numbering system) on either sides were recruited from the Department of Periodontics and Implantology of our institute. Prior to their participation, written informed consent was obtained from all study participants.

The inclusion criteria for the trial were as follows: participants were required to be systemically healthy with stable soft tissue morphology, demonstrate cooperation and a commitment to oral hygiene, present with root stumps or non-restorable teeth, fractured teeth, and possess approximately 4mm or more of apical bone to ensure primary stability. Additionally, sites were included if a minimum torque of 35N cm was achieved during implant insertion. Patients were excluded from the study if they exhibited general contraindications to implant surgical procedures, had a history of radiotherapy and/or chemotherapy, were undergoing or had previously undergone treatment with intravenous amino-bisphosphonates, were smokers or exhibited poor oral hygiene, or displayed para-functional habits.

Suitable sites in patients requiring dental implants and meeting the inclusion criteria were randomly assigned using computer-generated random tables and the simple randomization method. Allocation concealment was ensured through the use of sequentially numbered, opaque, sealed envelopes (SNOSE) technique. Each envelope contained a piece of paper indicating the assigned randomization group, and these envelopes were labelled with serial numbers to maintain anonymity and prevent bias. Once the patient provided consent to participate in the study, the investigator opened the sealed envelope and assigned the treatment group accordingly. This clinical trial employed a double-blinded approach, wherein both the patients and the assessor were blinded to treatment allocation. Selection bias was mitigated through randomization, ensuring equal distribution of participants across treatment groups. Performance bias was minimized as all patients received treatment from the same operator. To further control for potential confounding variables, patients were matched for demographics such as age and gender, as well as for relevant risk factors. Additionally, the reliability of the data collected was assessed through a test-retest method, enhancing the robustness and accuracy of the findings.

Based on the specified criteria, the study population was divided into two groups:

Group 1: Immediate implants without DFDBA in the JS (n=20 sites)

- Group 1 Subgroup 1 (G1S1): JS less than 2mm.
- Group 1 Subgroup 2 (G1S2): JS more than 2mm.
- Group 2: Immediate implants with DFDBA in the JS (n=20 sites)
- Group 2 Subgroup 1 (G2S1)-JS less than 2mm.
- Group 2 Subgroup 2 (G2S2)-JS more than 2mm.

Pre-surgical therapy

Before undergoing surgery, all patients received thorough pre-surgical hygiene therapy. This included a comprehensive case history review, a detailed intraoral examination, personalized oral hygiene instructions, and professional scaling and root planning.

Evaluation of Clinical and Radiographic Parameters

The clinical data for all patients was meticulously recorded by a single examiner (MP), who underwent pre-calibration for precise measurements. Moreover, the assessor remained blinded to the treatment group information. All parameters were documented at two separate time points, and the intra-observer reliability of the measurements was assessed using the intra-class correlation coefficient. Plaque index (PI) (10), Gingival Index (GI) (11) was assessed at baseline and post operatively at 6 and 12 months. Probing depth (PD), Soft tissue assessment using Testori esthetic score (TS) (12) and VAS scale was used for post–operative pain evaluation. Following radiographic parameters were evaluated using CBCT at baseline and 12 months' post therapy. (Figure 1) Scans were performed with standardized scanning parameters at 85 kV, 7 mA, and 3.6 s of exposure time using a field of view of 5 cm × 5 cm and a resolution of 150 µ. Interactive CBCT Processing software (3Diagnosys 4.2) was used to obtain reformatted coronal, sagittal, cross-sectional, and panoramic views.

1. Crestal bone height (CBH) was assessed at mesial, midfacial and distal aspects as the distance between tooth CEJ/implant shoulder to the most coronal point of interproximal crestal bone using CBCT.
2. Ridge width (RW) was measured at 2mm and 4mm from the alveolar crest.
3. Radiolucent area (RA) was measured as area between shoulder of implant and bone crest.
4. Vertical distance (VD) was measured as the distance between the first BIC to the first thread of the implant on the mesial (VDM) and distal (VDD) sides.
5. JS was measured on buccal, palatal, mesial and distal aspects as the distance between inner aspect of the alveolar bone to the outer surface of the implant.

Patients were taken up for the surgical intervention by a single experienced clinician (AK) and atraumatic extraction was done followed by implant placement without raising the flap. The JS when more than 2mm was filled with DFDBA in the Group 2 and was left un-grafted in the Group 1 (Figure 2). Post-operative instructions, antibiotics and anti-inflammatory drugs were given to the patients and regular follow up appointments were scheduled.

Statistical analysis

The data collected from all groups was analysed using Statistical Package for Social Sciences (SPSS) for Windows software, version 26.0. (IBM SPSS Inc., Armonk, NY, USA). The standard descriptive methods such as the mean, standard deviation, median, frequency, were applied to determine the characteristics of the sample. The demographic characteristics like age and sex were summarized according to scale of measurement. The comparison of mean age between treatment groups was performed using t-test for independent samples, while sex distribution was compared using chi-square test. The parameters PI, GI, PD, RW, GT, VD, CBH and JS were summarized in terms of mean and standard deviation. The VAS score was compared using Mann-Whitney U test. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

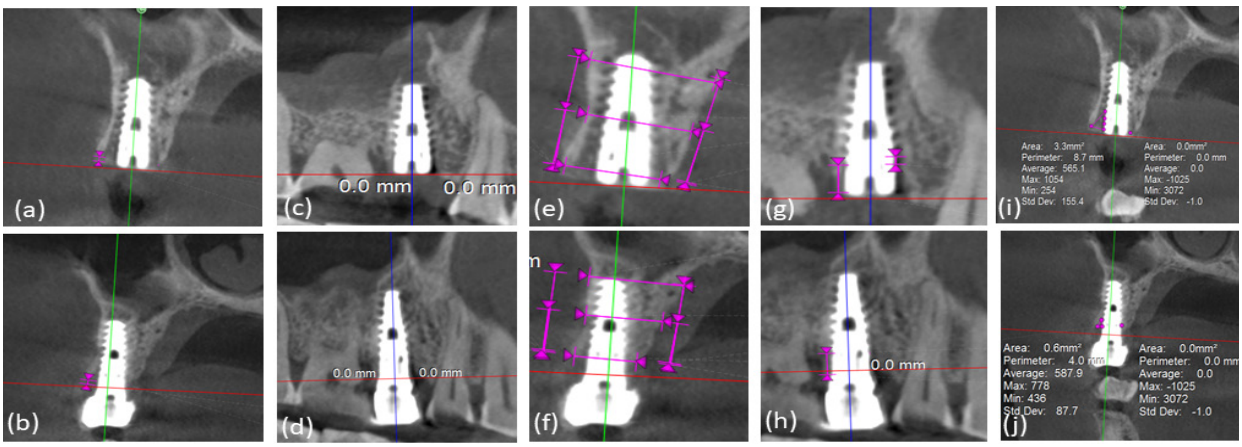

Figure 1. *(a): Measurement of Crestal bone height (CBH) baseline midcrestal, (b) Measurement of Crestal bone height (CBH) 12 months follow up midcrestal (c) Measurement of Crestal bone height (CBH) baseline mesial and distal, (d) Measurement of Crestal bone height (CBH) 12 months mesial and distal (e) Measurement of the Ridge Width (RW) baseline (f) Measurement of the Ridge Width (RW) 12 months follow up (g) Measurement of the vertical distance (VD) baseline, (h)Measurement of the vertical distance (VD) 12 months follow up, (i)Measurement of the Radiolucent area (RA) baseline, (j)Measurement of the Radiolucent area (RA) 12 months follow up.*

