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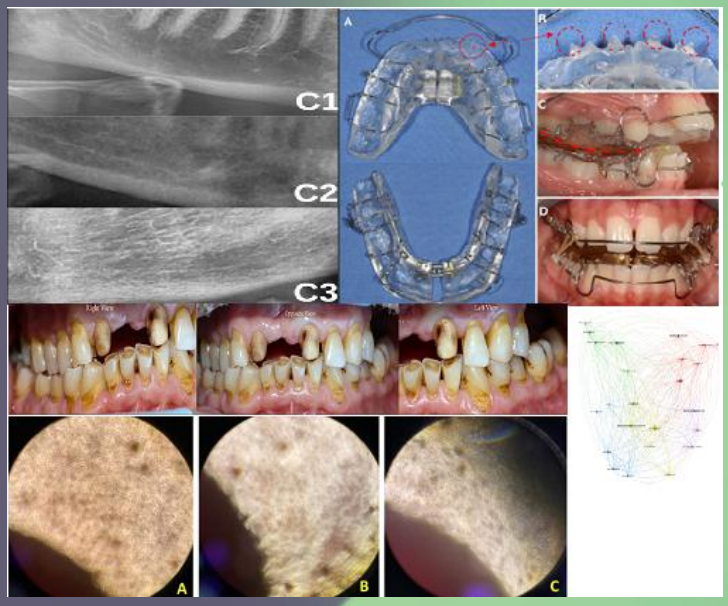

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Current Research in Dental Sciences

Current Research *in* Dental Sciences

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Radiomorphometric Analysis of Patients With Osseous Dysplasia

Osseöz Displazili Hastaların Radyomorfometrik Analizi

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ABSTRACT

Objective: The aim of this study was to examine the radiomorphometric parameters of trabecular and cortical bone of the mandible in patients of osseous dysplasia.

Methods: A total of 43 patients (40 females and 3 males) patients of osseous dysplasia and age-gender matched healthy 43 individuals (control group) were included in this study. The analyzed radiomorphometric parameters were fractal dimension, mandibular cortical width, and mandibular cortical index.

Results: There were not significant differences in the mean fractal dimensions of selected regions between the two groups (*P*>.005). However in mandible, especially bone quality of condyle region was significantly lower in patients of osseous dysplasia (*P*<.005). There were no significant differences in mandibular cortical width between the two groups (*P*> .05). The mean mandibular cortical width was 2.3 mm in the patients of osseous dysplasia and 2.4 mm in the control group. Therefore there were no significant differences in mandibular cortical index between the two groups (*P*> .05).

Conclusion: Especially fractal dimension of condyle region was significantly lower in patients of osseous dysplasia. Therefore, precaution is needed before surgical interventions in this region. It was also observed that the bone quality of the patients with the three variants of cemental dysplasia in the mandible was similar.

Keywords: Osseous Dysplasia, Panoramic radiography, Fractal, Jaw, Mandible

ÖZ

Amaç: Bu çalışmanın amacı osseöz displazi hastalarında mandibulanın trabeküler ve kortikal kemiğinin radyomorfometrik parametrelerini incelemektir.

Yöntemler: Toplamda 43 osseöz displazi hastası (40 kadın ve 3 erkek) ve yaş-cinsiyet açısından eşleşmiş 43 sağlıklı birey (kontrol grubu) bu çalışmaya dahil edilmiştir. Analiz edilen radyomorfometrik parametreler fraktal boyut, mandibular kortikal genişlik ve mandibular kortikal indeks idi.

Bulgular: İki grup arasında değerlendirilen dört bölgenin ortalama fraktal boyutları arasında anlamlı fark yoktu (*P*>,005). Ancak mandibulada, özellikle kondil bölgesinin kemik kalitesi osseöz displazi hastalarında anlamlı derecede daha düşüktü (*P*< ,005). İki grup arasında mandibular kortikal genişlikte anlamlı bir fark bulunmamıştır (*P*> ,05). Osseöz displazi hastalarında ortalama mandibular kortikal genişlik 2.3 mm, kontrol grubunda ise 2.4 mm olarak ölçülmüştür. Bu nedenle, iki grup arasında mandibular kortikal indekste de anlamlı bir fark bulunmamıştır (*P*>,05).

Sonuç: Mandibulada, özellikle kondil bölgesinin kemik kalitesi osseöz displazi hastalarında anlamlı derecede düşüktü. Bu nedenle, bu bölgeye yapılacak cerrahi müdahalelerden önce önlem alınması gerekmektedir. Ayrıca, mandibulada sementa displazinin üç varyantına sahip hastaların kemik kalitesinin benzer olduğu gözlendi.

Anahtar Kelimeler: Fraktal, Mandibula, Osseöz Displazi, Panoramik radyografi

INTRODUCTION

Osseous dysplasia, a medical entity alternatively recognized as cemento-osseous dysplasia (COD), represents a relatively rare and benign fibro-osseous lesion primarily localized within the osseous structures of the jaw. This pathological condition is typified by an aberrant proliferation of bone and fibrous tissue, which often supplants the physiological architecture of normal bone. Osseous dysplasia exhibits a predilection for tooth-bearing regions of the jawbones, and it is frequently discernible through the utilization of routine dental radiographic imaging or during comprehensive dental examinations.[1] The application of fractal dimension analysis introduces an innovative computational methodology for the quantitative assessment of the intricate and inherently irregular structural characteristics frequently encountered within osseous dysplasia lesions.

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Within the scope of osseous dysplasia, three cardinal subtypes merit investigation. One of these, periapical osseous dysplasia, primarily localizes to the anterior mandible and typically manifests as circumscribed radiolucent lesions enveloped by radiopaque margins. It is noteworthy that this subtype predominantly affects middle-aged African and American women and generally remains clinically asymptomatic. In contradistinction, focal osseous dysplasia preferentially affects the posterior regions of the mandible and maxilla. Its identification often stems from serendipitous discovery during routine dental radiographic assessments. Analogous to periapical osseous dysplasia, focal osseous dysplasia tends to present as an asymptomatic condition, with a higher prevalence among the female population. Conversely, florid osseous dysplasia, comprising the third variant, is hallmarked by its dissemination across multiple quadrants of the jaws. This subtype primarily affects middle-aged African and American women. However, it presents with symptoms such as infection and pain.[2]

Although the precise etiological underpinnings of osseous dysplasia remain enigmatic, prevailing conjecture posits an association with perturbations in the normative bone remodeling processes. Diagnosis conventionally relies on radiographic imaging modalities complemented by meticulous clinical evaluation, and interventions are typically reserved for instances where complications ensue.[1]

The present introduction aspires to elucidate the multifaceted dimensions of osseous dysplasia, encompassing its various subtypes, clinical attributes, diagnostic paradigms, and potential avenues for therapeutic intervention. Notably, it underscores the imperative for individuals diagnosed with osseous dysplasia to undertake periodic dental follow-up regimens, thereby facilitating the vigilant monitoring of potential evolving pathology and ensuing complications.

Fractal dimension, an enthralling mathematical construct that emanates from the field of fractal geometry, confers a novel paradigm for the quantitative assessment of intricate, irregular phenomena and configurations prevalent in the natural world. This mathematical parameter, fractal dimension, serves as a robust analytical tool when applied to the complex and multifaceted nature of osseous dysplasia lesions. Through fractal dimension calculations, scholars and practitioners alike attain profound insights into the intricate patterns and self-similarities embedded within these pathological entities. This computational approach holds substantial promise in fostering a deeper understanding of osseous dysplasia, thus culminating in its enhanced characterization, diagnosis, and the refinement of therapeutic stratagems.[3]

These divergent observations underscore the exigency of further scholarly inquiry aimed at elucidating the intricate relationship between mandibular fractal dimension analysis and the presence or progression of cemental, focal, and florid osseous dysplasia. In the interim, fractal dimension analysis remains an auspicious investigative tool for the evaluation of mandibular osseous structures across a spectrum of maladies, encompassing cemental, focal, and florid osseous dysplasia. Possessing attributes characteristic of a non-invasive, quantitative modality, it harbors substantial potential for the early detection and dynamic monitoring of osseous changes. Such capabilities augur considerable contributions to the management and prognostication of cemental, focal, and florid osseous dysplasia, along with other mandibular pathologies. The overall aim of this retrospective pilot study was to clarify whether osseous dysplasia leads to noticeable structural changes in the mandible.

METHODS

This retrospective cross-sectional study was conducted in accordance with the provisions of the Declaration of Helsinki and informed consent was obtained from the individuals included in the study. A comparative analysis was conducted on panoramic radiographs obtained from patients diagnosed with cemento-osseous dysplasia, focal osseous dysplasia, and florid osseous dysplasia, alongside age and gender-matched controls, at the Oral and Maxillofacial Radiology Department of the Faculty of Dentistry, during the period spanning 2019 to 2023. Each group comprised 2 male and 41 female subjects, ensuring gender parity across the study cohorts. Ethical clearance for the study was obtained from the Ethics Committee of Van Yüzüncüyıl University, under approval number 2023/13-07, date 08/12/2023.

The inclusion criteria were no systemic disease affecting bone development and metabolism except any osseous dysplasia and sufficient image quality. Lesions that are well-defined and appear as radiolucent, mixed, or radiopaque areas around the tips of lower front teeth were categorized as cemento osseous dysplasia (COD). Similar lesions affecting only one area around the back teeth were labeled as focal COD, whereas those found in multiple areas across different sections were termed florid COD.[4]

The study's exclusion criteria comprised individuals with a history of pharmaceutical intervention or systemic illnesses that may influence bone development or metabolism, images containing artifacts, the presence of syndromes affecting the oral or maxillofacial region, and cases where the diagnosis of osseous dysplasia was uncertain.

Panoramic radiographic images were captured utilizing an ORTHOPHOS XG device (Sirona, USA), with exposure parameters set at 60 kV, 3 mA, and a duration of 14.1 seconds. Subsequent to image acquisition, conversion to DICOM format was undertaken, followed by meticulous analysis employing ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA). The analysis was executed by two proficient oral and maxillofacial radiologists, each possessing ten and six years of clinical experience, with inter-examiner reliability being evaluated through an additional examiner proficient in the field.

For the assessment of fractal dimension, following the methodology outlined by Kato et al.,[5] nine distinct regions of interest (ROIs) were delineated for fractal analysis. These ROIs were manually drawn using ImageJ's Rectangle tool, encompassing the condyle (1), angulus of mandible (2), molar (3), premolar (4) regions bilaterally, along with the anterior (5) region (Figure 1). Additionally, ROIs were demarcated in the cortical bone bilaterally, extending from the mental foramen to the posterior region of the third molar. Fractal analysis was conducted according to the protocol established by White and Rudolph,[6] involving a series of steps including image duplication, Gaussian filtering, image subtraction, binarization, noise elimination, sharpening of external lines, inversion, and skeletonization (Figure 2). The fractal dimension was determined by counting 2–30 pixel-sized boxes on the skeletonized image using the Fractal box count plugin of ImageJ. Interexaminer reliability was assessed by analyzing 20% of the total sample.

Mandibular cortical width (MCW) was measured bilaterally surrounding the mental foramen, with two parallel lines drawn from the upper and lower borders of the cortex, and a third line perpendicular to these lines passing through the center of the mental foramen. The length of the third line between the other two lines was recorded as the mandibular cortical width (Figure 3). Interexaminer reliability was again assessed through analysis of 20% of the total sample.

The mandibular cortical index (MCI) was determined in accordance with the classification proposed by Klemetti et al.[7], categorizing erosion patterns of mandibular cortical bone into three groups: C1, C2, and C3. Each side was evaluated independently, with the highest category recorded (Figure 4). Inter- and intra-examiner reliability were evaluated using Cohen's kappa coefficient based on analysis of 20% of the total sample.

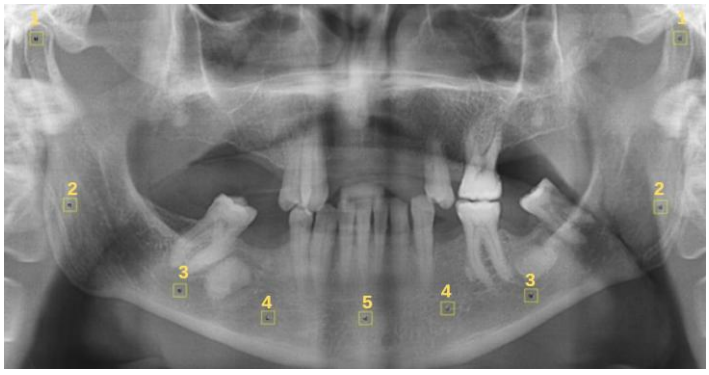

Figure 1. Selected ROIs for FA. Manually drawn squares of 30 × 30 pixels in the condyle, angulus of mandible, molar, premolar, and anterior region. Also, manually drawn bilateral ROIs of cortex.

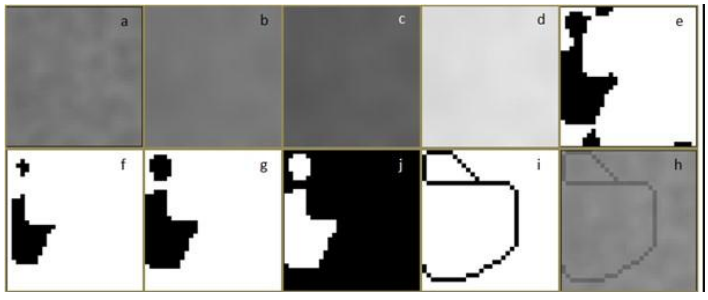

Figure 2. The process begins with the duplication of the original image post-cropping (a). Subsequently, a Gaussian filter is applied to induce blur with a sigma value of 35 (b). This blurred image is then subtracted from the original (c), followed by the addition of 128 Gy values (d). Binarization of the resulting image follows suit (e), succeeded by erosion (f) and dilation (g) operations. An inversion step is then performed (h), followed by skeletonization (i). Finally, the skeletonized image is superimposed onto the cropped image (j).

Figure 3. Two parallel lines passing from the upper and lower borders of the cortex were drawn. Then, a third line passing through the center of the mental foramen perpendicular to the two lines was drawn. The length of the third line between the other two lines was considered the MCW .