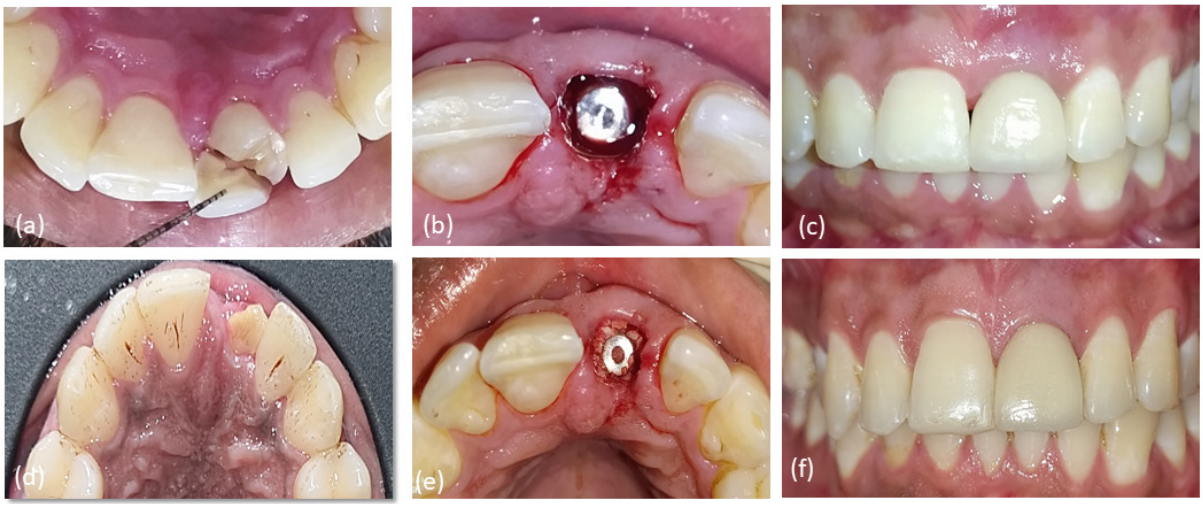

Figure 2. *(a): Pre-operative Group 1, (b): Non grafted Jump Space in Group 1 (c): Follow up of Group 1 after 12 months (d): Pre-operative Group 2, (e): DFDBA graft in Jump Space in Group 2, (f): Follow up of Group 2 after 12 months.*

Results

Table 1 presents the demographic characteristics of the patients, revealing a mean age of 44 ± 12.22 years in Group 1 and 42.33 ± 11.6 years in Group 2. In Table 2, a comparison of CBH at baseline and 12 months is provided for the G1S1, G1S2, G2S1, and G2S2. The comparison encompassed the mesial, midfacial, and distal sides at both time points. Interestingly, a statistically significant difference ($p < 0.0001$) was observed only in the midfacial region at the 12-month mark between the subgroups G1S1-G2S1 and G1S2-G2S2.

Table 3 provides the comparison for RW at baseline and 12 months in G1S1, G1S2, G2S1 and G2S2 categories. The difference of means at 2mm and 4mm from crest, showed statistically insignificant differences between the subgroups G1S1-G2S1 and G1S2 –G2S2 at baseline and 12 months. Table 4 gives the comparison of RA at baseline and 12 months in G1S1, G1S2, G2S1 and G2S2 categories. The difference of means between the two treatment groups was statistically significant on buccal aspect at 12 months with ($p<0.004$) in case G1S1 and G2S1. While on palatal and distal aspect it was found to be statistically insignificant. However, in case of G1S2 and G2S2 the mean differences were found to be statistically significant on buccal and mesial aspect with ($p<0.0001$).

Table 1*. Demographic characteristics of patients in study groups.*

	Parameter	With DFDBA	Without DFDBA	P-value
Age in years	N	20	20	0.704
	Mean	42.33	44	
	SD	11.6	12.22	
	Median	42	42	
	Minimum	28	28	
	Maximum	65	65	
Sex	Male (No. (%))	16(80%)	11 (53.3%)	0.245
	Female (No. (%))	4 (20%)	9 (46.7%)	

Table 5 gives the comparison of VD at baseline and 12 months in G1S1, G1S2, G2S1 and G2S2 categories. The paired differences were statistically insignificant all the groups on mesial aspect; except for the distal aspect of G1S1 and G2S1 which was found to be highly significant ($p<0.0001$).

Table 6 depicts comparison of Jumping Space (JS) in both the treatment groups at baseline and 12 months. On comparison of JS in G1S1 a significant difference was observed at the buccal ($p =0.002$) and mesial site ($p=0.007$) while in G1S2 significant difference was observed on buccal site ($p=0.004$). In G2S1 significant differences were observed after 12 months on buccal ($p<0.0001$) mesial ($p=0.001$) and distal ($p=0.003$) site whereas in G2S2 significant differences were observed on all sites buccal ($p<0.0001$), palatal ($p=0.029$), mesial ($p=0.037$), distal ($p =0.014$) with time. Table 7 shows the comparison of Testori Score (TS) in both the

Table 2*. Comparative statistics for crestal bone height (CBH) at baseline and 12 months.*

Groups	Site	Time period	Mean ± Standard deviation (in mm)	Significance (p value)
G1S1-G2S1 (JS<2mm)	Mesial	Baseline	0.20 ±0.40	0.15
		12 months	0.04±0.13	0.33
	Midfacial	Baseline	-	-
		12 months	0.34±0.19	**<0.0001***
	Distal	Baseline	-	-
		12 months	0.05±0.37	0.64
G1S2-G2S2 (JS>2mm)	Mesial	Baseline	-	-
		12 months	0.10±0.24	0.34
	Midfacial	Baseline	-	-
		12 months	0.75±0.26	**<0.0001***
	Distal	Baseline	-	-
		12 months	0.38±0.45	0.06

CBH: Crestal bone height; G1S1: Group 1subgroup 1, G1S2: Group 1subgroup 2; G2S1: Group 2 subgroup1, G2S2: Group 2 subgroup 2; JS: Jumping Space; P value is significant if <0.05; p- Probability.

***Table 3**. Comparative statistics for ridge width (RW) at baseline and 12 months.*

Groups	Level from the crest	Time period	Mean ± Standard deviation (in mm)	Significance (p value)
G1S1-G2S1 (JS<2mm)	2 mm	Baseline	0.01±0.50	0.957
		12 months	0.47±0.33	0.068
	4 mm	Baseline	0.07±0.52	0.748
		12 months	0.50±0.40	0.052
G1S2-G2S2 (JS>2mm)	2 mm	Baseline	0.55±1.04	0.191
		12 months	0.63±1.15	0.153
	4 mm	Baseline	0.30±0.99	0.441
		12 months	0.36±1.03	0.382

G1S1: Group 1subgroup 1, G1S2: Group 1subgroup 2; G2S1: Group 2 subgroup1, G2S2: Group 2 subgroup 2; JS: Jumping Space; P value is significant if <0.05; P-probability

***Table 4.** Comparative statistics for radiolucent area (RA) at baseline and 12 months.*

Groups	Side	Time period	Mean ± Standard deviation (in mm)	Significance (p-value)
G1S1-G2S1 (JS<2mm)	Buccal	Baseline	0.12 ± 0.20	0.42
		12 months	0.27 ± 0.25	**0.004***
	Palatal	Baseline	0.75 ± 1.24	0.09
		12 months	-	-
	Mesial	Baseline	0.37 ± 1.61	0.49
		12 months	0.04 ± 0.69	0.86
	Distal	Baseline	1.73 ± 3.24	0.10
		12 months	0.06 ± 0.98	0.82
G1S2-G2S2 (JS>2mm)	Buccal	Baseline	2.13 ± 0.25	**<0.0001***
		12 months	0.87 ± 0.41	**<0.0001***
	Palatal	Baseline	1.54 ± 2.28	**0.007***
		12 months	-	-
	Mesial	Baseline	1.05 ± 2.72	0.18
		12 months	0.92 ± 0.93	**0.010***
	Distal	Baseline	2.37 ± 2.29	**0.001***
		12 months	0.30 ± 1.52	0.68

G1S1: Group 1subgroup 1, G1S2: Group 1subgroup 2; G2S1: Group 2 subgroup1, G2S2: Group 2 subgroup 2; JS: Jumping Space; P value is significant if <0.05; P-probability

treatment groups at all the time points. Significant differences (p=0.001) were found after 12 months when G1S1-G2S1 and G1S2 –G2S2 were compared.