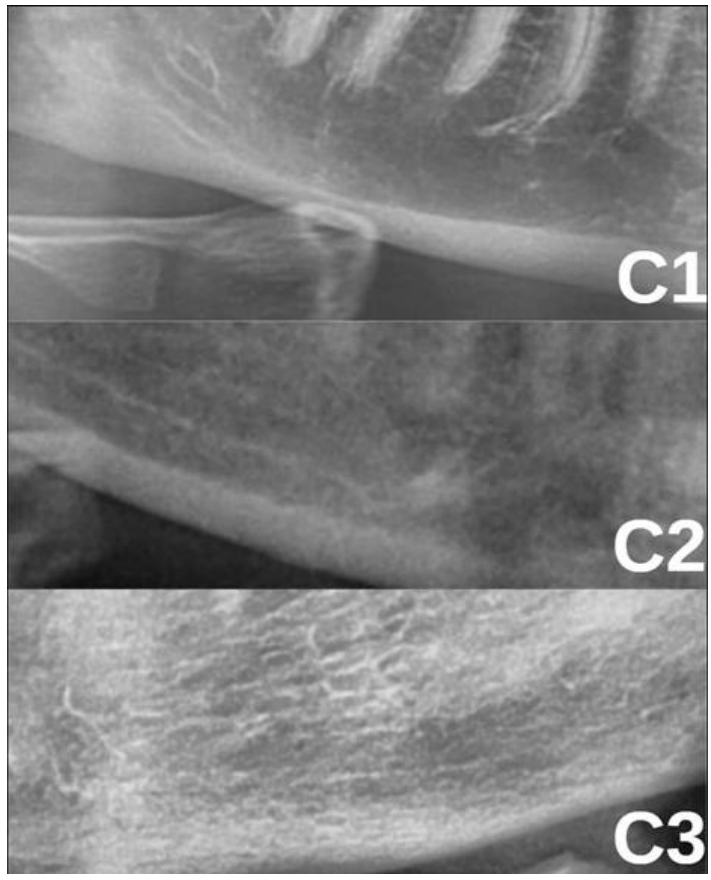

Figure 4. Radiographic presentation of mandibular cortical index classification. C1, C2, and C3.

Statistical analysis involved descriptive statistics to evaluate the distribution of each variable in each group. The McNemar–Bowker test was utilized to compare MCI distributions between osseous dysplasia patients and control groups. Student's t-test was employed to compare mean mandibular cortical width and fractal dimension values between osseous dysplasia and control groups, as well as to compare mean mandibular cortical width across different MCI subgroups. Differences in fractal dimension and mandibular cortical width values among patients with different types of osseous dysplasia were analyzed using the One-Way ANOVA test. All statistical analyses were performed using IBM SPSS V23 (IBM Co., Armonk, NY) software with a confidence interval of 95% and statistical significance was set at $P < .05$.

RESULTS

The study encompassed individuals with an average age of 40.6 years (± 11.4 years), ranging from 19 to 68 years, inclusive of patients diagnosed with various forms of osseous dysplasia and a control group. Specifically, among the cases 20 (46.5%) were afflicted with cemento-osseous dysplasia, 6 (14%) with focal osseous dysplasia and 17 (39.5%) with florid osseous dysplasia. Table 1 delineates a comparative analysis of fractal dimension (FD) values across various anatomical regions between the two cohorts. Noteworthy is the observation that patients with osseous dysplasia exhibited comparatively lower mean FD values in the condyle and anterior regions, whereas higher mean values were discerned in other regions when juxtaposed with the control group. However, these differences did not achieve statistical significance,

except in the condyle regions, as indicated in Table 1. In mandible, especially bone quality of condyle region was significantly lower in patients of osseous dysplasia. Additionally, Table 1 presents a comparative examination of mandibular cortical width (MCW) between the two cohorts, illustrating a slightly greater mean MCW in the control group, albeit lacking statistical significance (*P> .05*). Moreover, Table 1 also depicts the distribution of mandibular cortical index (MCI) classifications between patients with osseous dysplasia and the control group, indicating no statistically significant variance (*P> .05*) across the observed C1, C2, and C3 categories. Furthermore, Table 2 provides an overview of FD and MCW values stratified by osseous dysplasia types, revealing no statistically significant differences in any of the radiomorphometric parameters concerning the various osseous dysplasia types.

Reliability analysis indicated commendable inter-examiner agreement for FD assessment (intraclass correlation coefficient [ICC]= 0.743), excellent inter-examiner reliability for MCW measurement (ICC= 0.902), and good inter-examiner reliability for MCI determination employing Cohen's kappa coefficient (κ=0.659).

Table 1. Comparison of fractal dimensions of anatomic regions, mandibular cortical width, mandibular cortical index between the patients of osseous dysplasia and control groups.

Region	Groups	N	Mean	Std. deviation	Std. error mean	*P**
Anterior	Osseous	43	.84	.31		
	Dysplasia				.069	.453
	Control	43	.94	.33		
Premolar	Osseous	43	.97	.22		
	Dysplasia				.056	.057
	Control	43	.91	.29		
Molar	Osseous	43	.95	.24		
	Dysplasia				.55	.427
	Control	43	.89	.27		
Angulus	Osseous	43	.95	.19		
	Dysplasia				.041	.815
	Control	43	.95	.19		
Condyle	Osseous	43	.8	.36		
	Dysplasia				.069	.007
	Control	43	.93	.28		
MCW	Osseous	43	22.58	12.11		
	Dysplasia				1.15	.919
	Control	43	24.05	13.06		
		C1	C2	C3	N(%)	*P***
MCI	Osseous	23	16	4	43 (100)	
	Dysplasia					
	Controls	29	11	3	43 (100)	
						.413
	Total	52	27	17	86 (100)	

*N, number of measurements, MCW, mandibular cortical width, MCI, mandibular cortical index, P * Student's t test, P** McNemar–Bowker Test.*

Table 2. Comparison of anterior, premolar, molar, angulus, condyle region FD and MCW by osseous dysplasia type.

	Cemento Osseous Dysplasia (M±Sd)	Focal Osseous Dysplasia (M±Sd)	Florid Osseous Dysplasia (M±Sd)	*P***
Premolar	1.03±.076	.87±.23	.93±.31	.243
Molar	1.02±.17	.75±.38	.94±.21	.062
Angulus	.93±.16	.81±.30	1.02±.15	.060
Condyle	.85±.34	.71±.37	.79±.38	.718
MCW	24.37±5.05	22.37±3.93	21.07±6.31	.280

***One-way analysis of variance, M±Sd : Mean ± Standart deviation, P<.05*

DISCUSSION

Panoramic radiographs offer multiple forms of information despite their limitations in distortion and image overlap.[8] The panoramic radiograph, due to its low-dose X-radiation and affordability, is often requested in clinical practice to assess the oral and maxillofacial region. It serves as a dependable and cost-effective means of screening for low bone mineral density (BMD) in patients, especially as the high cost of DXA limits its evaluation to select female cases.[9] Furthermore, aside from their fractal dimension of radiographs non-invasive nature and ease of application, these methods offer crucial clinical insights into significant bone changes.[5]

Eser et al.[10] assessed changes in fractal dimension by identifying triangular-shaped regions of interest (ROIs) on the mesial and distal interradicular surfaces of the first molar teeth in panoramic radiographs of individuals with gingivitis and periodontitis. Korkmaz et al.[11] evaluated the effects of vitamin D deficiency on mandibular bone patterns using fractal analysis, assessing a total of eight distinct regions: the condyle, the angulus and the areas anterior and posterior to the mental foramen.

Balkan et al.[12] evaluated changes in fractal dimension observed in the mandibular condyle, ramus, and angulus regions on panoramic radiographs following botulinum toxin injection into the masseter muscle in individuals with bruxism.

Yavuz et al.[13] compared imaging methods for trabecular bone fractal dimension analyses, with each group consisting of 15 radiographs (digital periapical radiographs, panoramic radiographs, and cone-beam computed tomography). They found no statistically significant difference between the digital periapical radiography technique and the panoramic radiography technique. However, they concluded that the cone-beam computed tomography method did not correlate with the other methods in the fractal dimension analysis of trabecular bone.

Software and studies are available for conducting fractal dimension analyses on both two-dimensional and three-dimensional radiographs. Each type of radiograph has its own advantages and disadvantages. A significant disadvantage is the radiation dose administered to the patient. However, the major advantage is that they provide information about the three-dimensional structure of tissues.[14] Therefore, radiographic indications are established considering clinical benefit. Two-dimensional radiographs are commonly used in bone assessments with fractal analysis, which is why this study evaluated changes in fractal dimensions across nine distinct regions of interest (ROIs). This approach aims to represent changes occurring in each anatomical region of the mandible.

De Oliveira et al.[15] conducted a fractal analysis on cone-beam computed tomography images of six individuals diagnosed with fibrous dysplasia and six individuals diagnosed with ossifying fibroma. In their study, they found that the bone quality of fibrous dysplasia patients was higher in both two-dimensional and three-dimensional radiographs compared to those with ossifying fibroma. However, in this study, no significant differences were observed among the FD, MCW, and MCI values in three different types of osseous dysplasia.

In Arsan's[16] study, a fractal analysis was conducted on cone-beam computed tomography images of 28 patients diagnosed with cemento osseous dysplasia in five different regions. There was no statistically significant difference observed between the cemento osseous dysplasia group and the control group. Similarly, Kato et al.[5] performed fractal analysis on panoramic radiographs of 30 female patients with cemento osseous dysplasia. The FD value, similar to Arsan's study,

did not show a statistically significant difference, while the MCW and MCI values were found to be lower in the cemento osseous dysplasia group compared to the healthy group.Unlike Kato's study, in our research, although cortical bone thickness and porosity in COD patients were similar to the healthy group, it was observed that the bone quality in the condylar region of COD patients was significantly weaker.

When looking at the pathogenesis of Cemento Osseous Dysplasia (COD), Haefliger et al.[17] observed "pathogenic hotspot mutations involving the RAS-MAPK signaling pathway" in 28% of the 30 COD cases they studied.[17] However, they argued that these mutations still do not explain the mechanism of spontaneous growth.In a study by Lv et al.[18], they discovered an "ANO5 missense mutation" in a family with three generations of florid COD. The authors emphasized that this discovery could lead to better clinical management and genetic counseling for FCOD cases.

Klemm et al.[19] reported that in the case of a very rare disease known as "Camurati–Engelmann disease" or alternatively referred to as "progressive diaphyseal dysplasia," mutations in the transforming growth factor-beta 1 gene were observed. It was noted that in this disease, bilateral osteosclerosis can be observed in the cranial base and mandible.

Cheng et al.[20] have identified that Kenny-Caffey syndrome is a bone dysplasia related to the FAM111A protein. In this syndrome, they observed features such as frontal bossing, relative macrocephaly, triangular face, narrow nasal ridge and micrognathia or microretrognathia. Additionally, dental manifestations including oligodontia, enamel hypoplasia, retention of primary dentition, and delayed eruption of secondary dentition were also noted. When all of these studies analyzing osseous dysplasias are interpreted, it can be concluded that there is a possibility of pathogenesis related to gene mutations in these respective bone dysplasias.

In a review where Kato et al.[21] analyzed 66 cases of Cemento Osseous Dysplasia, they found that the most common cause of COD-related local infections was the extraction of adjacent teeth. Additionally, in this review, the factors most commonly reported to contribute to the development of COD-related osteomyelitis were, in order, tooth extraction, prosthesis trauma, and root canal treatment. Therefore, it was emphasized that clinicians should differentiate between periapical pathology and COD to prevent such complications.

Consolaro et al.[22] have emphasized that orthodontic treatment can be contraindicated in some cases of Cemento Osseous Dysplasia. The reason for this is the potential for bacterial contamination from oral microbiota due to surgical procedures such as the installment of mini-implants or mini-plates and the application of traction to impacted teeth in the hypovascular bone tissue. Additionally, it was mentioned that tooth movement is not possible in this disorganized bone area, and remodeling does not occur in this abnormal mineralized and unorganized bone tissue. Whitt et al.[23], in their follow-up study on cases of segmental odontomaxillary dysplasia, have reached the opposite conclusion. They found that there were no problems with orthodontic tooth movement or dental implant osseointegration in these patients.

Colak[24] found that in patients with ectodermal dysplasia, another type of dysplasia observed in the jaws, the bone mineral density was lower in cone-beam computed tomography images. Leet et al.[25] reported that in patients with polyostotic fibrous dysplasia, the incidence of bone fractures is higher in the first decades of life, and in cases where "phosphaturia" is observed, there is a higher likelihood of bone fractures occurring earlier and more frequently.

Apolinário et al.[26] conducted radiomorphometric analysis before and after the use of pamidronate (PAM) in 62 children with osteogenesis imperfecta. They observed a statistically significant increase in FD and MCW values after the use of the medication. This indicates that fractal analysis can be used as an effective, reliable, and easy analysis method in diseases causing resorption and during medication usage.

Limitations of our study is the need to examine the changes caused by osseous dysplasias and other bone-affecting diseases in the jawbones from both a fractal dimension and genetic perspective using larger sample sizes. Additionally, similar studies can be conducted using both two-dimensional and three-dimensional radiographs to compare the results. This would allow for an investigation into the adequacy of two-dimensional radiographs in this conditions.

CONCLUSION

In conclusion, it has been determined that cortical bone porosity and bone thickness in COD patients are similar to those in healthy individuals. However, it should be kept in mind that the quality of bone, especially in the condylar region of COD patients, may not be equivalent to that of healthy individuals. Clinicians should approach surgical procedures in the joint area of these patients more cautiously. Additionally, patients with the three variants of segmental dysplasia in the mandible were found to have similar bone quality.

Ethics Committee Approval: Approval for this study was obtained from Van Yüzüncü Yıl University Non-Interventional Clinical Research Ethics Committee (Approval number: 2023/13-07, date 08/12/2023).

Informed Consent: In this study, panoramic radiographs used during routine radiologic examination of patients admitted to our clinic were used. Therefore, no consent form was obtained.