Discussion

Various biomaterials have been suggested in existing literature for effectively filling the JS (9,13,14). However, there's a notable concern regarding residual particles potentially

***Table 5**. Comparative statistics for vertical distance (VD) at baseline and 12 months.*

Groups	Side	Time period	Mean ± Standard deviation (in mm)	Significance (p)
G1S1-G2S1 (JS<2mm)	Mesial	Baseline	1.50±2.33	0.05
		12 months	0.45±0.90	0.17
	Distal	Baseline	3.12±2.01	**<0.0001***
		12 months	0.36±2.02	0.55
G1S2-G2S2 (JS>2mm)	Mesial	Baseline	0.18±3.12	0.88
		12 months	1.37±2.41	0.18
	Distal	Baseline	0.87±3.34	0.48
		12 months	0.57±0.85	0.17

G1S1: Group 1subgroup 1, G1S2: Group 1subgroup 2; G2S1: Group 2 subgroup1, G2S2: Group 2 subgroup 2; JS: Jumping Space; P value is significant if <0.05; P-probability

***Table 6.** Comparison of Jumping Space (JS) in both the treatment groups at baseline and 12 months.*

Groups	Side	Time period	Mean ± Standard deviation (in mm)	Significance (p value)
Group 1 (JS<2mm)	Buccal	Baseline-12 months	0.43 ±0.28	**0.002***
	Palatal/ Lingual	Baseline-12 months	0.94±2.94	0.36
	Mesial	Baseline-12 months	0.42±0.35	**0.007***
	Distal	Baseline-12 months	0.14±0.33	0.23
Group 1 (JS>2mm)	Buccal	Baseline-12 months	1.73±0.85	**0.004***
	Palatal/ Lingual	Baseline-12 months	0.52±0.80	0.17
	Mesial	Baseline-12 months	0.08±0.68	0.77
	Distal	Baseline-12 months	0.30±0.39	0.12
Group 2 (JS<2mm)	Buccal	Baseline-12 months	0.71±0.35	**<0.0001***
	Palatal/ Lingual	Baseline-12 months	0.42±0.55	0.053
	Mesial	Baseline-12 months	0.52±0.30	**0.001***
	Distal	Baseline-12 months	0.73±0.53	**0.003***
Group 2 (JS>2mm)	Buccal	Baseline-12 months	2.41±0.29	**<0.0001***
	Palatal/ Lingual	Baseline-12 months	1.01±0.83	**0.029***
	Mesial	Baseline-12 months	1.13±0.98	**0.037***
	Distal	Baseline-12 months	0.8±0.58	**0.014***

G1S1: Group 1subgroup 1, G1S2: Group 1subgroup 2; G2S1: Group 2 subgroup1, G2S2: Group 2 subgroup 2; JS: Jumping Space; P value is significant if <0.05; P-probability

Table 7. *Comparison of Testori Score (TS) in both the treatment groups at all the time points.*

Groups	Time period	Mean ± Standard deviation (in mm)	Significance (p-value)
G1S1-G2S1 (JS<2mm)	Baseline	1.11±0.93	**<0.0001***
	6 months	0.44±0.73	0.10
	12 months	0.55±0.53	**0.01***
G1S2-G2S2 (JS>2mm)	Baseline	0.67±0.82	0.17
	6 months	0.17±0.75	0.55
	12 months	1.33±0.82	**0.001***

G1S1: Group 1subgroup 1, G1S2: Group 1subgroup 2; G2S1: Group 2 subgroup1, G2S2: Group 2 subgroup 2; JS: Jumping Space; P value is significant if <0.05; P-probability

impeding efficient BIC and the ability to fill both the vertical distance and the radiolucent area. DFDBA bone graft was preferred to be used in the present study because it contains bone morphogenetic protein (BMP), which induces new bone formation during healing process. DFDBA offers several advantages over other biomaterials used in bone grafting procedures: DFDBA contains growth factors and proteins that stimulate the recruitment and differentiation of osteogenic cells, promoting bone formation. This osteoinductive property enhances the bone regeneration process, leading to more predictable outcome. DFDBA undergoes gradual resorption over time as new bone forms, eventually being replaced by the patient's own bone tissue. This process mimics natural bone remodelling, resulting in long-term stability and integration with the surrounding anatomy. DFDBA is readily available in freeze-dried form, allowing for easy storage, handling, and use in clinical settings. Its availability reduces surgical time and complexity, contributing to overall procedural efficiency. Many clinical trials have reported effective bone augmentation and intrabony defect fill using DFDBA bone grafts (15,16,17). Overall, DFDBA bone graft offers a combination of biological, structural and clinical advantages hence we used it as preferred choice of bone graft over other biomaterials. The current study aimed to assess whether DFDBA bone grafting around immediate implants in the JS has any impact on enhancing both hard and soft tissues.

The outcome of this clinical trial revealed a notable enhancement in the PI within the G1S1 group compared to G2S1. This suggests that when the JS is less than 2mm, it favours oral hygiene procedures more effectively, as the surrounding soft tissues tend to heal faster and without deformity. Indeed, the observed improvement in the JS < 2 mm group may be attributed to several factors. Grafting in JS less than 2 mm helps preserve and stabilize the blood clot, aiding in bleeding control and preventing soft tissue collapse. Moreover, it serves as a protective barrier, guarding the wound area against food residue and bacteria. Similarly, the GI demonstrated significant improvement in the G1S1 group compared to G2S1 and other groups, further supporting the benefits of grafting in this context.

The inevitable crestal bone resorption following extraction is largely attributed to the loss of blood supply when the periodontal ligament is removed. Consequently, the disparity in CBH was deemed non-significant in the mesial and distal sides of both Group 1 and Group 2, regardless of the JS. However, a notable finding emerged; CBH was significantly reduced in G1S2 at the 12-month mark compared to other groups. This underscores the importance of grafting in JS greater than 2mm to mitigate crestal bone resorption. Chen *et al*. (18) conducted a study assessing the outcomes of immediate implants in the maxilla, comparing three treatment approaches. The authors in Control group left the gap unfilled, while in the other two groups the gap was filled with deproteinized bovine bone mineral (DBBM) alone or in combination with a native bilayer collagen membrane (CM). Interestingly, both experimental groups exhibited comparable outcomes, demonstrating a significant decrease in horizontal crestal bone resorption compared to the Control group (19).