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Evaluation of Dental Treatments in Adult Patients with Special Needs: A Retrospective Study

Özel Gereksinimli Erişkin Hastalarda Diş Tedavilerinin Değerlendirilmesi: Retrospektif Bir Çalışma

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ABSTRACT

Objective: Patients with special needs are at a higher risk of inadequate oral care, leading to dental caries or periodontal diseases. Therefore, this study aimed to retrospectively analyze the dental records of patients with special needs who received dental treatments under general anesthesia or sedation.
Methods: A total of 186 cases (170 patients) of dental general anesthesia and sedation in adult patients with special needs were included in the study from January 2015 to April 2022. Demographic data, restorative treatments, tooth extractions performed under general anesthesia/sedation, secondary diseases, type of anesthesia, and American Society of Anesthesiologists (ASA) classification were obtained from patient files. Data were recorded as mean ± standard deviation (SD) or numerical value.
Results: Of the 186 cases (mean age 28.55 ± 11.91 years), 134 (72 %) were performed under general anesthesia and 52 (28 %) under sedation. A total of 396 fillings, 775 extractions, 14 root canal treatments, and 93 scaling procedures were performed in 186 cases. On average, each case received 5.5 ± 3.4 teeth fillings, 4.5 ± 4.0 teeth extractions, and 1.6 ± 1.0 root canal treatments. Patients were classified as ASA I (n = 100) at most and ASA III (n = 5) at least.
Conclusion: Patients with special needs have a high demand for dental treatment. In dental treatment of patients with special needs under general anesthesia/sedation, dentists prefer radical treatments such as tooth extraction.
Keywords: Anesthesia; Conscious Sedation; Deep Sedation

ÖZ

Amaç: Özel bakıma ihtiyaç duyan hastalar, diş çürüğü veya periodontal hastalıklara yol açan yetersiz ağız bakımı açısından daha yüksek risk altındadır. Bu çalışma da genel anestezi veya sedasyon altında dental tedavileri yapılan özel bakıma ihtiyaç duyan hastaların dental kayıtlarının retrospektif olarak analiz edilmesi amaçlandı.
Yöntem: Ocak 2015 - Nisan 2022 tarihleri arasında özel bakıma ihtiyaç duyan yetişkin hastalardan dental genel anestezi veya sedasyonla tedavi edilen toplam 186 vaka (170 hasta) çalışmaya dahil edildi. Demografik veriler, restoratif tedaviler, genel anestezi/sedasyon altında yapılan diş çekimleri, sekonder hastalıklar, anestezi tipi ve Amerikan Anestezistler Derneği (ASA) sınıflandırması hasta dosyalarından elde edildi. Veriler ortalama ± standart sapma (SS) veya sayısal değer olarak kaydedildi.
Bulgular: 186 vakanın (ortalama yaş 28,55 ± 11,91 yıl) 134'ü (%72) genel anestezi altında, 52'si (%28) sedasyon altında gerçekleştirilmiştir. 186 vakada toplam 396 dolgu, 775 çekim, 14 kanal tedavisi ve 93 diş taşı temizliği yapılmıştır. Ortalama olarak her vakaya 5,5 ± 3,4 diş dolgusu, 4,5 ± 4,0 diş çekimi ve 1,6 ± 1,0 kök kanal tedavisi uygulanmıştır. Hastalar en fazla ASA I (n = 100) ve en az ASA III (n = 5) olarak sınıflandırılmıştır.
Sonuç: Özel bakıma ihtiyaç duyan hastaların dental tedaviye olan talepleri oldukça yüksektir. Özel gereksinime sahip hastaların genel anestezi/sedasyon altında yapılan dental tedavilerinde diş hekimleri daha çok diş çekimi gibi radikal tedavileri tercih etmektedirler.
Anahtar Kelimeler: Anestezi; Bilinçli Sedasyon; Derin Sedasyon

INTRODUCTION

Most of the studies among these methods lead to the preference of fixed prostheses due to the problems with removable prostheses Oral implantation which is one of Individuals with special needs refers to patients with mental, physical, psychiatric and complex medical problems.[1] They often struggle to maintain effective oral hygiene due to motor, sensory, or mental disabilities, resulting in serious periodontal problems and a high incidence of dental caries.[2-4] Additionally, these patients often display

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weak cooperation and high anxiety, making routine dental procedures challenging or impossible.[5,6] As healthcare professionals, it is our responsibility to ensure that patients with special needs receive optimal medical treatment. However, as it is known, in order to carry out dental treatments efficiently, effective communication with the patient is necessary.[7] Unfortunately, it is not possible to communicate effecttively with these patients. Due to these reasons, dental treatments should be personalized for such special needs patients and the most appropriate medical treatment should be provided.[8]

General anesthesia and sedation are viable treatment alternative for patients who are uncooperative and require extensive dental treatment.[9] These methods offer advantages, such as completing the procedure in a single appointment and requiring little or no cooperation with the patient. Even so, the possibility that general anesthesia may pose a risk to the general health of the patient should not be ignored.[10,11] Because, a significant portion of special needs patients has other medical problems and these problems can cause various complications.[12] On the other hand, many of this patient group has central nervous system diseases such as epilepsy, poor motor control, paralysis, and spasticity.[13] Furthermore, General Dental Council of United Kingdom noted in its guidelines that "General anesthesia is a procedure which is never without a risk".[14]

The American Society of Anesthesiologists physical status classification (ASA) is a classification developed to categorize the preoperative condition of patients.[15] This classification, the first version of which was published in 1941, has now become a scale from 1 to 6.[16] The purpose of ASA classification is to assess a patient's medical comorbidities before anesthesia and to predict perioperative risks.[17]

This study focused specifically on dental treatments to, assess patients with special needs. In addition, the aim of this study was to retrospectively examine dental treatments for adults with special needs to contribute to the literature.

METHODS

This study presented to you has been conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Non-Interventional Clinical Research Ethics Committee of Recep Tayyip Erdogan University (Date: 08.09.2022, Number: 2022/145). General anesthesia/ sedation treatment was applied to 910 adult and pediatric patients at the Recep Tayyip Erdogan University Faculty of Dentistry between January 1, 2015, and April 1, 2022. Patients treated under general anesthesia or sedation due to dental anxiety, major surgical procedures, and patients younger than 15 years of age were not included in the study. Only adults and patients with special needs were included in this study. Therefore, anonymized data from 170 patients were obtainned from digital patient files. The anonymized data collected was as follows:

1. Gender
2. Date of birth
3. Date of general anesthesia/sedation
4. Teeth undergoing restorative treatment (filling)
5. Extracted teeth
6. Teeth undergoing root canal treatment
7. Scaling and tooth polishing (yes/no)
8. Secondary diseases
9. Type of anesthesia (general anesthesia/sedation)
10. ASA classification

The patient's age at the time of general anesthesia/sedation was obtained using the patient's date of birth and general anesthesia/ sedation date.

Statistical analysis

Statistical analyses were performed using SPSS (version 23.0, IBM SPSS Corp., Armonk, NY, USA). Continuous data were tested for normality. Normally distributed data are summarized as mean and standard deviation. Data that were not normally distributed were summarized using the median (minimum-maximum). Categorical data are summarized using numbers.

RESULTS

One hundred seventy patients were treated under general anesthesia/sedation (186 cases). Fourteen patients were treated twice and one patient was treated thrice. Of the 186 cases, 134 (72 %) were conducted under general anesthesia and 52 (28 %) were performed under sedation. The mean age and gender distribution of the patients are given in Table 1. The ages of the patients ranged from 15 to 66 years and the mean age was 28.55 ± 11.91. 99 patients were male and 71 were female.

A total of 396 fillings, 775 extractions, 14 root canal treatments, and 93 scaling procedures were performed in 186 cases. On average, each case received 5.5 ± 3.4 fillings, 4.5 ± 4.0 extractions, and 1.6 ± 1.0 root canal treatments (Table 2). Moreover, a maximum of 13 fillings, 22 extractions, and 4 root canal treatments were performed at one time, in different patients (Fig. 1). The distribution of filled, extracted, and root canal treated teeth per case (0, 1 – 5, 6 – 10, 11 – 15, and > 15 teeth) is shown in Table 3. Accordingly, 114 (61.3%) of 186 cases had no filling, 14 (7.5%) had no extraction, 177 (95.2%) had no root canal treatment and 93 (50%) had no scaling. Under general anesthesia/ sedation, the majority of filling and extraction treatments were applied to molars, while the least were applied to premolars. Root canal treatment was mostly performed on the anterior teeth and least frequently on the molars (Table 4).

Epilepsy is the most common secondary disease in patients with special needs. One hundred patients had no secondary diseases (Table 5). The patients were mostly classified as ASA I (n = 100) and least as ASA III (n = 5) (Table 6).

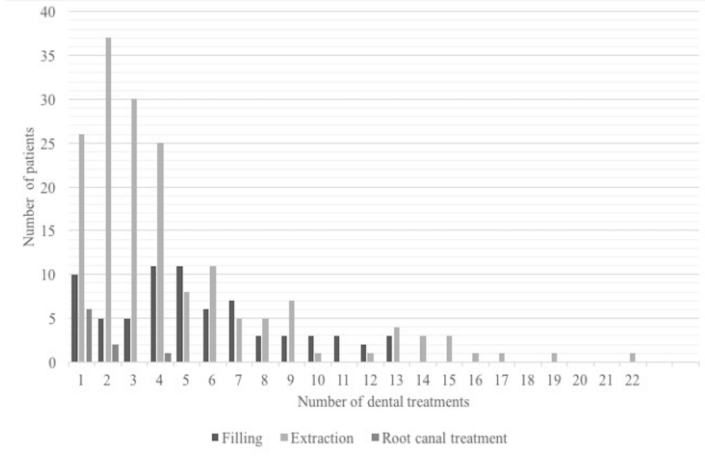

Figure 1. Distribution of the number of dental treatments performed

Table 1. *Age and gender of patients*

Age (Mean ± SD)	28.55 ± 11.91
Gender (n (%))	
Male	99 (58.2)
Female	71 (41.8)

Table 2. *Dental treatments according to the number of teeth and case*

	Total number of teeth	Total number of case	Mean (number of treatments per case)
Filling	396	72	5.5 ± 3.4
Extraction	775	172	4.5 ± 4.0
Root canal treatment	14	9	1.6 ± 1.0
Scaling	-	93	-

Table 3. *Number of dental treatments performed under general anesthesia/sedation*

Number of treatment teeth	Number of case (Filling)	Number of case (Extraction)	Number of case (Root canal treatment)
0	114 (61.3 %)	14 (7.5 %)	177 (95.2 %)
1 - 5	42 (22.6 %)	128 (68.8 %)	9 (4.8 %)
6 - 10	22 (11,8 %)	29 (15.6 %)	0
11 - 15	8 (4.3 %)	11 (5.9 %)	0
> 15	0	4 (2.2 %)	0
Total	186	186	186

Table 4. *Number of dental treatment according to tooth groups*

	Anterior n (%)	Premolar n (%)	Molar n (%)	Total
Filling	123 (31.1)	121 (30.5)	152 (38.4)	396
Extraction	184 (23.7)	163 (21.1)	428 (55.2)	775
Root canal treatment	8 (57.2)	5 (35.7)	1 (7.1)	14

Table 5. *Secondary diseases of patients*

Secondary diseases	**n (%)**
Epilepsy	25 (14.7)
Autism	9 (5.2)
Down syndrome	6 (3.5)
Hypertension	2 (1.2)
Type 2 diabetes	2 (1.2)
Cerebral palsy	2 (1.2)
Hydrocephalus	2 (1.2)
Microcephaly	2 (1.2)
Visual impairment	1 (0.6)
Renpenning syndrome	1 (0.6)
Paralysis	1 (0.6)
Epilepsy + hydrocephalus	3 (1.7)
Epilepsy + Schizophrenia + Visual impairment	2 (1.2)
Epilepsy + Type 2 diabetes	1 (0.6)
Epilepsy + Heart failure	1 (0.6)
Epilepsy + Autism	1 (0.6)
Epilepsy + Autism + Speech impediment	1 (0.6)
Epilepsy + Autism + Visual impairment	1 (0.6)
Epilepsy + Osteoporosis	1 (0.6)
Epilepsy + Cerebral palsy	1 (0.6)
Physical disability + Hypothyroidism	1 (0.6)
Hypertension + Asthma	1 (0.6)
Hypertension + Bipolar disorder	1 (0.6)
Down syndrome + Hypothyroidism	1 (0.6)
Down syndrome + Goiter	1 (0.6)
Without secondary diseases	100 (58.7)
Total	170

Table 6. *ASA classification of patients*

ASA classification	Definition	n (%)
ASA I	A normal healthy patient	100 (58.8)
ASA II	A patient with mild systemic disease	65 (38.3)
ASA III	A patient with severe systemic disease	5 (2.9)
ASA IV	A patient with severe systemic disease that is a constant threat to life	-
ASA V	A moribund patient who is not expected to survive without the operation	-
ASA VI	A declared brain-dead patient whose organs are being removed for donor purposes	-

DISCUSSION

This study provides information about the oral health status of patients with special needs undergoing dental treatment under general anesthesia/sedation. As the special needs population ages and life expectancy increases in many countries, it is crucial to provide regular and high-quality dental care to prevent disease and enhance the quality of life for these individuals.[18,19] In addition, the increasing demand for dental treatment among patients with special needs and the provision of appropriate treatments under optimal conditions demonstrate the quality of healthcare services and experience of healthcare professionals.

This study did not include patients under the age of 15. This is because the Republic of Türkiye Social Security Institution covers the treatments performed by the pediatric dentistry department for patients under the age of 15 years. Patients over the age of 15 years are treated in departments such as restorative dentistry and endodontics. Additionally, there are many differences between pediatric and adult patients, including age, height, weight, and others.[20] Furthermore, due to the different treatment approaches used for permanent and deciduous teeth, patients under the age of 15 years were not included in this study.

In this study, the number of male patients was higher than the number of female patients. Many studies in the literature, similar to our study, have also shown that male patients outnumbered female patients.[21-24] On the contrary, the study by de Nova Garcia et al.[23] showed that the number of female patients was higher than that of male patients. The reason for this discrepancy between studies could be the differences in age, race, gender, ethnicity, and demographic characteristics of the study populations.

There are studies that have found restorative procedures to be more demanded in patients with special needs treated under general anesthesia, especially in adolescents and adults, compared to extraction treatment.[25,26] Conversely, Ali et al.[27] indicated that extractions are performed more frequently than restorations in patients with special needs between the ages of 16 and 75 years. In the present study, extraction was the most preferred treatment. More aggressive treatment approaches have been preferred in patients with special needs to prevent the need for re-general anesthesia and to reduce the risk of complications by reducing the procedure time. Although root canal treatment is performed in some cases with a conservative approach, extraction has been preferred for deeply decayed teeth because of the difficulties in taking X-rays under general anesthesia and the potential complications that may occur after treatment. It is noteworthy that in this study, the number of extractions and fillings was low in the 9 patients who underwent root canal treatment.

In this study, scaling was not performed on half of the patients. In most patients with special needs, multiple dental treatments are simultaneously performed at the same time. The remaining number of teeth in the mouth was low because of the high number of planned tooth extractions. Additionally, dental calculus is less commonly seen in patients with a high incidence of dental caries.[28] Therefore, in patients with special needs, the existing plaque was easily removed by polishing without the need for scaling.

In this study, the majority of patients with special needs were treated under general anesthesia. When choosing the type of anesthesia, many factors such as patient cooperation, the extent of the treatment plan, and treatment duration play an important role. Sedation is generally preferred for simple and few tooth extractions.