A prospective cohort study by Cardaropoli *et al*. (20) documented the soft tissue contour changes between implant placement and 1 year later of 26 single dental implants inserted in fresh extraction sockets which were immediately provisionalized, where the JS was grafted with a bovine bone mineral. The results showed reduction in the crestal bone changes and horizontal bone width stability after grafting in the bone implant gap. Our study findings corroborate the previously mentioned research outcomes. However, our study provides additional insights by comparing sites with JS less than and greater than 2 mm. Regardless of the JS extent, augmenting the defect was shown to preserve CBH, particularly in the midfacial region. While statistically significant reductions in crestal bone resorption were not achieved on all sides, our results indicate partial preservation of CBH. Preserving CBH in the midfacial region holds particular significance, as it contributes to improved hard and soft tissue healing post-therapy.

Clinical studies have consistently noted a high rate of spontaneous closure of JS at immediate implant sites, particularly in JS wider than 2 mm (21) with over 90% exhibiting this phenomenon. Moreover, the median percentage fill was reported to be 100% (22). However, despite these observations, recent recommendations advocate for filling marginal gaps with a bone replacement graft to enhance esthetic outcomes (23). Discrepancies in findings across studies regarding JS fill can be attributed to variations in the gap sizes, differences in buccal plate thickness, implant positioning, and diverse surgical techniques employed.

Our study stands out as one of the pioneering investigations to comprehensively evaluate bone fill around dental implants from all four sides. Notably, we observed that the greatest bone fill occurred on the buccal side for both Group 1 and Group 2. This phenomenon can be attributed to the implants being positioned more towards the palatal aspect. After a meticulous 12-month follow-up, our findings revealed a noteworthy observation: a significant disparity was observed solely in the filled buccal side of the JS between G2S1 and G2S2. This observation underscores the importance of our study in uncovering subtle yet critical differences in bone fill dynamics surrounding dental implants. Our findings align closely with the insights provided by Novaes Jr. *et al*. (24), whose animal studies illuminated the process of new bone growth within the JS, ultimately facilitating osseointegration. Notably, their research underscores that the

percentage of bone-to-implant contact diminishes notably when the space width exceeds 1.0 mm.

Remarkably, our study sheds new light on the significance of a 2.0 mm JS, a critical factor that has been overlooked in prior reports, which have typically relied on directed bone regeneration to achieve such fill. This novel observation highlights the intrinsic capacity for bone fill within this specific gap width, independent of additional interventions.

Furthermore, our findings substantiate earlier observations in one of the trials (25), which demonstrated that defects grafted with either bovine bone mineral or autograft exhibited significantly larger amounts of BIC compared to defects left without grafting. This underscores the pivotal role of grafting materials in promoting osseointegration and underscores the multifaceted nature of bone regeneration dynamics in implant dentistry.

Intriguingly, our investigation revealed no notable discrepancies in RW between the two groups. However, a crucial aspect to highlight is that throughout our study, the labial bone plate remained unexposed, and JS grafting was performed without the necessity of reflecting the periosteum. Moreover, a compelling finding emerged, a consistent decrease in VD was observed across both groups, indicative of vertical bone formation extending from the initial thread of the implant to the first point of BIC. Notably, Group 2 exhibited greater values, implying an enhanced benefit of bone augmentation in this cohort. This underscores the efficacy of our approach in fostering vertical bone growth, thus contributing to the overall success and stability of the implant site. Due to the anatomical difference between implant and socket wall, the horizontal and vertical defects were created, which was seen radiographically as a triangular RA. In the present study the difference in mean values of RA was significant when G1S1 was compared with G2S1 on the buccal side and in case of G1S2 and G2S2 on the buccal and mesial side after 12 months. This indicates that there was a certain amount of bone fill which occurred in the JS leading to reduction in RA. Additionally, our results align closely with those of a previous study (22), wherein the authors employed a flapless technique and used Tricalcium phosphate to fill the JS. The present study measured the RA throughout all four sites: buccal, palatal, mesial and distal and revealed that there was significant difference on all four sites during intragroup analysis in Group 2. While the Group 1 showed a significant difference only on the buccal side as compared to the baseline dimensions. Intergroup comparison revealed that the Group 2 had a greater reduction in RA on the buccal aspect thereby indicating substantial bone fill. Polyzois *et al.* (26) observed increased BIC and more bone within the threads in grafted areas relative to nongrafted areas with defects of the same diameter. Limited osseointegration was observed in the defect region in locations where no grafting was used. The wider grafted defects, on the other hand, managed to illustrate BIC more coronally than locations with defects of similar magnitude that did not contain grafts. The current study's results are consistent with these findings and found minimal RA across implants in Group 2, denoting more BIC and more JS fill implying a greater bone fill at the grafted sites.

When the Testori score for the soft tissue changes was compared, better scores were observed in Group 2 as opposed to Group 1. Amongst the Group 2 subgroups, the scores were better in G2S2 implying that grafting of JS with DFDBA not only restores the buccal bone but also conserves and improves the soft tissue architecture. The results of the present study confirmed that better bone levels with enhanced soft tissue contours appear to have been achieved when DFDBA was used. This modality can be considered as an effective and predictable option for replacing teeth with added advantage of improved esthetics. However, there are certain limitations such as smaller sample size and inability to conduct histological examination of the restored tissue and further studies are desired to improve our understanding and substantiate the results.

Conclusion

Within the study constraints it can be inferred that use of DFDBA showed significant CBH augmentation in the midfacial region leading to enhanced soft tissue levels. Also, a significant reduction in RA indicating radiographic bone fill was observed in G2S2. In spite of the small differences observed between the approaches, the overall results seem to indicate a trend towards better outcomes with the use of DFDBA. Future research endeavours should aim for more homogeneous study designs with extended follow-up periods to confirm and elucidate this observed tendency. Such studies will not only enhance our understanding but also provide valuable insights into optimizing treatment strategies for improved clinical outcomes in dental implantology.

Türkçe öz: *Demineralize dondurulmuş kurutulmuş kemik allogrefti kullanılan ve kullanılmayan immediat implantlarda kemik boşluğunun yönetimi: randomize kontrollü bir çalışma. Amaç: Bu çalışma, demoralize edilmiş dondurularak kurutulmuş kemik allogrefti (DFDBA) kullanılan ve kullanılmayan anında implantlarda atlayış boşluğunun (JS) yönetimini flepsiz yaklaşım ile değerlendirmeyi ve karşılaştırmayı amaçlamaktadır. Bireyler ve Yöntem: Bu çalışmaya, üst çene ön bölgesinde anında implant yerleştirilen 40 bölge dahil edilmiştir. Grup 1 hastaları herhangi bir augmentasyon yapılmadan tedavi edilirken, Grup 2 hastalarında JS içinde DFDBA kullanılmıştır. Her iki grup, yatay boyutlarına göre JS 2 mm'den az (G1S1, G2S1) veya fazla (G1S2, G2S2) olacak şekilde alt gruplara ayrılmıştır. Plak indeksi (PI), Dişeti İndeksi (GI), Sondalama derinliği (PD), Testori estetik skoru (TS), VAS skoru, Crestal Kemik yüksekliği (CBH), Sırt genişliği (RW), Dikey mesafe (VD) ve radyolüsent alan (RA), başlangıçta ve tedaviden 12 ay sonra CBCT ile radyografik olarak değerlendirilmiştir. Bulgular: G1S1-G2S1 ve G1S2-G2S2 gruplarında orta yüz bölgesinde CBH'de sırasıyla 0.34±0.19mm ve 0.75±0.26 mm ortalamaları ile 12 ayda anlamlı farklılıklar gözlenmiştir. G1S1 ve G2S1 gruplarında TS'de ortalama 0.55±0.53 iken, G1S2 ve G2S2 gruplarında 1.33±0.82 değerleri ile anlamlı farklılıklar gözlenmiştir. Sonuç: DFDBA, orta yüz bölgesinde daha iyi CBH korunumu, RA'da azalma göstererek JS'nin daha iyi çözünmesi ve dolayısıyla daha iyi sert ve yumuşak doku iyileşmesine yol açmaktadır. Anahtar Kelimeler: immediat implantlar, kemik boşluğu, peri-implant dokular, kemik grefti, doku iyileşmesi*

Ethics Committee Approval: The study was carried out between February 2021 and March 2022. Approval for this clinical trial was obtained from the Institutional Ethics Committee of our institute, aligning with the updated principles of the Helsinki Declaration for biomedical research. The Institutional Ethics Committee (IEC) provided approval under the registration number IEC/VSPMDCRC/06/2019. Furthermore, the trial was registered with the Clinical Trials Registry of India under registration number CTRI/2021/01/030620 and adhered to the CONSORT statement and EQUATOR guidelines for reporting.