Because filling or scaling procedures are performed under pressurized water cooling and can prolong the procedure time, sedation is not a suitable anesthesia type for these patients. Therefore, in this study, multiple treatments and time-consuming procedures were performed under general anesthesia.[29]

Not all patients with special needs have the same disability or need for dental treatment. Therefore, if possible, treatment of some patients was attempted in a clinical setting with or without local anesthesia. In addition to preferring less traumatic techniques, such as chemo-mechanical caries removal agents or lasers in the treatment of these patients, the treatment times were kept short to prevent disruption of the patient's compliance with the treatment. Thus, the need for general anesthesia/sedation was eliminated in patients with special needs who were able to comply with simple and few treatments. However, the treatments of some patients with special needs can be challenging and time-consuming.[30] In fact, even intraoral examination may not be possible. Therefore, general anesthesia and sedation may be the only treatment options. As mentioned before, to minimize potential complications that may arise in these patients, all treatments performed under general anesthesia/sedation should be carried out in comprehensive healthcare institutions by experienced healthcare personnel.[31]

Regular follow-up appointments are crucial for long-term success of dental treatment under general anesthesia/sedation. Oral hygiene and dietary education provided to parents or caregivers of patients with special needs can prevent new caries and/or periodontal disease in these patients. In some studies in the literature, follow-up appointments were scheduled after general anesthesia.[22,24] However, the lack of a regular follow-up program for patients treated under general anesthesia/sedation in this study is a significant limitation. Regular follow-up appointments, oral hygiene and diet education provided to caregivers and parents of patients with special needs can significantly reduce the need for repeated general anesthesia for dental treatments.[32]

Isolation is essential for successful restorative treatment. This also applies to restorative treatments performed under general anesthesia/sedation. Although the patient's saliva flow is reduced due to general anesthesia/sedation, it is very difficult to exclude oral tissues, especially the tongue, from the operation area. Gingival bleeding is also more common in patients with special needs due to poor oral hygiene.[33] Despite all these, preventive and restorative treatment of patients with special needs under general anesthesia/sedation has an important advantage in terms of preventing tooth loss and allowing conservative treatments.[34]

General anesthesia and sedation should be considered as the last treatment option for dental treatments of patients with special needs.[35] However, successful dental treatments can be performed with general anesthesia/sedation practices performed by experienced healthcare professionals in comprehensive healthcare institutions in mandatory cases.

The demand for dental treatment in patients with special needs is continuously increasing. Patients with special needs may have an additional systemic disease. Furthermore, dentists often prefer for more radical treatment options, such as tooth extraction, in the dental treatment of patients with special needs.

Ethics Committee Approval: Approved by Recep Tayyip Erdoğan University, Non-Interventional Clinical Research Ethics Committee (Date: 08.09.2022, Number: 2022/145).

Informed Consent: Since the study was retrospective, written permission was obtained from the Dean of Recep Tayyip Erdoğan University, Faculty of Dentistry to obtain the patient data recorded in the system.

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Comparison of the Vas and Anxiety Inventory After Periodontal Surgery

Periodontal Cerrahi Sonrasının Vas ve Kaygı Envanteri ile Karşılaştırılması

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ABSTRACT

Objective: Oral surgery, though rarely life-threatening with a short recovery time, can be stressful due to its physical and psychological impacts. This study aimed to examine the relationships between periodontal surgery type and postoperative anxiety and visual analog scale (VAS) scores.

Methods: Patients (n=42) aged 19-65 years who visited the Periodontology Clinic were divided into three groups according to their indications. Patients who underwent mucogingival-1 (coronally advanced flap, laterally closed tunnel, and free gingival graft), mucogingival-2 (frenectomy, gingivectomy, and vestibuloplasty), and flap surgeries completed the State Anxiety Inventory (STAI-S) and Trait Anxiety Inventory (STAI-T), and Modified Dental Anxiety Scale (MDAS). At the 3rd hour and on the 1st, 3rd, 7th, and 14th days after surgery, pain, sensitivity, eating, and speaking difficulty were determined using the VAS. The statistical significance level was set at $P<.05$.

Results: There was a statistically significant difference in the STAI-S scores of the participants according to the type of surgery ($P<.05$). While the mucogingival surgery-1 group had a low STAI-S score, only the flap surgery group had a high STAI-S score. There was no statistically significant difference between the surgery groups in terms of MDAS scores ($P>.05$). A statistically significant difference between the participants' levels of difficulty eating on the 7th day was noted according to the type of surgery ($P<.05$).

Conclusion: The study revealed a correlation between state anxiety and periodontal surgery, whereas no association with trait anxiety, pain, or sensitivity was observed. To improve patient care, it is essential to use these findings to customize preoperative counseling and develop effective postoperative pain management strategies.

Keywords: Dental anxiety, oral surgery, visual analog pain scale

ÖZ

Amaç : Ağız cerrahisi, kısa iyileşme süresiyle nadiren hayatı tehdit etse de, fiziksel ve psikolojik etkileri nedeniyle stresli olabilir. Bu araştırmanın amacı periodontal cerrahi tiplerinin ameliyatlar sonrası anksiyete ve görsel analog skalası ile ilişkisini incelemektir.

Yöntemler : 19-65 yaş arasında Periodontoloji Kliniği'ne başvuran hastalar (42 hasta) endikasyonlarına göre üç gruba ayrılmıştır. Mukogingival-1 (koronale pozisyone flep, lateral kapalı tünel ve serbest dişeti grefti), mukogingival-2 (frenektomi, gingivektomi ve vestibüloplasti) ve flep ameliyatları geçiren hastalar Durumluk Kaygı Envanteri (STAI-S) ve Sürekli Kaygı Envanteri (STAI-T) ile Modifiye Dental Kaygı Ölçeğini (MDAS) doldurmuştur. Ameliyattan sonra 3. saat, 1. gün, 3. gün, 7. gün ve 14. gün ağrı, hassasiyet, yemek yemede güçlük ve konuşmada zorluk skorları Vas skalası ile tespit edilmiştir. Araştırmada istatistiksel anlamlılık düzeyi $P<,05$ olarak belirlenmiştir.

Bulgular : Katılımcıların STAI-S değerlendirmelerinde ameliyat türüne göre istatistiksel olarak anlamlı bir fark bulunmuştur ($P<,05$). Mukogingival cerrahi-1 grup düşük STAI-S değerlendirmesinde yer alırken, flep cerrahisi hastaları yüksek STAI-S grubunda yer almıştır. Katılımcıların ameliyat türleri arasında modifiye dental anksiyete skorları açısından istatistiksel olarak anlamlı bir fark yoktur ($P >,05$). Katılımcılara uygulanan ameliyat türlerine göre sadece 7. günde yemek yeme güçlüğü skorları arasında istatistiksel olarak anlamlı bir fark bulunmuştur ($P<,05$).

Sonuç : Bu çalışma, durumluk kaygı ile periodontal cerrahi arasında bir korelasyon olduğunu ortaya koyarken, sürekli kaygı, ağrı veya hassasiyet ile herhangi bir ilişki gözlemlenmemiştir. Hasta bakımını iyileştirmek, ameliyat öncesi kişiye özel yaklaşım ve etkili ameliyat sonrası ağrı yönetimi stratejileri geliştirmek için bu bulguları kullanmak önemlidir.

Anahtar Kelimeler: Ağız cerrahisi, dental anksiyete, görsel analog ağrı skalası

INTRODUCTION

Anxiety refers to an emotional reaction to stress, apprehension, unease, and perceived threats and is often accompanied by activation of the autonomic nervous system.[1] Dental anxiety is a multifaceted construct shaped by diverse factors, including age, sex, educational attainment, socioeconomic status, previous

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experiences, residential environment, and individual personality traits.[2] Dental anxiety is assessed through a set of established and validated measurement tests.[3] The Anxiety Inventory evaluates both state and trait anxiety using a total of 20 questions. Specifically, the State Anxiety Inventory (STAI-S) assesses an individual's temporary emotional conditions, which can vary over time and are typically triggered by specific situations or stimuli, whereas the Trait Anxiety Inventory (STAI-T) assesses an individual's general tendencies to experience anxiety across many situations over time.[4] The Modified Dental Anxiety Scale (MDAS) evaluates patients' apprehension concerning dental procedures and the professionals conducting them.[5]

The Pain assessments might indicate that there is not only a physiological aspect but also a psychological dimension of pain. Not only the intensity but also the unpredictability of pain leads to a difference in grading.[6] Additionally, pain perception depends on a person's pain threshold, which might be used as a baseline to compare pain intensity.[7] The variables influencing the level of pain reported include the expectation of discomfort, age, the administration of sedatives, and the quantity of analgesics consumed.[6] Oral surgery induces stress in both patients and surgeons, impacting vital signs and anxiety levels.[8] Postoperative pain after oral surgery has been extensively studied in pharmacological and pain research. Disruption of tissue homeostasis during surgery triggers physiological changes that affect pain perception through autonomic, hormonal, and chemical systems, which are especially sensitive in craniofacial and oral areas, necessitating meticulous pain control strategies for optimal treatment outcomes. [9] Periodontal therapy induces discomfort in a considerable proportion of patients.[10] The Visual Analog Scale (VAS) is commonly used in dental or oral surgery investigations due to its enhanced reliability, validity, sensitivity, and convenience.[7] Subjective experiences such as pain, craving, or well-being can fluctuate in intensity and are difficult for individuals to articulate precisely. The VAS offers a dependable and valid method to address this complicated measurement issue.[11] One study examined the relationship between periodontal surgery types and Vas scores.[12] Hence, it is imperative for dentists to evaluate pain responses both during and post-treatment.[10]

This study aimed to investigate the relationship between the types of periodontal surgical operations and postoperative anxiety and the visual analog scale.

METHODS

Research and Publication Ethics

The Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University approved this study (Date: July 5, 2022, No: 2022/175). Before participation, all patients were informed about the purpose of the questionnaire, and written informed consent was obtained from the patients following the Helsinki Declaration. The study was conducted at the Faculty of Dentistry's Periodontology Department between July 2022 and July 2023.

Sample Size Calculations

According to the results of the power analysis performed with the G Power program (G * Power 3.1 software; Heinrich Heine University, Düsseldorf, Germany), the F test, repeated-measures ANOVA, and between factor analysis, a minimum of 33 participants was required to carry out the study based on five repeated measurements for three subgroups with α (margin of error) = 0.05, an effect size of 0.60 (f), and a power of level 0.85 power (1-β).

Inclusion Criteria

Individuals aged 19-65 years who could answer the questions were included in the study. Individuals who were systemically healthy or had controlled systemic disease were also included.

Exclusion Criteria

Patients requiring preoperative sedation, those who used long-term analgesics preoperatively, individuals with mental retardation, severe suicidal tendency, dementia, or psychosis, and those with a history of surgical procedures and long-term corticosteroid use; were excluded from the study. Additionally, patients who were pregnant, had anxiety disorders, or experienced paresthesia were not included in the study population.

Study Settings

Individuals aged 19 to 65; 42 patients who underwent surgical procedures at the Bolu Abant İzzet Baysal University Periodontology Clinic, specifically those who underwent mucogingival surgery, flap surgery, frenectomy, gingivectomy, and vestibuloplasty, were assessed using the STAI-S, STAI-T, and MDAS. Each patient was asked about the following: income level, the marital status of his or her parents, medication use, history of oral surgery, and whether he or she found dental treatment frightening. The patients did not have any systemic disorders or medication use that would affect anxiety. The STAI-T, STAI-S, and MDAS were completed after the surgeries. Pain, sensitivity, difficulty eating, and difficulty speaking were determined using the VAS at the 3rd hour and 1, 3, 7, and 14 days after the operation. Eight patients who did not report their Vas values in the postoperative days dropped out of the study. The study participants were divided into three groups based on the type of surgical intervention they received. The mucogingival surgery-1 group included patients who underwent procedures involving a coronally positioned flap, a laterally closed tunnel, and a free gingival graft. The flap surgery group underwent flap surgery, while the mucogingival surgery-2 group underwent frenectomy, gingivectomy, or vestibuloplasty. The surgical procedures included at least three to five teeth (with the exception of frenectomy). All surgeries and tests were performed by a single clinician. Each surgery was performed with the same technique. The same postoperative care and diet were recommended after all surgeries (mouthwash was prescribed twice in the first week).

Anxiety Inventory

The Anxiety Inventory was used to analyze the patients' various anxiety states. The inventory comprises two subsections: the STAI-S and the STAI-T. Responses are scored on a scale ranging from 1 (not at all) to 4 (very much).[13] The total score ranges from 20 to 80 points, with higher scores correlating with more significant anxiety. This means that the STAI-S only measures state anxiety and that the STAI-T only measures trait anxiety, which was the ultimate goal of this test.[14] Low scores indicate mild anxiety, and high scores indicate severe anxiety. Both scales have questions regarding the absence and presence of anxiety. Anxiety-present questions are used to determine the absence of anxiety in statements such as, “I feel secure.” Anxiety-present questions are used to determine the presence of anxiety in statements such as “I feel worried.” Each measure has a different rating scale[4]. The low-trait-anxiety group had a score of 40 points or below, while the high-trait-anxiety group had scores of 51 points or above. The medium-trait anxiety group had scores between 41 and 50 points.[15]

The MDAS was used to assess the patients' anxiety about dental procedures and the people performing dental procedures. The MDAS includes five questions about local injections, and all given questions are answered using a simplified and consistent answer scheme.

Patients answer each question indicating whether they feel "not anxious", "slightly anxious", "fairly anxious", "very anxious" or "extremely anxious". The MDAS is scored using a Likert-type scoring system (1-2-3-4-5), where a high score indicates high anxiety. The item scores are summed to derive the total score.[16] In the Turkish population, the cutoff score for the MDAS is 15 points or above.[17] A single physician (T.Ş.) conducted each measurement.

Vas

Pain, sensitivity, eating, and difficulty speaking were assessed using a chart with an 11-point VAS over a long horizontal line. When assessing pain, 0 indicates no pain, and 10 indicates severe pain. A number from zero to ten is chosen to indicate the intensity of pain.[18] The scale ranges from 0 to 10.[19]

Statistical Analysis

The data of 34 patients were collected in this study. Statistical analyses were performed with the SPSS version 26 (IBM SPSS Corp., Armonk, NY, USA) statistical program. Basic descriptive statistics are given as the mean and standard deviation for measurement data and as the frequency percentage for categorical data. Independent sample t-tests were used to compare the measurements of the participants according to the STAI score. The chi-square test was used to compare STAI scores according to surgery type. One-way ANOVA was used to compare dental anxiety assessment scores according to surgery type. The statistical significance level was set at $P<.05$.

RESULTS

Demographic Characteristics

The mean age of the 34 participants in the study was 40.06 years, with a standard deviation of 13.99 years. The participants' ages ranged from 19 to 65 years. In terms of sex distribution, 38.2% of the participants identified as male, while 61.8% identified as female. According to their financial status, 5.9% of the participants had an income below the minimum wage, 70.6% had an income at the minimum wage, and 23.5% had an income above the minimum wage. Although 94.1% of the participants were living together, 5.9% were divorced.

Health Status

In total, 23.5% of the participants had systemic diseases. Regarding regular medication use, 38.2% of the participants answered yes. 52.9% of the participants stated that they had previously undergone surgery under local anesthesia. A total of 23.5% of the participants feared dental treatment.

Results of the Participants' Anxiety Assessments

The average total dental anxiety score of the participants was 27.71±12.36 points, ranging from a minimum of 20 to a maximum of 75. According to the State-Trait Anxiety Inventory, the mean STAI-S score was 34.47±7.81 points, with values ranging from 20 to 51. The mean STAI-T score was 36.44±7.28 points, with scores ranging from 21 to 52.

The classification of the participants as having low, moderate, or high scores on the STAI-S, STAI-T, and MDAS is given in Table 1.

Regarding the types of surgeries, no statistically significant difference was observed in terms of MDAS scores ($P>.05$). However, patients who underwent mucogingival surgery-2 were found to have higher levels of dental anxiety (Table 2).