Informed Consent: Participants provided informed consent.

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Author contributions: APK, PVB participated in designing the study. MP participated in generating the data for the study. MP, RAK participated in gathering the data for the study. MP, PVB participated in the analysis of the data. PVB wrote the majority of the original draft of the paper. APK participated in writing the paper. MP has had access to all of the raw data of the study. RAK has reviewed the pertinent raw data on which the results and conclusions of this study are based. RAK have approved the final version of this paper. PVB guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Invited review

Inflammatory mediators' essence in apical periodontitis

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Abstract

Apical periodontitis (AP) represents chronic inflammatory reaction of periradicular tissues of teeth with necrotic pulp. Although AP has been considered as a multifactorial disease, different microorganisms and their virulence factors from infected root canals are considered to be the primary cause of periradicular inflammatory process. The interplay between microbes and host leads to an inflammatory cascade of events that includes activation of innate and adaptive components of immunity. Activation of different immune cells in AP is intermediated by different molecules known as mediators of inflammation. These molecules establish various network interrelationships in the inflamed periapical area and induce alveolar bone resorption. This narrative review aimed to explore and present the current knowledge of selected inflammatory mediators, including cytokines, matrix metalloproteinases, bone resorption regulators and components of oxidative stress involved in the alveolar bone resorption in AP.

Keywords: *Periapical periodontitis, cytokines, oxidative stress, matrix metalloproteinases, bone resorption*

Introduction

Apical periodontitis (AP) represents chronic inflammatory reaction of periradicular tissues of teeth with necrotic pulp (1). Although AP has been considered as a multifactorial disease, in most of the cases it is a consequence of dental caries. Therefore, different microorganisms and their virulence factors from infected root canals are considered to be the primary cause of periradicular inflammatory process (2). The leading radiographic characteristic of AP is the destruction of periradicular tissues, manifested as a radiolucency surrounding the apex of the roots of the affected tooth (1). Infectious agents, along with the toxins they produce, and metabolic waste products mediate an array of immunological responses within the host's dental pulp and periradicular tissues. Such kind of interplay between microbes and the host leads to an inflammatory sequence of events, including the activation of innate (polymorphonuclear leukocytes, macrophages, and endothelial cells) and adaptive components (T – and B – lymphocytes) of immunity (3). Activation of different immune cells in AP is intermediated by different molecules known as mediators of inflammation. It is due to these molecules that different network interrelationships are established in the inflamed periapical area. However, the arrangement and extent of their gene expression are different among individuals and determined by the origin of the stimulatory agent (4). Bearing in mind the importance of proinflammatory mediators' essence in the pathogenesis of AP this narrative review aimed to explore and present the current knowledge of selected inflammatory mediators, including cytokines, matrix metalloproteinases, bone resorption regulators and components of oxidative stress involved in the alveolar bone resorption in AP.

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Cytokines

Cytokines are low molecular weight polypeptides or glycoproteins originating from hematopoietic and/or structural cells. They regulate many important events in human body including cell growth, differentiation, inflammation, immune defense, tissue remodeling and repair, etc. (5). Cytokines exhibit a pleiotropic effect on the target cells and can function through autocrine, paracrine, and/or endocrine pathways. They can act as pro- and anti-inflammatory mediators showing their synergistic and/or antagonistic effects as well. Cytokines encompass a wide range of categories, i.e., interleukins (ILs), tumor necrosis factors (TNFs), colony stimulating factors (CSFs), monokines, lymphokines interferons (IFNs), and transforming growth factors (TGFs) (5). Making the complex interrelationship networks in periradicular tissues proinflammatory cytokines are able to stimulate osteoblasts differentiation into osteoclasts and promote alveolar bone resorption (3, 6). This subsection will present a brief overview of the most important proinflammatory cytokines involved in that process.

Tumor necrosis factor – alpha

Tumor necrosis factor – alpha (TNF-α), which is also known as cachexin or cachectin, belongs to the TNF superfamily, which comprises various transmembrane proteins with a homologous TNF domain. TNF-α represents an inflammatory cytokine generated during acute inflammation as a by-product of macrophages/monocytes. It induces a variety of signaling events within cells, eventually resulting in either necrosis or apoptosis (7). TNF-α, as an endogenous pyrogen, can cause fever, apoptotic cell death, cachexia, inflammation, inhibit cancer genesis and viral replication, and respond to septic conditions (7). An extensive range of human conditions has been associated with dysregulation of TNF-α production. These include cancer, inflammatory bowel disease, Alzheimer's disease, psoriasis, major depression, etc. (8). Moreover, TNF-α belongs to the group of pro-inflammatory cytokines that have a prominent role in alveolar bone resorption in AP (3). Several *in vitro* studies showed that inflammatory cells of harvested periapical lesions produced TNF- α (9, 10). Gazivoda and co-workers (9) documented a heightened level of TNF-α within the inflammatory cells of larger periapical lesions when compared to smaller ones. The authors also revealed a positive relationship when it comes to the levels of TNF-α and increased presence of inflammatory cells, e.g., monocytes, macrophages and dendritic cells in periapical lesions (9). In addition, Artese *et al.* (10) showed that mononuclear cells cultivated from harvested AP lesions are able to secrete TNF-α. Furthermore, the involvement of TNF-α in the pathogenesis of AP has been shown in animal experimental model. In 2001, Graves *et al.* (11) notified that TNF-α modulates fibroblast apoptosis, polymorphonuclear recruitment and osteoclast formation in mice as a consequence of *Porphyromonas gingivalis* (*P. gingivalis*) infection. The authors employed *in vivo* calvarial model in mice with targeted deletion of TNF receptors p55 and p75 and matched wild-type mice. In conclusion, the authors stated that TNF represents a major mediator when it comes to *P. gingivalis*-induced apoptosis and inflammation in AP (11). Moreover, Samuel *et al.* (12) noted that the multiple AP in rats can affect overall health by increasing lymphocyte and TNF-α levels in the blood. Noteworthy, 30 years ago, Safavi and Rossomando (13) identified detectable levels of TNF in periapical tissue exudates in chronic AP. Most recently, Nunez *et al.* (14) also identified significantly elevated concentrations of TNF-α in gingival crevicular fluid (GCF) from diseased teeth with AP compared to the healthy controls. Previously conducted studies in humans analyzed the association between the levels of TNF-α and different clinical, radiographic, and pathohistological features of AP (15-19). Although some studies reported that the levels of TNF-α were elevated in larger in comparison with smaller lesions (15), and in the case of radicular cysts compared to periapical granulomas (16), there were no significant differences between different clinical presentations of analyzed AP (17-19).