Table 1. STAI-S and STAI-T and MDAS Assessments

		f	%
STAI-S	**Low**	29	85.3
	Moderate	4	11.8
	High	1	2.9
STAI-T	**Low**	27	79.4
	Moderate	5	14.7
	High	2	5.9
MDAS	**Low**	32	94.1
	High	2	5.9

STAI-S: State Anxiety Inventory; STAI-T: Trait Anxiety Inventory; MDAS: Modified Dental Anxiety Scale

Table 2. MDAS of Participants According to Surgery Types

	N	Mean Value	Standard Deviation	*P*
Flap surgery	12	7.33	2.61	.07
Mucogingival surgery-1	14	7.21	3.12	
Mucogingival surgery-2	8	9.00	4.11	
Total	34	7.68	3.20	

MDAS: Modified Dental Anxiety Scale

There was a statistically significant difference in the STAI-S scores of the participants according to the type of surgery ($P<.05$). Although the mucogingival surgery-1 group had a low STAI-S score, only the flap surgery patients group had a high STAI-S score. According to the analysis, there were no statistically significant differences observed in the participants' STAI-T scores across the different types of surgeries ($P>.05$). Compared with the other groups, mucogingival surgery-1 group had a greater proportion of individuals in the low category for the STAI-T assessment, while the mucogingival surgery-2 group had a greater proportion of individuals in the middle category for the STAI-T evaluation (Table 3).

Table 3. Comparison of State-Trait Anxiety Values of Participants According to Surgery Types

			STAI-S			Total	*P*
			low	moderate	high		
Surgery	Flap surgery	n	11	0	1	12	.003*
		%	91.70%	0.00%	8.30%	100.00%	
	Mucogingival surgery-1	n	14	0	0	14	
		%	100.00%	0.00%	0.00%	100.00%	
	Mucogingival surgery-2	n	4	4	0	8	
		%	50.00%	50.00%	0.00%	100.00%	
	Total	n	29	4	1	34	
		%	85.30%	11.80%	2.90%	100.00%	
			STAI-T			Total	*P*
			low	moderate	high		
Surgery	Flap surgery	n	10	2	0	12	.58
		%	83.33%	16.67%	0.00%	100.00%	
	Mucogingival surgery-1	n	12	1	1	14	
		%	85.71%	7.14%	7.14%	100.00%	
	Mucogingival surgery-2	n	5	2	1	8	
		%	62.50%	25.00%	12.50%	100.00%	
	Total	n	27	5	2	34	
		%	79.41%	14.71%	5.88%	100.00%	

STAI-S: State Anxiety Inventory; STAI-T: Trait Anxiety Inventory

There were no statistically significant differences in pain levels at the 3rd hour or on the 1st day, 3rd day, 7th day, or 14th day were observed among the surgical procedure groups (P >.05). However, the pain levels of individuals who underwent mucogingival surgery-1 were greater. Based on the surgical procedures, no statistically significant differences were observed in the participant's sensitivity levels at the 3rd hour; or on the 1st day, 3rd day, 7th day, or 14th day (P >.05). However, the sensitivity levels of individuals who underwent flap surgery at the 3rd hour were greater. On days 1, 3, 7, and 14, the sensitivity levels were greater in the mucogingival surgery-1 group.

Regarding the surgical interventions, no statistically significant differences were observed in the levels of difficulty encountered while eating at the 3rd hour; or on the 1st day, 3rd day, or 14th day (P >.05). However, individuals who underwent flap surgery reported high levels of difficulty eating at 3 hours; however, these levels were greater in individuals who underwent mucogingival-1 surgery on Days 1, 3, and 14. Regarding the type of surgery, a statistically significant difference was detected in the levels of eating difficulty experienced on the 7th day (P<.05). Individuals who underwent mucogingival surgery-1 reported greater difficulty eating, and the difference from the other two groups was significant.

There was no statistically significant difference in the proportion of patients who experienced difficulty speaking at the 3rd hour, 1st day, 3rd day, 7th day, or 14th day after surgery (P >.05). However, individuals who underwent mucogingival surgery-1 had greater difficulty speaking (Figure 1).

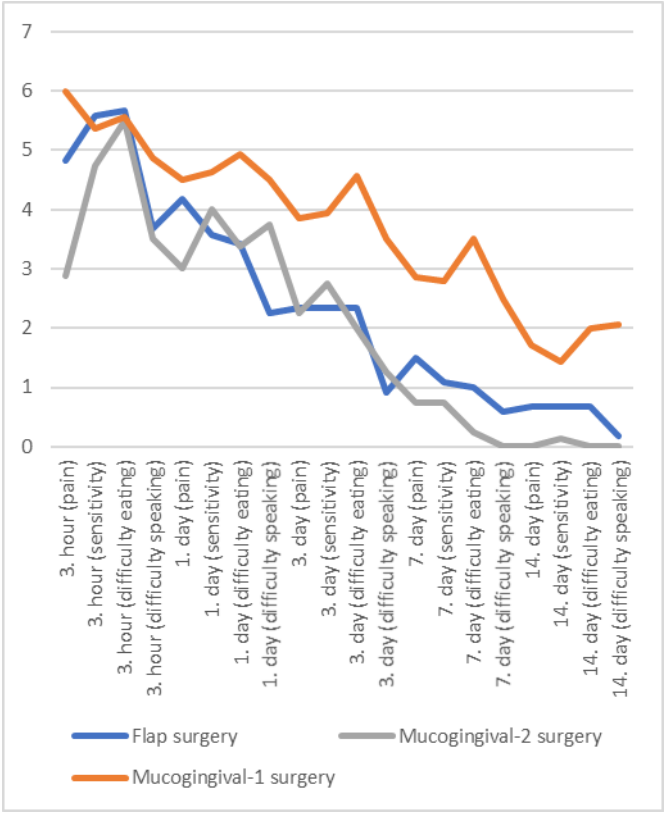

Figure 1. Comparison of Vas values of oral surgeries according to hours and days

DISCUSSION

This study sought to assess the impact of different types of periodontal surgery on patients' anxiety levels and VAS scores. There was a notable association among the State-Trait Anxiety Inventory, STAI-S score, difficulty eating on the seventh day after surgery, and the type of periodontal surgery performed. However, no significant correlation was detected between the STAI-T score and the MDAS score.

Muglali et al.[20] reported that following oral surgery, 30% of patients experienced mild anxiety, 40% experienced moderate anxiety, 14% experienced high anxiety, 11% experienced very high anxiety, and only 5% reported no anxiety. Uslu et al.[21] reported elevated anxiety levels among patients who underwent gingivectomy, frenectomy, mucogingival surgery, and sinus surgery, particularly among younger individuals. The heightened anxiety associated with advanced periodontal procedures such as sinus surgery suggests a need for more comprehensive patient education and communication regarding the surgical process in patients who undergo gingivectomy, frenectomy, mucogingival surgery, or sinus surgery. This finding was explained by the fact that the gingivectomy and frenectomy patients were young. Presurgical anxiety scores exhibited a notable increase in patients who underwent implant surgery compared to those who underwent periodontal surgery.[22] Çanakçı et al.[10] found no statistically significant differences in discomfort levels among patients who underwent modified Widman flap surgery, flap surgery with osseous resection, or gingivectomy during periodontal treatment. However, postoperative pain was notably greater following osseous resection and gingivectomy than following compared to scaling and root planing, as well as following modified Widman flap procedures. Fillingim[23] reported that patient anxiety might be contingent upon individual characteristics. In this study, the STAI-S scores of the flap, mucogingival surgery-1, and mucogingival-2 immediate surgery groups differed, but the greatest was found in the flap surgery.

Dental anxiety is multifaceted and influenced by diverse factors, including age, sex, educational attainment, socioeconomic status, experience.[24] Türer et al.[2] reported findings supporting the conclusion that sociodemographic characteristics have limited effects on anxiety. In support of this finding, no change was observed in anxiety levels in terms of age in studies.[13,25] In contrast, in another study, no significant relationship was observed between pain after periodontal surgery and sex, while a significant decrease in the Vas score was observed with increasing age.[6,10] Bell et al.[3] observed a high level of anxiety in one-eighth of the population, including older adults. It was concluded that age and sex should be taken into consideration when providing information on periodontal surgeries.[21]

Anxiety was correlated with the perception of pain following periodontal flap surgery.[25] Postoperative factors such as swelling, difficulty eating, and pain should be taken into account to alleviate patient anxiety.[20] The study revealed a positive association between pain anticipation and preoperative anxiety, as well as between pain encountered during surgery and postoperative anxiety. Preoperative anxiety was correlated with patients' trait anxiety scores and their past dental encounters. Although the complexity of the procedure did not correlate with immediate postoperative anxiety, it correlated with anxiety during subsequent follow-up visits.[20] Mounssif et al.[12] reported that out of 155 participants, 25.8% reported eating discomfort, and 67.7% reported speech discomfort, with an average VAS score of 2.96±2.39 points (range 0–9). They noted that postoperative

discomfort usually subsided within a week. During the operation, patients who underwent the implant installation had the lowest median VAS score, and there was no significant correlation between the type of surgery and the VAS score.[26] No significant differences were observed in VAS scores regarding discomfort perception between periodontal surgery and implant surgery.[22] Patients typically experience the most pain on the day of surgery, followed by a notable reduction in pain each subsequent day post-surgery.[6] Approximately 80% of the subjects reported experiencing pain on the first day after surgery, while approximately half and one-third of the subjects reported experiencing pain on the second and third days, respectively.[27] A significant difference was observed in the experience of pain and acute anxiety between the first and second days following surgery in trait anxiety research. This situation persisted until the fifth day in the context of trait anxiety.[25] Patients generally reported low levels of discomfort following surgery, according to assessments using the VAS, but patients with high anxiety levels reported increased difficulty.[22] Following periodontal surgery, both pain intensity and quality of life showed immediate reductions, reverting to baseline levels within one month.[28] While most patients experienced only minimal discomfort following surgery, some patients experienced more pain after difficult surgery, periodontal plastic surgery, surgical extension, and anesthetic volume. Seventy percent of the participants experienced minimal or nonexistent discomfort following implant or periodontal treatments.[27] A study involving 200 patients who underwent periodontal surgery revealed that 73.5% of patients experienced pain, with 21.0% reporting severe pain.[29] Mucogingival surgery showed a significant association with pain, 3.5 times more likely to induce pain than osseous surgery and six times more likely to induce pain than plastic soft tissue surgery, with 51.3% of patients reporting minimal or no postoperative pain.[30] Although there was no significant difference, pain levels were greater in the mucogingival surgery-1 group in this study. In addition, eating difficulty was found to be greater in this group.

This study is notable for its rigorous methodology, employing validated tools such as the STAI and the MDAS to accurately assess anxiety and the VAS to evaluate pain. This study fills a critical gap in the literature by examining the relationship between different types of periodontal surgeries and postoperative anxiety and pain, offering a comprehensive analysis of various procedures. This study has several limitations that should be acknowledged. First, the sample size was relatively small, with only 34 participants completing the study, which may limit the generalizability of the findings. Additionally, the study population was restricted to a single university clinic, potentially introducing selection bias. Another limitation is the lack of long-term follow-up, which could provide insights into the sustained impact of anxiety and pain associated with periodontal surgery. To enhance the robustness of future research, it is recommended to include a larger and more diverse sample, conduct multicenter studies to increase generalizability, and consider long-term follow-up to assess the lasting effects of periodontal surgical procedures.

Despite the anxious and painful nature of periodontal surgery, there was no significant difference in pain, sensitivity, or difficulty speaking based on the type of surgery, although a partial change in anxiety levels was noted. Therefore, it is essential to consider factors such as anxiety, pain, sensitivity, difficulty eating, and difficulty speaking during periodontal procedures. To improve patient care, these findings should inform tailored preoperative counseling and effective postoperative pain management strategies, and further research with larger cohorts is necessary to refine these approaches and enhance surgical outcomes.

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Evaluation of Antibacterial and Antibiofilm Effect of Laurel Leaf Essential Oil Against *Prevotella intermedia*: An in vitro Study

Defne Yaprağı Esansiyel Yağının Prevotella intermedia'ya Karşı Antibakteriyel ve Antibiyofilm Etkisinin Değerlendirilmesi: İn vitro Bir Çalışma

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ABSTRACT

Objective: This study aims to evaluate the antimicrobial and antibiofilm activity of laurel leaf oil against *Prevotella intermedia* (*P. intermedia)* DSM20706.

Methods: The components of laurel leaf oil were detected by gas chromatography-mass spectroscopy (GK-KS). The agar diffusion cytotoxicity method was used to detect the cytotoxicity of laurel leaf oil to fibroblast cells, and the minimum inhibitory concentration (MIC) assay was used to determine antibacterial activity. The effect of laurel leaf oil on bacterial morphology was evaluated by scanning electron microscopy (SEM) and fluorescence microscopy. In addition, the effect of laurel leaf oil on gingival fibroblast cell morphology was studied. The effect of laurel leaf oil on biofilm formation was evaluated numerically using a microplate reader.

Results: 1,8-cineole (64.43%), terpinenyl acetate (11.56%) and pinene (5.62%) were found to be the main constituents. No cytotoxic effect was observed in the cell groups treated directly and with 50% concentration of laurel leaf oil and no zone diameter was observed. The MIC value of the laurel leaf oil for *P.intermedia* was determined to be 6.25µl. Moreover, no biofilm formation was observed when laurel leaf oil was applied at a concentration of 3.125µl. The images obtained from SEM showed that the laurel leaf oil applied to the microorganism destroyed the bacteria. In fluorescence microscope, it was observed that the bacteria were fragmented and the particles radiated. When fibroblast cells stained with double staining solution were examined under the fluorescence microscope, it was found that the cells had not died.

Conclusion: As a result, it was determined that laurel leaf oil has antibacterial and antibiofilm effect against *P.intermedia* DSM20706.

Keywords: Antimicrobial activity, in vitro, laurel leaf oil, *P. intermedia*

ÖZ

Amaç: Bu çalışmada defne yaprağı yağının *Prevotella intermedia (P. intermedia)* DSM20706'ya karşı antimikrobiyal ve antibiyofilm aktivitesinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Defne yaprağı yağının bileşenleri gaz kromatografisi-kütle spektroskopisi (GK-KS) ile tespit edildi. Defne yaprağı yağının fibroblast hücrelerine olan sitotoksisitesini tespit etmek için agar difüzyon sitotoksisite yöntemi kullanıldı ve antibakteriyel aktiviteyi belirlemek için minimum inhibitör konsantrasyon (MİK) testi kullanıldı. Defne yaprağı yağının bakteri morfolojisi üzerindeki etkisi taramalı elektron mikroskobu (SEM) ve floresan mikroskobu ile değerlendirildi. Ayrıca defne yaprağı yağının dişeti fibroblast hücre morfolojisi üzerindeki etkisi incelendi. Defne yaprağı yağının biyofilm oluşumu üzerindeki etkisi bir mikro plaka okuyucu kullanılarak sayısal olarak değerlendirildi.

Bulgular : 1,8-sineol (%64,43), terpinenil asetat (%11,56) ve pinen (%5,62) ana bileşenler olarak bulundu. Defne yaprağı yağının doğrudan ve %50 konsantrasyonuyla tedavi edilen hücre gruplarında sitotoksik etki gözlenmedi ve zon çapı gözlenmedi. Defne yaprağı yağının *P.intermedia* için MİK değeri 6,25 µl olarak belirlendi. Ayrıca defne yaprağı yağı 3,125 µl konsantrasyonda uygulandığında biyofilm oluşumu gözlenmedi. SEM'den elde edilen görüntüler, mikroorganizmaya uygulanan defne yaprağı yağının bakterileri yok ettiğini gösterdi. Floresan mikroskobunda bakterilerin parçalandığı ve parçacıkların ışınlandığı görüldü. Çift boyama solüsyonuyla boyanan fibroblast hücreleri floresan mikroskobu altında incelendiğinde hücrelerin ölmediği görüldü.

Sonuç: Sonuç olarak, defne yaprağı yağının *P.intermedia* DSM20706'ya karşı antibakteriyel ve antibiyofilm etkisine sahip olduğu belirlendi.

Anahtar Kelimeler: Antimikrobiyal aktivite, in vitro, defne yaprağı yağı, *P. intermedia*

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INTRODUCTION

The approaches to periodontal treatment mainly include cleaning of the tooth surface and mechanical cleaning in planning the root surface.[1] In the treatment of periodontal disease, local and systemic antimicrobial agents are used in addition to mechanical cleaning.[2] The increasing resistance to antimicrobials used in addition to mechanical therapy, which have some side effects, have led scientists to investigate the antimicrobial and antifungal effects of plants.[3] Plants and their extracts have been used in traditional medicine for centuries.[4] The phenolic compounds of plants are often used in the treatment of infectious diseases caused by bacteria.[5] Essential oils are produced by special cells or groups of cells found in plant parts such as roots, bark, seeds, flowers, leaves, fruits and stems. Essential oils are characterized by two or three major constituents (terpenes and terpenoids, 20-70%) present in very high concentrations, and other minor constituents (phenylpropanoids) present in very high concentrations that determine their important biological properties. Essential oils are known for their anti-inflammatory, antimicrobial and antioxidant properties. Oxygenated terpenoids and some hydrocarbons present in essential oils are responsible for most of their antimicrobial activity. The main mode of action of essential oils is based on disrupting the membrane integrity of gram (+) and gram (-) bacteria.[6-8]

Laurel, a plant of the maquis flora is a perennial, non-shooting, rounded and densely branched plant that grows about 78 inch high. The homeland of laurel, which can be called a characteristic plant of the Mediterranean climate, is Anatolia. About 97% of the world production comes from Turkey. Used are the leaves and fruits of the plant. The leaves are rich in essential oils. Because of its traditional use and commercial value, the chemical composition of its leaves has been studied more than that of any other part. The essential oil of laurel leaves has antimicrobial properties and is used as an antirheumatic, antiseptic, diaphoretic, digestive and diuretic.[9,10] Phytochemical studies conducted in all parts of the laurel plant characterized the presence of the monoterpene 1,8-cineole as the predominant essential oil compound 1,8-cineole usually accounts for 50% to 70% of the parent compound. 1,8-cineole is a saturated monoterpene found in several plant species.[11] It has been shown to be effective in the treatment of respiratory diseases, cancers, digestive system disorders, dysphoria, cardiovascular diseases, and bacilli.[12-16] Various studies have shown that 1,8-cineole has antibacterial activity and inhibits some strains of bacteria.[17,18] The aim of this study is to investigate the antimicrobial and antibiofilm effects of laurel leaf oil against periodontal pathogens *P. intermedia* in an in vitro study.

METHODS

These study analyzes were conducted at Kırıkkale University Scientific and Technological Research Application and Research Center and Hitit University Scientific and Technical Application and Research Center. 20 mL laurel leaf essential oil used in our study was obtained from NATIVITAL Natural Life and Health Products. *P. intermedia*, a Gram-negative anaerobic bacterium, was obtained from DSM 20706 Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures.

Chemicals

Primary gingival fibroblast cells were provided from Kırıkkale University Scientific and Technological Research Application and Research Center. 89% DMEM (Capricorn), 10% the complete culture medium for the cells was fetal bovine serum (FBS; Capricorn), 1% L-glutamine (Capricorn), 1% penicillin/streptomycin antibiotic (Biological Industries).

For removal of cells from culture flasks, 0.05% Trypsin–EDTA solution (Trypsin–Ethylenediamine Tetraacetic Acid, Capricorn) and 1X concentration Calcium/Magnesium-free phosphate buffer (PBS, Biological Industries) were used for the removal of cells from the culture flasks, a 0.05% trypsin-EDTA solution (trypsin-ethylenediamine tetraacetic acid, Capricorn) and a 1X concentration of calcium/ magnesium- free phosphate buffer (PBS, Biological Industries) were used for washing and cleaning procedures. For cell counting (1:1 ratio), trypan blue was used. Tetrazolium salt MTT (serva, Israel) was used to determine cell viability in the cytotoxicity assay. For cell culture studies, serology pipettes, culture dishes and multiple well plates (Corning, USA) were used.

Cell Culture

The frozen cells were rapidly thawed at 37°C and the cells were dissolved in a sterile class II laminar flow cabinet and transferred to a 15 mL Falcon tube. Centrifuged at 250G for 5 minutes. 3 mL of complete culture medium was added to the Falcon and, after homogenisation, 25 cm^2 flasks were cultured. The flasks were incubated at 37°C in an incubator containing 5% CO_2. Freezed cells were passaged until the proliferating cycle was stabilised.

Cytotoxicity Test (MTT)

EN ISO 10993-5, which is used to determine cell viability and the effects of materials on cell viability: This is the reference standard for medical materials. 96-well plates were used to test. Cells were counted and the number of viable cells in each well calculated. Cells were added to 100 µl of complete medium in each well of the 96-well plates and incubated for 24 hours. Cell adherence to the surface of the well plate, morphology and viability were checked at the end of 24 hours. The medium in the wells which were in accordance with the criteria under investigation was drained. Bay leaf oil was added at 50% of the total volume and its lower dilutions (25%, 12.5%, 6.25%, 3.125% and 1.56%). In the negative control group, only complete culture medium was used. The cells were incubated for a period of 24 hours with the materials used. MTT (1 mg/mL) solution was pipetted into the well. The MTT solution was drained from the wells and 100 µL MTT solvent (isopropanol) was added after incubation for 2-2.5 hours at 37°C. The absorbance density values of the 96-well plate were read at 570 nm in a Multiplaka plate reader to determine cell viability. The percentage of cell viability of each group was calculated as in Erdem et al.[19], assuming control cell viability as 100%.

Agar Diffusion Cytotoxicity Test

This assay was designed to demonstrate non-specific cytotoxicity of the test material after diffusion from agar or agarose. This method is preferred in cases where the material has a cytotoxic effect directly on the cell or the chemical marked in cytotoxicity tests such as MTT, XTT WST is not effective. Materials such as essential oils, dental filling materials, tissue scaffolds, wound dressings interact with the cell and sometimes lyse the cell by showing a disintegrating effect. and its toxicity cannot be measured by classical methods, and sometimes it can interfere with the colorimetric chemicals used in the measurement of cystotoxisten and prevent it from radiating. For the test method, the agar diffusion cytotoxicity method specified in TS EN ISO 7405:2018 Section 6.2 was used.[20] In this experimental method, L929 fibroblast cells were used as the cell line. Complete culture medium containing 90% DMEM, 10% FBS and 1% antibiotics is used for the selected cell line. The prepared medium was sterilized by passing through 0.2 µm membrane filters. Two layers of concentrated culture medium were used to prepare the agar. 3-5% agar or 3% agarose is prepared. It was sterilized by autoclave. Vital paint was prepared just before use by diluting 1% neutral red aqueous stock solution with 0.01 mol/L

phosphate buffered saline at a ratio of 1:100. Neutral red solutions were stored protected from light. The experiment was carried out in 6-well culture dishes. The cells were cultured until they reached the end of the logarithmic growth phase (frozen cells were subjected to at least two passages before the experiment). The study was performed according to the "TS EN ISO 7405/Agar Diffusion Test" standard. Cells seeded in 6-well plates were counted as $20x10^4$/well and incubated for 24 hours to achieve 80% confluency. At the end of 24 hours, the medium on the cells was discarded, and 3-5% Agar medium mixed with fresh medium was added to the cells. The agar culture medium mixture was allowed to solidify at room temperature (approximately 30 min). Add 10 mL of neutral reddish solution and keep in the dark for 15-20 minutes. Excess neutral red solution was aspirated. Then, by impregnating the blind membranes with bay leaf oil directly and diluted 50%, the cells were exposed to the agar surface for 24 hours. At the end of the procedure, the wells were washed with 1X PBS and the 6-well plate was closed so that no light could be seen. At the end of incubation, cells were evaluated using an inverted microscope. Tables specified in the standard were used for evaluation (ISO 7405:2018).

Minimum Inhibition Concentration (MIC) Test

Gram negative bacteria *P. intermedia* DSM 20706 was used in the study. Cultures stocked for the determination of antibacterial activity are augmented by incubation at 37°C for 18-24 hours in Muller Hinton Agar (MHA) and Brain Heart Infusion Agar (BHIA). After 24 hours of incubation, it was adjusted to 0.5 McFarland value (10^8 CFU/mL) (absorbance values range from 0.08 to 0.13). Following these steps, 100 µl of Muller Hinton broth (10.5 mg Muller Hinton was added into 500 ml of distilled water and prepared by sterilizing in an autoclave) medium was added to sterile, U-bottomed, 96 well-plates to be used in the microdilution method. The prepared bay leaf oil was added to the wells at the determined concentrations (50 µl, 25 µl, 20 µl, 12.5 µl, 10 µl, 6.25 µl, 3.125 µl and 1.625 µl concentrations in the well) with a total volume of 100 µl in each well. Then, the microorganisms adjusted at 0.5 McFarland value were diluted 1/100 with physiological saline and 10 µl was added (the added microorganism concentration corresponds to 105). Sterility control was made by adding only MHA medium to the 6 wells of the plate used, and growth control was performed by applying MHA medium containing microorganisms to 6 wells. The prepared test system was left to incubate at 37°C for 24 hours. After incubation, the first well without growth was determined as the MIC value. For this process, growth control was performed by measuring the absorbance in both visual growth control and spectrophotometer. In addition, the sample taken from the first well in which no growth was observed and the MIC value was determined, was planted in sheep blood agar and left to incubate for 24 and 48 hours. Reproduction control test was done. Since all microorganisms used were anaerobic, all incubation processes were carried out in an oxygen-free environment, in a jar.

Examining the morphology of bacteria

P. intermedia DSM 20706 was incubated with appropriate concentrations of bay leaf oil, the concentration of which was determined by microorganism MIC and cytotoxicity test, for 24 hours and without any application substance. It was fixed in 2.5% glutaraldehyde overnight at 4°C to allow fixation prior to SEM imaging. Gluteraldehyde was removed from the medium and washed twice with PBS. Samples coated with a gold sputter coating were observed with a scanning electron microscope (Quanta FEG 250, Kastamonu University). In addition, fluorescent marking was performed to view the parts of the bacteria and to evaluate the membrane integrity, if any, and both the activation of the biofilm layer and the morphology of the bacteria was examined. PI (Propidium Iodide) dye and hoechst 33342 dye will be used for this process.

Determination of Cell Morphologies

PI was used to stain the nucleus and hoechst 33342 dye was used to stain the viable cell membrane in order to check the membrane integrity of the cells to which bay leaf oil has been applied. 6-well plates was used for this process. The gingival fibroblast cells, which were also used in the cytotoxicity test, were seeded on the plates at a rate of 500,000 per well. Bay leaf oil prepared in different concentrations interacted with the cells for 24 hours. After the end of the application, the cells were evaluated in DAPI and FITC filters, and both their membrane structures, cell morphologies and the cause of apoptosis/necrosis deaths was evaluated.

RESULTS

Chemical Components of Laurel Leaf Oil

The components of laurel leaf oil added to the device GK-KS under suitable conditions were matched with the library research and their percentage values are shown in Table 1. According to the obtained result, 1,8-cineole (64.43%), alpha-terpinenyl acetate (11.56%) and alpha-pinene (5.62%) were defined as the main components.

Table 1. Percentage values of the components in the bay leaf oil sample

Component name	Area %
à-Thujene	0.29
α-Pinene	5.62
Camphene	0.80
Sabinene	4.91
2-β-Pinene	2.25
β-Myrcene	0.37
α-Terpinen	0.34
1,8-cineole (Eucalyptol)	64.43
cis-Ocimene	0.15
γ-Terpinen	0.41
CIS-Sabınene Hydrate	0.24
L-Lınalool	1.71
1-Terpıneol	0.13
Isopinocampheol	0.27
Pinocarvone	0.17
α-Terpineol	0.40
4-Terpineol	2.27
β-Phenylethanol	0.97
Myrtenal	0.27
Bornyl acetate	0.76
Ocimenyl acetate	0.58
α-Terpınenyl Acetate	11.56
β-Elemene	0.28
trans-Caryophyllene	0.63
Caryophyllene oxide	0.20

Cytotoxicity Analysis

The data on lysis and decolorization indices obtained after direct and 50% application of laurel leaf oil are shown in Table 2. No cytotoxic effect was observed in the treated cell groups and no zone diameter was observed around the disk (Figure 1). In addition, the morphology and the edges of the cells are smooth, indicating that there is no cytotoxic effect.

Table 2. Lysis and decolorization indices after bay leaf oil application

	Lysis index	Decolorization index
Bay Leaf Oil Directly	0	0
Bay Leaf Oil 50% Concentration	0	0
Negative Control	0	0

MIC Assay

Growth was observed in wells treated with a concentration of 3.156 µl (containing 3.156 laurel leaf oil). No growth was observed at the concentrations of 6.25 µl, 12.5 µl, 25 µl, and 50 µl of the laurel leaf

oil sample used (Figure 2). The experiment was performed in 3 separate plates and in 3 replicates for each plate, and no difference was observed in the results.

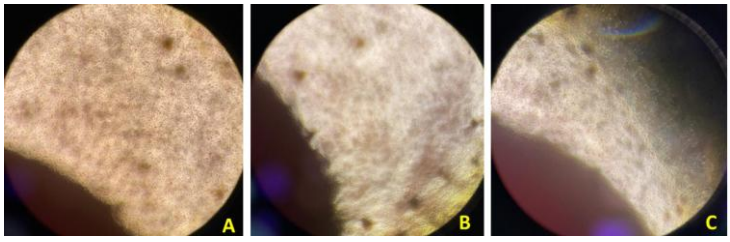

Figure 1. The image of the cells applied after the agar diffusion test in an inverted microscope
A: Images of cells with full concentration of laurel leaf oil
B: Images of negative control cells
C: Images of cells applied 50% concentration of laurel leaf oil
(Images were taken at 10X magnification)

The Effect of Laurel Leaf Oil on *P. intermedia* Biofilm Formation

After the decolorization process the absorbance was measured at 590 nm and evaluated numerically. While moderate biofilm formation was observed in the wells where laurel leaf oil was applied at a concentration of 1.562 µl, no biofilm formation was observed at a concentration of 3.125 µl. Low biofilm formation was observed when a medium concentration of 2 µl was applied. Severe biofilm formation was observed in the group of microorganisms that were not subjected to any treatment (Figure 3).