Interleukin – 1 beta

Interleukin – 1 beta (IL-1β), which is also known as a leukocytic pyrogen, lymphocyte activating factor, leukocytic endogenous mediator, and mononuclear cell factor , represents a proinflammatory cytokine that is encoded by the *IL1B* gene in humans (20, 21). This cytokine serves as a salient mediator in the inflammatory response and has a role in various cellular activities, including cell proliferation, differentiation, and apoptosis (20, 21). Earlier *in vitro* studies demonstrated that inflammatory cells from symptomatic AP lesions, which harbored a higher percentage of granulocytes, produced elevated concentrations of IL-1β in comparison with asymptomatic lesions (9). In another *in vitro* study Artese et al. (10) observed IL-1β positive cells were present in human periapical granulomas to a small extent, and the morphology of positive cells corresponded to monocytes/macrophages. Moreover, some animal studies also examined the role of IL-1β in alveolar bone resorption (22, 23). In 1995, Hamachi and co-workers demonstrated cells expressing IL-1β mRNA by *in situ* hybridization in periapical lesions in rats (22). They concluded that macrophages could play a role in IL-1β production and that they could significantly contribute to activating osteoclastic bone resorption in AP (22). This was also confirmed by Matsumoto *et al.* (23) who showed that macrophages expressing IL-1β might have a considerable influence on the activation and recrudescence of osteoclastic bone resorption in an AP rat model. In 1992, for the first time, IL-1β was detected in human AP lesions (24). An IL-1β enzyme-linked immunosorbent assay (ELISA), which relied on monoclonal antibodies specific for IL-1β, was used to measure its activity. In this study, AP samples demonstrated a considerable activity of IL-1β whereas healthy pulp tissue had no activity (24). The authors concluded that IL-1β is locally generated and released in inflammatory AP lesions and that it probably mediates alveolar bone loss (24). More recent studies investigated the correlation between IL-1β concentrations in human AP lesions and their clinical, radiographic and histopathological presentation (17, 19, 25, 26). Jakovljevic *et al.* (19) reported significantly elevated IL-1β concentrations in symptomatic lesions compared to asymptomatic lesions and control tissue samples (19). This is in line with previous investigations that also observed increased

IL-1β concentrations symptomatic lesions exudates (25, 26). Moreover, Jakovljevic *et al.* (19) revealed that IL-1β levels were significantly increased in radicular cysts in comparison with periapical granulomas. These results were partly in line with the investigation performed by Ataoğlu *et al.* (17) who also noted a significant increase of IL-1β levels in canals with larger compared to those with smaller radiolucent areas. Based on the reported data, it can be established that AP development is in close association with IL-1β expression and that IL-1β represents a powerful bone-resorptive cytokine that triggers osteoclast formation and activation (27).

Interleukin-6

Interleukin-6 (IL-6) can act as both pro- and anti-inflammatory cytokine (28). This interleukin is thought to act like a hormone that mobilizes extracellular substances and/or alters substrate delivery during physical activity. In addition, it is generated in the body on the site of either acute or chronic inflammation and may act as a pyrogen that can cause fever in autoimmune, infectious, or non-infectious diseases (28). Several *in vitro* studies investigated how IL-6 affects AP pathogenesis (9, 29). Namely, IL-6 has been detected in AP and IL-6 concentrations are proportional to the size of periapical lesions. Gazivoda *et al.* (9) reported that inflammatory cells from symptomatic and large-size lesions secreted higher concentrations of IL-6 compared to asymptomatic and small-size AP lesions. It is also important to stress that neutrophils and macrophages present in AP lesions can secrete IL-6 *in vitro* after different bacterial stimuli. Thus, Matsushita *et al.* (29) showed that *Prevotella melaninogenica* and *P. gingivalis* may be involved in the pathogenesis of AP by increasing levels of IL-6. In 1999, IL-6 was detected in experimentally induced murine AP lesions (30). Thereafter, several animal investigations confirmed its role in the development of AP (31, 32). Huang *et al.* (31) have reported that it took far less time for large AP lesions to develop in mice in whom IL-6 deletion was detected than in healthy one. On the other hand, it has been noted that the increased bone resorption in IL-6-deficient animals was in a correlation with an increase in osteoclast numbers and elevated expression of other bone-resorptive cytokines in AP lesions (32). IL-6 in human AP lesions was first described in an investigation by Barkhodar *et al.* (33). The authors reported the mean IL-6 concentrations were significantly higher in AP lesions when compared to healthy pulp tissue (33). Moreover, several clinical investigations observed significantly increased IL-6 concentrations in symptomatic lesions in comparison with asymptomatic lesions and the control group (18, 19, 34). Furthermore, a different investigation showed that IL-6 could be potentially used as a marker of pathologic inflammatory activities in chronic AP lesions (35). Therefore, it could be implied that pro-inflammatory cytokines are strongly involved in AP development and alveolar bone resorption.

Bone resorption regulators

Molecular mechanisms involved in alveolar bone loss in AP are regulated by the interaction of a group of molecules entitled bone resorption regulators (36-38). This group of molecules is presented by: receptor activator of NF-κB ligand (RANKL), its cellular receptor – RANK, and the decoy receptor osteoprotegerin (OPG) (36-38). They belong to the TNF receptor and ligand superfamilies (36-38). Initially, RANKL was recognized as a cell membrane-bound ligand capable of triggering osteoclast formation and bone resorption. Presently, it has been acknowledged that RANKL production can be attributed to different cells, including osteoblasts, fibroblasts, and activated T and B lymphocytes (36-38). By binding to its receptor which is found on the surface of precursor cells, i.e. the cells belonging to the lineage of monocytes/macrophages, RANKL initiates their transformation into fully developed osteoclasts (36-38). Besides, OPG acts as a soluble decoy receptor that interferes with RANKL, prevents it from binding with RANK, and thus inhibits osteoclast activation (36-38). Different cells produce RANKL and OPG and this is regulated by both systemic and local stimuli. These include cytokines from IL-1 family, virulence factors of different microorganisms, etc. (36-38). In 2005, Zhang and Peng examined the presence of RANKL in periapical areas and its role in alveolar bone loss using a rat model (39). In this study, osteoclast-like cells, which were identified owing to their tartrate-resistant acid phosphatase (TRAP) positivity, and cells positive for RANKL were found in the periapical region as early as one week after exposing the dental pulp. By the end of the second week, a notable increase in inflammatory cells was detected and bone resorption around the periapical area became evident. This was accompanied by a peak in the number of cells positive for RANKL and osteoclast-like cells. Following a four-week exposure, even though chronic inflammation persisted, the levels of these osteoclast-like and RANKL positive cells returned to their initial values, and the periapical bone resorption rate decelerated (39). Thereafter, Kawashima *et al.* (40) confirmed these findings at the mRNA expression level. Namely, the authors reported that the expression of RANKL in the periapical area reached its highest levels following a pulp exposure of 2 to 3 weeks and remained elevated above the baseline values for up to 8 weeks. The expressions of RANK and OPG also increased, although not as prominently as RANKL. The increase in OPG expression was seen as a response that was supposed to counteract the effects of an abrupt rise in RANKL levels. The relative expression ratio of RANKL/OPG reached its peak after 3 weeks and remained high throughout the 8 weeks of observation, indicating considerable potential in terms of bone resorption. During the 2- to 3-week period, when the RANKL/OPG ratio was at its highest, the expression of pro-inflammatory cytokines like IL-1α, IL-1β, and TNF-α, which can stimulate RANKL production, was also increased (40). In addition, Chuang *et al.* (41) also revealed that level of bone resorption regulators in animal experimental models can be stimulated by lipopolysaccharides from *Escherichia coli*. Following a 3-week exposure, the authors noticed a significant increase in RANKL expression, reaching a peak of 208% in comparison with the control group with unexposed pulp, by the end of the 8-week period. Moreover, the expression of OPG varied from week 1 to week 8; yet it consistently remained lower when compared to the control group (41). In 2004, Tay *et al.* (42) using immunohistochemistry, reported that RANKL was present in radicular cysts. The same findings were verified by Sabeti *et al.* (43) who showed an increased expression of RANKL in human AP lesions. Moreover, some

investigations confirmed that the presence of RANKL to OPG predominant ratio was in periapical granulomas compared to radicular cysts (44, 45). In addition, recent investigations reported significant difference in RANKL and OPG expression between AP lesions with different clinical presentation suggesting that these molecules could serve as discriminating biomarkers (46, 47). These findings are in accordance with Nikolic *et al.* (48) who reported that symptomatic AP lesions were more frequently detected in RANKL-predominant AP lesions than in OPG-predominant ones. The authors also revealed significant positive correlation between investigated pro-inflammatory cytokines and bone resorption regulators in AP lesions, suggesting their concomitant role in complex process of alveolar bone resorption (48).