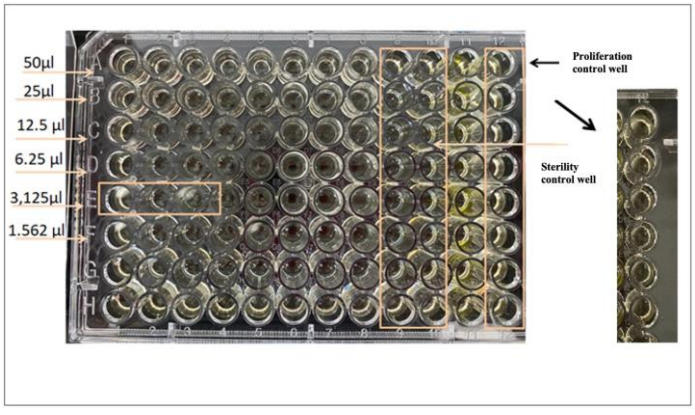

Figure 2. MIC plate image after application of leaf essential oil to *P. intermedia*
Sterility control well, growth control wells and applied groups are shown on the picture

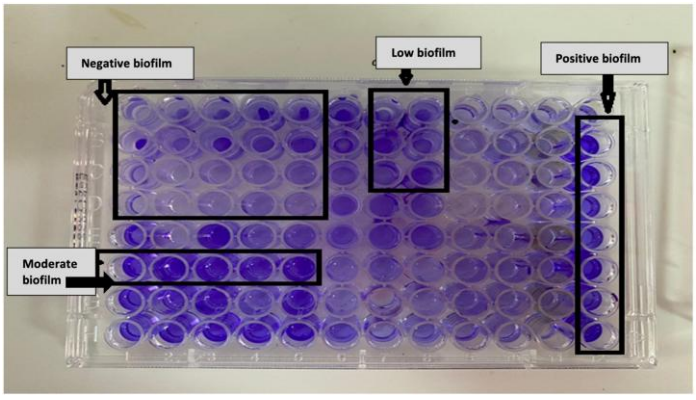

Figure 3. Image of biofilm staining plate after application

Effects of Laurel Leaf Oil on the Morphology of *P. intermedia*

Images from SEM show that laurel leaf oil applied to the microorganisms destroys the bacteria. Complete morphology of the microorganism was not observed in the group where the full concentration was applied. The parts of the bacteria are shown in Figure 4. It was found that the bacteria in the groups where 50 µl and 25µl of laurel leaf oil were applied lost their membrane structure and started to decompose. As a result of the fluorescence microscope examination, no biofilm layer was observed in the treated groups, but it was found that a biofilm layer formed, bacteria radiated and vitality was present in the untreated group. It was observed that the bacteria were fragmented and the particles radiated in the FITC filter, while the absence of radiation in the DAPI filter indicated that the microorganisms were dead (Figure 5).

Effects of Laurel Leaf Oil on Fibroblast Cell Morphology

When the gingival fibroblast cells were examined under the fluorescence microscope, it was found that the cells did not die and that the minimal concentration of laurel leaf oil that could be used did not damage the gingival fibroblast cells (Figure 6).

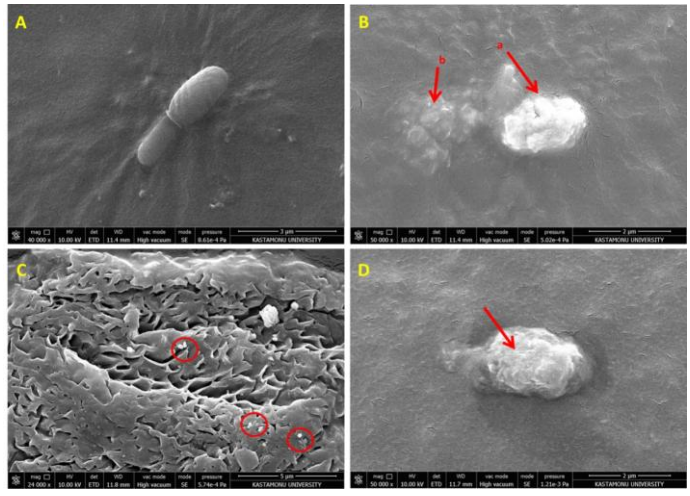

Figure 4. SEM image of *P.intermedia* after laurel leaf oil application
A: *P.intermedia* image without application
B: *P.intermedia* image with 50 µl laurel leaf oil applied, arrow sign (a) shows *P.intermedia* with impaired membrane structure arrow pointing (b) indicates fragmented *P.intermedia* residues
C: *P.intermedia* image applied full concentration laurel leaf oil, marked areas show *P.intermedia* residues
D: *P.intermedia* image applied 25 µl laurel leaf oil, arrow sign indicates microorganism shows its deteriorated outer surface

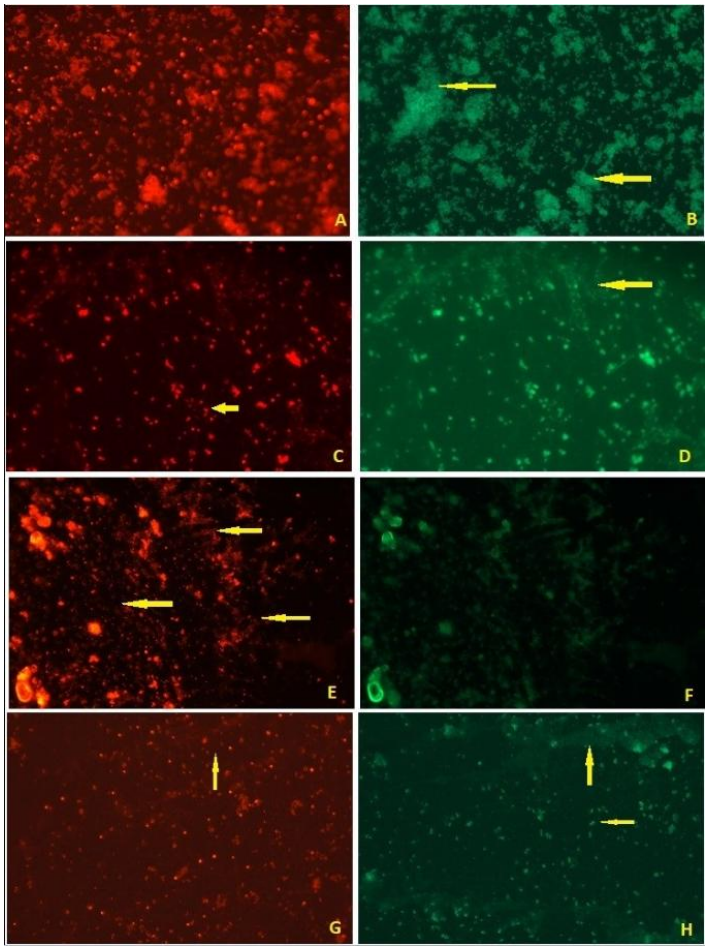

Figure 5. Imaging of bacteria under fluorescence microscopy after double staining
A: control group in FITC filter
B: Control group in DAPI filter, arrow indicates biofilm layer
C: 12.5 µl of laurel leaf oil applied microorganism image on FITC filter, arrow indicates chromosome fragments
D: The image of the microorganisms applied 12.5 µl of laurel leaf oil on the DAPI filter, the arrow indicates the inactive biofilm layer
E: The images of microorganisms applied 50 µl of laurel leaf oil in FITC filter, arrows indicate chromosome fragments
F: The images of microorganisms in the DAPI filter, in which 50 µl of laurel leaf oil was applied, no microorganism with membrane integrity could be detected
G: Microorganisms applied 6.25 µl of laurel leaf oil appear on FITC filter, arrows indicate chromosome fragments
H: The images of the microorganisms applied 6.25 µl of laurel leaf oil in the DAPI filter, the biofilm layer was shown to be inactive, rare bacterial growths

Figure 6 Imaging of gingival fibroblast cells under fluorescent microscope after double staining
A: 6.25 µl of laurel leaf oil applied fibroblast cells image on FITC filter
B: 6.25 µl of laurel leaf oil applied fibroblast cells image on DAPI filter
C: Untreated gingival fibroblast cells

DISCUSSION

The treatment of periodontal disease remains challenging due to its multifactorial etiology and complex disease course. Mechanical and surgical approaches to periodontal disease have been combined with antimicrobial therapy for many years. However, success rates are not at the desired level due to difficulties in application and undesirable side effects. The high cost also has a negative impact on access to treatment. Herbal products are preferred over conventional drugs due to their broad biological efficacy, high safety, and low cost and are used for both prevention and treatment of periodontal disease.

Laurel is one of the plants that have long been studied in medicine. Studies have shown that laurel oil has antimicrobial activity against various microorganisms.[21-23] In reviewing the literature, the effect of laurel leaf oil on no periodontopathogen was evaluated. Therefore, in this study, the antimicrobial and antibiofilm effects of laurel leaf oil against *P. intermedia* were investigated in vitro. In studies conducted in different years, it was found that the proportion of 1,8-cineole in the essential oil of laurel is higher than the other components.[21-23] In accordance with the results of the studies in the literature, it was found in our study that the proportion of 1,8-cineole (64.43%) is significantly higher than the other components in the essential oil of laurel leaf. 1,8-cineole is a saturated monoterpene found in various plant species and its main source is eucalyptus leaves.

As an active ingredient, 1,8-cineole has been shown to play an important role in the treatment of respiratory diseases, cancers, digestive system disorders, dysphoria, cardiovascular diseases, and bacilli[11]. It is suggested that the antibacterial effect of laurel leaf essential oil used in our study is mainly due to 1,8-cineole. It is believed that 1,8-cineole, the main component of some essential oils, acts on plasma membranes, which are the target of chlorhexidine. Şimşek & Duman[24] investigated the antimicrobial properties of 1,8-cineole alone or in combination with chlorhexidine in their study. They concluded that chlorhexidine in combination with 1,8-cineole showed synergistic antimicrobial activity in some microorganisms tested. The synergistic activity between chlorhexidine and 1,8-cineole resulted in the elimination of some microcolonies that had developed resistance to chlorhexidine. The lowest concentration tried at various concentrations at which the antimicrobial component completely stops microbial growth is defined as the MIC. MIC is considered the gold standard for determining the susceptibility of microorganisms to antimicrobial agents. Park et al.[25] evaluated the antibacterial activity of ginger extract against *P. gingivalis, P. endodontalis,* and *P. intermedia*, which are Gram-negative anaerobic bacteria that cause periodontal disease, in vitro. They showed that the growth of these oral pathogens was effectively inhibited by ginger compounds 6-30 in the range of µg/mL MIC. Izui et al.[26] investigated the antibacterial effect of curcumin extracted from turmeric root on *F. nucleatum, P. gingivalis, T. denticola,* and *P. intermedia* and found that curcumin inhibited the growth of these bacteria in a dose-dependent manner. MIC values were determined to be 10 µg/mL, 15 µg/mL, 10 µg/mL, and 5 µg/mL for *F. nucleatum, P. gingivalis, P. intermedia,* and *T. denticola*, respectively. Ramak and Talei[27] evaluated the antibacterial and antibiofilm effects of Deliçay plant (Stachys koelzii) essential oil against *P. intermedia* in vitro. Thus, the essential oil extracted from this plant was shown to have acceptable antibacterial and antibiofilm activity against *P. intermedia* at low levels (0.1 and 0.2 mg/ml MIC). In our study, no bacterial growth was observed at the concentrations of 6.25

µl, 12.5 µl, 25 µl and 50 µl of laurel leaf essential oil against *P. intermedia* DSM 20706. Therefore, the MIC of laurel leaf oil used in our study was set at 6.25 µl. In our study, the cytotoxic effect of laurel leaf oil was evaluated using the agar diffusion cytotoxicity method. This method is preferred in dental studies when the material has a direct cytotoxic effect on the cell. Essential oils lyse the cell by exhibiting a disintegrating effect, making their toxicity unmeasurable by classical methods.

When the effect of laurel oil on bacterial morphology was studied, the microorganism fragments obtained in the SEM images supported each other with fluorescence microscopy. In the SEM images, it was found that the bacterial morphology was deteriorated, the capsule structure had changed and fragments were present. Staining of the nuclear fragments under fluorescence microscope showed that the bacterial membrane structure was ruptured and the genetic material was scattered. Thus, it was confirmed that the laurel leaf oil used kills the bacteria by dissolving them. The results obtained with the MIC test were confirmed by SEM and the fluorescence microscopy.

When evaluating the effect of laurel leaf oil on gingival fibroblast cells, it was found that cell morphology did not deteriorate and membrane integrity was maintained. In addition, it was observed that the cells did not show necrotic death in the staining method, indicating that there was no inflammatory potential. The fact that laurel leaf oil was not compared with chlorhexidine (control group), which is considered the gold standard can be considered a limitation of the study. As a result, in this study, the antibacterial effect of laurel leaf oil against periodontal pathogen *P. intermedia* DSM 20706 was investigated and found to have antibacterial activity. In vivo and clinical studies are needed to better understand the antibacterial activity of laurel leaf oil. The antibacterial effect of this oil on various periodontal pathogens can also be evaluated. Moreover, this vegetable oil is one of the active ingredients that can be used as an agent for topical application such as toothpaste, mouthwash, gel and in addition. For this reason, this topic can be supported by various scientific studies.

Ethics Committee Approval: Since there was no human/animal substrate in this study, no ethical approval was obtained.
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The Relationship Between COVID-19 And Periodontitis Severity: A Retrospective Cohort Study

COVID-19 ile Periodontitis Şiddeti Arasındaki İlişki: Retrospektif Kohort Çalışması

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ABSTRACT

Objective: Coronavirus disease-2019 (COVID-19) is a severe acute respiratory infection. The severity of symptoms and mortality rate vary according to age, gender, and other existing systemic diseases. Periodontitis is a chronic inflammation, and it has relations to other systemic diseases. The aim of this study is to examine the relationship between periodontal disease and COVID-19 severity by retrospectively evaluating patient records and periodontal status.

Methods: 344 patients were included in the study. The severity of periodontal disease was determined as healthy, mild, moderate and severe according to National Health and Nutrition Examination Survey (NHANES) criteria. The COVID-19 severity was grouped as asymptomatic/mild, moderate and severe according to whether the patients required hospitalization and intensive care. Kruskal-Wallis test was used for independent samples between gender-age-periodontal status and gender-age- COVID-19 relationships.