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) represent a broad category of calcium dependent zinc containing endopeptidases which contribute to remodeling and degradation of extracellular matrix components. These proteolytic enzymes are also involved in different physiological and pathological processes regulated by hormones, growth factors and cytokines (49).

According to their substrate specificity and domain structure, MMPs can be classified into 6 groups (50): 1) collagenases (MMP-1, MMP-8 and MMP-13, mostly digesting collagen types I, II, III, soluble proteins and extracellular matrix components); 2) gelatinases (MMP-2 and MMP-9, cleaving collagen types IV, V, XI, laminin); 3) stromelysins (MMP-3, MMP-10 and MMP-11, whose characteristics are similar to those of collagenases, but not to degrading interstitial collagen); 4) matrilysins (MMP-7 and MMP-26, interacting with cell surface proteins); 5) membrane-type MMPs (MMP-14, with collagenolytic action) and 6) others (MMP-12).

The domain structure of MMPs is common. There are four such domains and they include the signal domain, pro-peptide, catalytic domain, and hemopexin-like C terminal domain, which is connected to the catalytic domain by a flexible hinge region. Under normal physiological conditions, the catalytic activity of MMPs is closely monitored and this is achieved at four different levels : 1) gene expression with transcriptional and post-transcriptional regulation; 2) extracellular localisation and tissue or cell type of MMP release, called compartmentalization; 3) pro-enzyme activation by pro-domain removal and 4) inhibition by specific inhibitors, i.e. tissue inhibitors of matrix metalloproteinases (TIMPs), and by non-specific proteinase inhibitors (e.g. α2-macroglobulin) (51).

MMPs, providing they have become active, can modulate the global proteolytic potential in the extracellular milieu through zymogen (i.e. MMP pro-form) activation and inhibitor degradation or inactivation of other proteases (49, 51). MMPs and TIMPs are normally expressed in low concentrations under physiological conditions. Nevertheless, it has been noted that during the developmental stages of various human diseases, diverse types of MMPs are overexpressed in particular tissues and distinct processes, such as malignancy and inflammation (49, 51).

Alveolar bone resorption in AP is encompassed by MMPs as one of their modulators (49, 52). Breakdown of the extracellular matrix by MMPs is initiated and even further enhanced by low pH level caused by endodontic pathogens (49, 52 - 54). A number of *in vitro* studies investigated MMPs production by human pulp cells cultures (55-57). Panagakos *et al.* (55) treated pulp cells of humans and pulp cells RPC-C2A of clonal rats with IL-1α, IL-1β, TNF-α, and LPS for 24 hours, while conditioned medium and cell lysates were gathered and examined by gelatin zymography method. The authors revealed that pulp cells of humans that had been treated with either cytokines or LPS's did not demonstrate any changes concerning the MMP pattern that was generated or secreted in either cellular or conditioned medium fractions (55). A few years later, O'Boskey and Panagakos (56) investigated MMP production by human pulp cells both in the presence and absence of IL-1α and TNF-α in long-term cultures (2 to 16 days) applying the same method. This time, the authors concluded that MMP production in human pulp cells in long-term cultures is stimulated by cytokines. Another conclusion implied that these MMPs could contribute to pulp inflammation (56). Thereafter, it has been reported that MMPs are also produced by mast cells of human AP lesions (57). Further experiments on animals showed a moderate expression of MMP-2 and MMP-9 during the chronic stage of the AP lesion in rats suggesting that a decreasing number of polymorphonuclear cells during the chronic stage may interfere with IL-1α and IL-6 expression (58). Based on these findings the authors concluded that MMP-2 and MMP-9 have an essential role regarding the development of AP lesions. This could be accounted by extracellular matrix degradation that occurs during the beginning stage of lesion development (58). Having explored the levels of different MMPs and their tissue inhibitors in the process of AP lesion development, Wan *et al.* (59) reported that the MMP-1, MMP-2, MMP-9, TIMP-1, and TIMP-2 mRNA and protein expression values rose in the acute and chronic phases of AP lesions, with the values of MMP-2 and MMP-9 expression being lower during the chronic phase, which supports previous results. In 2002, Shin *et al.* (60) using the ELISA method showed that MMP-3 values were significantly higher in the periapical lesion than in healthy pulps. Further study demonstrated that MMP-13 expression pattern contributes to a periapical granuloma with epithelium transforming into a radicular cyst (61). Martinho *et al.* (62) investigated the significance of MMPs and TIMPs in clinical settings with regard to AP lesions. The authors reported that in teeth with larger-size radiolucent lesions higher mean values of MMP1, -2, and -9 were recorded in comparison with the smaller ones. They also elucidated the association of MMP-9 with higher risk of pain on palpation, while MMP-1 was correlated with lower chance of tenderness to percussion (62). Investigating the clinical relevance of MMPs and their tissue inhibitors Letra *et al.* (63) showed that significantly higher TIMP-1 was observed in asymptomatic AP cases than in the cases with a chronic apical abscess, while, in turn, the cases with a chronic apical abscess demonstrated higher MMP-2, MMP-7, and MMP-9 mRNA values. Finally, Hadziabdic *et al.* (64) found no significant difference in the mRNA expression of MMP-1, MMP-2, TIMP-1, and TIMP-2 between periapical granulomas and radicular cysts. On the other hand, Pereira Faustino *et al.* (65) reported that in periapical granuloma and in cases associated with pain MMP-2 expression is increased. All these

data stress the significance of MMPs and their tissue inhibitors in relation to AP pathogenesis.

Oxidative stress

Reactive oxygen species (ROS) represent highly reactive by-products of oxygen metabolism, serving as essential signaling molecules in various cellular processes (66). ROS, including oxygen-derived free radicals (e.g. superoxide (O–) and hydroxyl anion (•OH)), as along with non-radicals that can easily convert into radicals (e.g. nitric oxide (NO•), hydrogen peroxide (H2O2), and hypochlorous acid (HOCl)), have a key role in cell signaling, metabolism, and the regulation of cellular functions, e.g., in gene expression, proliferation, cell death, migration, and inflammation (67-70). In normal cellular physiology, ROS and antioxidant mechanisms maintain a delicate balance to sustain physiological processes. However, during inflammation, there is an excessive production of ROS that overwhelms the available antioxidants, leading to oxidative stress (OS) (66). OS is defined as a disturbance in the pro-oxidant-antioxidant balance in favor of the former, resulting in disruptions to redox signaling and possible molecular damage (71). Those alterations in oxidative metabolism are often referred to as the "respiratory burst" (72).

It has been reported that OS could be a contributing factor in a few chronic inflammatory diseases, including cancer, rheumatoid arthritis, diabetes, atherosclerosis, etc. (73). Additionally, recent investigations indicated its role in the pathophysiology of AP (74). Frazão and co-workers (75) investigated whether AP alters systemic values of the antioxidant and pro-oxidant parameters in different periods in Wistar rats. The authors revealed that glutathione (GSH) and Trolox equivalent antioxidant capacity (TEAC) were increased after 14 days and lipid peroxidation (TBARS) was significantly elevated following 28 days of AP induction. Thus, they concluded that the oxidative biochemistry response was modulated based on the progression of alveolar bone resorption (75). Moreover, several studies explored the influence of AP on OS parameters in animals with general health impairment (76-78). Prieto *et al.* (76) revealed that uric acid, and malondialdehyde (MDA) levels, as well as inflammatory infiltration were more increased in the periapical region of diabetic rats with induced AP than in rats with AP but without diabetes mellitus (DM) suggesting that DM may change the antioxidant status. Also, Milojevic Samanovic *et al.* (77) conducted an investigation involving rats which showed a correlation between AP and impaired cardiodynamics, disturbed cardiac OS, antioxidant defense, and cardiac pathologic alterations in the conditions of hypertension. Finally, Tsosura *et al.* (78) presented that maternal AP modulates the antioxidant defense system (i.e. attenuating lipid peroxidation) in the investigated tissues of their adult offspring. These results suggest that a maternal chronic oral inflammatory process may aggravate the damage of oxidative tissue in their offspring during the postnatal phase.

In parallel, numerous studies in humans have been conducted to investigate the impact of OS on AP (66). These investigations have explored OS both systemically in blood and saliva (79 - 83) and locally in GCF and root canal contents (83, 84).

Another recent investigation conducted by Cotti *et al.* (79) explored the role of ROS in the pathogenesis of AP systematically in blood. Their findings not only revealed significantly elevated ROS levels in AP patients overall compared to healthy patients, but also highlighted a notably greater increase in ROS levels among female patients (79). This observation aligns with the outcomes of a separate study that investigated potential sex differences in prooxidant and antioxidant status (85). In study performed by Miller *et al.* (86) it was proposed the existence of sex-dependent variations in both the production and metabolic deactivation of ROS. In the pioneering study by Vengerfeldt *et al.* (83) that comprehensively investigated both local and systemic levels of OS in various endodontic pathologies, it was established that OS not only serves as a crucial pathogenetic mechanism in several endodontic conditions including AP, but also exhibits a significant association with certain clinical indicators, including pain and bone destruction (83). Similar results, indicating alveolar bone loss, were obtained by Dezerega *et al.* (84) which examined ROS in both, GCF and AP tissue. This study revealed an imbalance favoring ROS in both apical lesions and GCF from AP-affected teeth when compared to healthy controls and teeth that underwent endodontic treatment (84), once again establishing a clear connection between ROS and degradation of extracellular matrix components that occurs in connective tissue during inflammatory conditions (87). Furthermore, the study found a positive association between the Total Oxidant Status (TOS) and the extent of bone resorption, as well as a negative association between TOS and Total Antioxidant Status (TAS) in AP lesions, which was not the case in healthy periodontal ligaments (PDL). Such an imbalance favoring ROS could potentially encourage the loss of alveolar bone. Latest findings also suggest that ROS may contribute to osteolysis by inhibiting bone formation by means of suppressing osteoblastic differentiation and through promoting osteoclast differentiation and bone resorption (88), primarily by inducing the RANKL. Additionally, increased MMP-2 expression and activation in response to ROS have been reported highlighting a critical association between the production of ROS, MMP-mediated proteolysis, and bone resorption, which may have a vital role when it comes to the progression of apical lesions (89, 90).

The evidence presented underscores the pivotal role of ROS in the intricate cascade of immunologic responses, highlighting their significance in both the initiation and perpetuation of inflammatory reactions. Notably, phagocytic cells produce ROS in response to bacterial pathogens, which serves as a critical host defense mechanism (74). AP, with its microbial etiology, including viruses (91) further accentuates the relevance of ROS. Certain endodontic pathogens, e.g., *E. faecalis* and/or *Epstein-Bar virus*, have been identified as inducers of ROS production (92) and certain microorganisms are also capable of independently generating ROS (93). Consequently, locally produced ROS in the context of AP can emanate from either human or microbial sources. Nevertheless, the current limitations prevent the definitive discrimination between the origins of these ROS. It is important to stress that AP can also influence both local and systemic antioxidant activities. Markers assessing antioxidant status in the blood have shown significantly lower levels in individuals with AP compared to healthy control groups (94). Inchingolo *et al.* (95) reported that, in comparison with the posttreatment values, higher concentrations of blood ROS

and lower blood antioxidant levels were recorded prior to treatment.

In light of the aforementioned considerations, a potential strategy to disrupt this cycle of oxidative stress and inflammation lies in the systemic administration of antioxidants. Insights from animal studies suggest that systemic antioxidants, such as vitamin C, have the capacity to mitigate oxidative stress triggered by injuries (96). Beyond their role in pain reduction, antioxidants may also serve as a valuable tool for enhancing and expediting the scale of the inflammatory response. Specifically, once the microbial infection within the root canals has been effectively eradicated, the administration of antioxidants could potentially contribute to fostering the resolution of AP.

Conclusion

This narrative review presents state-of-the-art related to the role of pro-inflammatory cytokines, bone resorption regulators, matrix metalloproteinases and oxidative stress parameters concerning alveolar bone resorption in apical periodontitis. It is evident that all of these molecules make a complex interrelationship network, triggered by different stimuli (mostly by different microorganisms), induce osteoclastogenesis and promote alveolar bone loss. Interaction between inflammatory mediators and activated cells is regulated by different molecular pathways. The potential clinical implications of these findings may be further explored in future investigations. These studies could examine the effects of inhibiting these mediators and their associated pathways in the context of preventing alveolar bone loss. Acquiring knowledge about all these processes will allow scientists to understand the pathogenesis of apical periodontitis and therefore guide and shape future investigations in this field.

Türkçe öz: *Apikal periodontitis (AP), dişlerin periradiküler dokularının nekrotik pulpa ile kronik inflamatuar reaksiyonunu ifade eder. AP çok faktörlü bir hastalık olarak kabul edilmesine rağmen, enfekte kök kanallarından farklı mikroorganizmalar ve bunların virülans faktörlerinin periradiküler inflamatuar sürecin birincil nedeni olduğu düşünülmektedir. Mikroplar ve konakçı arasındaki etkileşim, bağışıklığın doğuştan gelen ve adaptif bileşenlerinin aktivasyonunu içeren inflamatuar bir olaylar dizisine yol açar. AP'de farklı bağışıklık hücrelerinin aktivasyonuna, inflamasyon aracıları olarak bilinen farklı moleküller aracılık eder. Bu moleküller iltihaplı periapikal bölgede çeşitli ağ ilişkileri kurar ve alveolar kemik emilimini indükler. Bu derleme makalesi, sitokinler, matriks metalloproteinazlar, kemik rezorpsiyon düzenleyicileri ve AP'de alveoler kemik rezorpsiyonunda rol oynayan oksidatif stres bileşenleri dahil olmak üzere bazı inflamatuar mediatörlerle ilgili mevcut bilgileri sunmayı amaçlamaktadır. Anahtar kelimeler: periapikal periodontitis, sitokinler, oksidatif stres, matriks metalloproteinazlar, kemik rezorpsiyonu*

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