Results: The mean age of all patients was 36±13; the mean age of those with systemic disease was 52±15, and the mean age of those without it was 32±9. The mean age of those with systemic disease was greater than that of those without it (*P*<.001). The relationship between systemic disease and periodontal status was corrected for age, and since no statistical significance was observed (*P*>.05) There was statistically significant relationship was observed between COVİD-19 severity and periodontal status (*P*=.029).

Conclusion: Periodontitis and the severity of COVID-19 increases, regardless of systemic diseases and age.

Key words: COVID-19, Periodontal Diseases, SARS-CoV-2

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) ciddi bir akut solunum yolu enfeksiyonudur. Semptomların şiddeti ve ölüm oranı yaşa, cinsiyete ve diğer mevcut sistemik hastalıklara göre değişir. Periodontitis kronik bir inflamasyondur ve diğer sistemik hastalıklarla ilişkileri vardır. Bu çalışmanın amacı, COVİD-19 hasta kayıtlarını ve periodontal durumu retrospektif olarak değerlendirerek periodontal hastalık ile COVID-19 şiddeti arasındaki ilişkiyi incelemektir.

Yöntemler: Çalışmaya 344 hasta dahil edildi. Periodontal hastalığın şiddeti Ulusal Sağlık ve Beslenme İnceleme Anketi (NHANES) kriterlerine göre sağlıklı, hafif, orta ve şiddetli olarak belirlendi. COVID-19 şiddeti, hastaların hastaneye yatırılması ve yoğun bakıma ihtiyaç duyup duymamasına göre asemptomatik/hafif, orta ve şiddetli olarak gruplandırıldı. Cinsiyet-yaş-periodontal durum ve cinsiyet-yaş-COVID-19 ilişkileri arasındaki bağımsız örnekler için Kruskal-Wallis testi kullanıldı.

Bulgular : Tüm hastaların yaş ortalaması 36±13; sistemik hastalığı olanların yaş ortalaması 52±15, olmayanların yaş ortalaması 32±9 idi. Sistemik hastalığı olanların yaş ortalaması, olmayanlardan daha büyüktü (*P*<,001). Sistemik hastalık ile periodontal durum arasındaki ilişki yaşa göre düzeltildi ve istatistiksel olarak anlamlı bir ilişki gözlenmediğinden (*P*>,05) COVID-19 şiddeti ile periodontal durum arasında istatistiksel olarak anlamlı bir ilişki gözlendi (*P*=,029).

Sonuç: Periodontitis ve COVID-19 şiddeti, sistemik hastalıklardan ve yaştan bağımsız olarak artmaktadır.

Anahtar kelimeler: COVID-19, Periodontal

INTRODUCTION

Coronavirus disease-2019 (COVID-19) is a severe acute respiratory infection caused by respiratory syndrome coronavirus 2 (SARS-CoV-2) that emerged in Wuhan Province, China, in late 2019.[1] In COVID-19, contamination occurs through respiratory aerosols or body fluids. Symptoms such as fever, nasal congestion, sore throat, joint pain, headache, abdominal pain, skin rashes, diarrhea, cough and shortness

of breath are observed.[2] Although the severity of symptoms and mortality rate vary according to age, gender and other existing systemic diseases (diabetes, obesity, cardiovascular diseases, cancer, etc.), but other unidentified risk factors may also affect the severity of COVID-19.[3]

Periodontitis is a condition that affects both periodontal tissues and causes systemic inflammation; and it is caused due to the unbalance between the host immune system and oral bacteria.[4,5] Periodontitis is thought to affect 45% to 50% of adults worldwide.[6] Therefore, associations between periodontitis and other chronic non-communicable diseases such as diabetes, cardiovascular diseases, adverse pregnancy outcomes and Alzheimer's disease have been widely investigated.[4]

T helper 17 cells (Th17) are effective adaptive immune response cells showing functions by releasing IL-17 (IL-17A), IL-17F, IL-21, IL-22 and TNF-α cytokines. High Th17-mediated responses have been observed in SARS-CoV and MERS-CoV patients.[7] Similarly Th17 cell population has been increased in patients with periodontitis compared to healthy individuals; and higher serum IL-17 levels have been observed in individuals with periodontitis.[8] In addition, higher levels of IL-2, IL-7, IL-10, G-CSF, MCP1, MIP1A and TNFα have been observed in patients requiring intensive care due to COVID-19.[7,9] These cytokines which are closely associated with periodontal disease might serve as a link between periodontal disease and COVID-19.

All this information suggests that there may be a relationship between COVID-19 and periodontal diseases and the severity of periodontal disease may be a determinant for the severity of COVID-19. The aim of this study is to examine the relationship between COVID-19 severity and periodontal disease retrospectively by evaluating the patient records and periodontal status.

METHODS

Study Design

The study was approved by Ataturk University School of Medicine's Ethics Committee and carried out in strict compliance with ethical guidelines of World Medical Association's Declaration of Helsinki (B.30.2.ATA.0.01.00/485; 25.11.2021). Prior to periodontal examination, each participant was informed about study methodology and written informed consent was obtained. Participants were enrolled among the patients applied to Ataturk University Department of Periodontology, between November 2021 and March 2022. Three hundred forty-four patients between the ages of 18-85, who were both diagnosed with periodontal disease and COVID-19 were included in the study. Systemic diseases such as diabetes, cardiovascular diseases, hypertension, asthma, chronic obstructive pulmonary disease (COPD) and epilepsy were recorded for all participants. Patients who did not have COVID-19, were pregnant, smoked, had any drug addiction, and did not agree to participate in the study were excluded from the study (Figure 1).

Determining COVID-19 Severity

The diagnosis of COVID-19 was determined by real time-polymerase chain reaction (Rt-PCR) test and the severity of the patients was questioned by taking verbal anamnesis and whether they required hospitalization and intensive care unit, which was further confirmed by comparing with the patients' health records. COVID-19 severity was grouped as follows:[10]

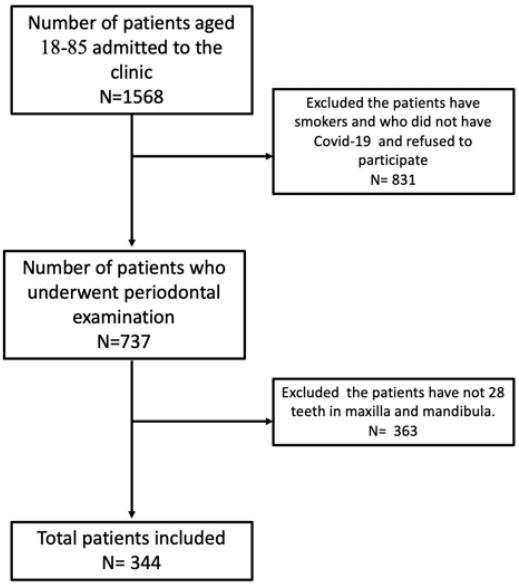

Figure 1. Flowchart of study sample. The patients were aged 18-85. Periodontal measurements were carried out according to NHANES III.

Asymptomatic/Mild group: Patients who had a positive Rt-PCR result but did not show symptoms of COVID-19, and patients who had symptoms such as fever, sore throat, muscle pain, joint pain and cough, but did not have respiratory distress, and whose chest radiography and/or lung tomography were normal.

Moderate group: Patients with symptoms such as fever, sore throat, muscle pain, joint pain and cough, respiratory rate <30 per minute, Oxygen saturation (SpO2) >90% under normal conditions, and patients showing signs of mild-moderate pneumonia on chest radiography or tomography.

Severe group: Patients with symptoms such as fever, sore throat, muscle pain, joint pain and cough respiratory rate ≥30 per minute, SpO2 ≤90% in room air, and findings of bilateral widespread pneumonia on chest radiography or tomography.

Periodontal Examination

Periodontal measurements were made according to NHANES III criteria.[11] One maxillary and one mandibular quadrant were included in each patient. Periodontal measurements were performed only on fully erupted teeth, and third molars were excluded. All measurements were made by a single clinician (YÖK). A maximum of 14 teeth were examined in each patient, including 7 teeth in the maxilla and 7 in the mandible. Patients were categorized according to their periodontal status:

1- Healthy patients (Gingivitis + healthy): Patients with no clinical attachment level (CAL) and probing depth (PD).

2-Mild periodontitis group: CAL between 3 and 4 mm in 2 or more interproximal areas, not on the same tooth; patients with 4 mm or more PD in 2 or more interproximal areas, not on the same tooth, or in a single area.

3- Moderate periodontitis group: CAL between 4 and 6 mm in 2 or more interproximal areas, not on the same tooth; patients with 5 mm or more PD in 2 or more interproximal areas, but not in the same tooth.

4- Severe periodontitis group: 6 mm or more CAL in 2 or more interproximal areas, not on the same tooth; patients with 5 mm or more PD in 2 or more interproximal areas, but not in the same tooth

Statistical Analysis

Based on the previous study results, the G* Power 3.1 Software Package's one-way ANOVA test was used to calculate the sample size.[12] Using an effect size of f = 0.980, it was determined that, with 80% power, each group should contain a minimum of 340 individuals, with a 95% confidence interval taking study Statistical analysis was performed using IBM SPSS Statistics v 20 (IBM SPSS Corp., Armonk, NY, USA). Kruskal-Wallis test was used for independent samples between age-periodontal status and age-COVID-19 relationships. All data are presented as mean, standard deviation, median, minimum-maximum, number and percentage. Statistical significance was determined as *P*<.05.

RESULTS

A total of 344 patients (204 men (59.3%) and 140 (40.7%) women) were evaluated. Of those who did not have a systemic disease, 166 (60.4%) were men and 109 (39.6%) were women. Of those with systemic disease, 38 (55.1%) were men and 31 (44.9%) were women. The average age of all patients was 36±13; the average age of those without systemic disease was 32±9, those with systemic disease was 52±15. Patients with systemic disease had a higher mean age than patients without systemic disease (*P* <.001) (Table 1). While 275 (79.9%) of the patients were systemically healthy, 69 (20.1%) had at least one systemic disease. Sixty-five (18.9%) of the participants were periodontally healthy (healthy and gingivitis); 103 (29.9%) had mild, 97 (28.2%) moderate, and 79 (23%) severe periodontitis. 290 (84%) of the patients had mild COVID-19, 48 (14%) had moderate and 6 (2%) had severe COVID-19 (Table 2)

Table 1. Age and Systemic health status

	Systemic disease -				Systemic disease +					
	Mean	Standard Deviation	Median	Min-Max	Mean	Standard Deviation	Median	Min-Max	Z	*P*
Age	32	9	31	18-66	52	15	55	22-81	-9.298	***<.001***

** Statistical significance was determined P <.05*
Systemic Disease - = Systematically healthy patients
Systemic Disease + = Patients having systemic disease

Table 2. Systemic disease, COVID-19 severity and periodontal status

		Systemic Disease		
		Systemic Disease – (%)	Systemic Disease +(%)	*P*
COVID-19 Severity	Asymptomatic/Mild group	258 (93.8%)	32 (46.4%)	**<.001**
	Moderate group	15 (5.5%)	33 (47.8%)	
	Severe group	2 (0.7%)	4 (5.8%)	
Periodontal Status	Healhty	62 (22.5%)	3 (4.3%)	**.262**
	Mild Periodontitis	94 (34.2%)	9 (13.0%)	
	Moderate Periodontitis	79 (28.7%)	18 (26.1%)	
	Severe Periodontitis	40 (14.5%)	39 (56.5%)	

** Statistical significance was determined P <.05*
Systemic Disease - = Systematically healthy patients
Systemic Disease + = Patients having systemic disease

The association between periodontal state and COVID-19 severity was determined independent of age, gender, and systemic disease by statistically correcting these factors. According to the results of this evaluation, a statistically significant relationship was observed between COVID-19 severity and periodontal status (*P* .=029) (Table 3).

The probability of individuals with periodontitis having severe COVID-19 was found to be 1.24 times higher than those without periodontitis (Odd ratio (OR): 1.24; 95% Confidence Interval (CI); (1.17-1.31).

Table 3. Covid-19 Severity and Periodontitis Status

		Periodontal Status					*kappa*	
		Healhty	Mild Periodontitis	Moderate Periodontitis	Severe Periodontitis	*Chi-Square*	*value*	*P*
COVID-19 Severity	Asymptomatic/Mild group	65	97	84	44	*<0,001*	*0.046*	**.029**
	Moderate group	0	5	12	31			
	Severe group	0	1	1	4			

** Statistical significance was determined P <.05*

DISCUSSION

In this study, we aimed to retrospectively evaluate the relationship between COVID-19 and periodontal disease severity retrospectively, independent of systemic diseases, gender and age. Our results showed that there was a relationship between COVID-19 and periodontal disease severity, and that COVID-19 severity and periodontal disease severity increased in parallel.

Periodontitis has general inflammatory risk factors which effect the severity of COVID-19.[13,14] The relationship between periodontal disease and COVID-19 severity has been explained through various mechanisms including the induction of angiotensin converting enzyme-2 (ACE2) overexpression by bronchial cells and alveolar epithelial cells, increase in IL-6 and IL-8 cytokine levels through aspiration of periodontal pathogens.[15–17] Additionally, increase in the inflammatory release capacity of Th17 cells, one of the central cells in the formation of periodontal disease, during COVID-19 which results in cytokine storm development and enhanced inflammatory environment has been reported.[18,19]

A previous study found that periodontitis was associated with serious complications caused by COVID-19, including intensive care unit admission, necessity of a ventilator, and death in more severe conditions.[1] Also according to the findings, patients with severe periodontitis had a higher likelihood of developing COVID-19-related issues compared to those with milder cases of the disease.[1] Another study suggests that there is a direct relationship between periodontitis and COVID-19-related outcomes.[20] In a study, periodontitis that the patient indicates, has been argued that the disease is a helpful marker to determine the risk of SARS-CoV-2 and claimed that these people show more symptoms at the beginning of COVID-19 disease.[21] In our study, we found that the severity of COVID-19 was increased in people with severe periodontitis, following up the previous studies.

Aging is one of the important factors that increases the severity of both COVID-19 and periodontal disease.[1,22,23] A study showed that older age may be associated with an increase in comorbidity of COVID-19.[24] Also, the relationship between the severity of COVID-19 and systemic diseases has been addressed in previous studies.[25,26] A meta-analysis found that the patients with pre-existing chronic diseases have a higher risk of severe COVID-19 disease.[27] Another meta-analyses found that SARS-CoV-2 infected patients with a chronic illness have a more severe risk of covid disease and higher mortality rate.[28] In our study, COVID-19 severity was determined regardless of any systemic disease or age. Our results showed that there was a parallelism between periodontal disease and COVID-19 severity, independent of these two cofounding factors.

The Covid-19 virus is more common in men. According to data collected at the start of the pandemic, men contributed about 60% of

all COVID-19 patients,.[29,30] 59 studies including 36,470 patients men had a higher risk than women in having infection, disease severity, intensive care unit (ICU) admission, and death. An additional investigation revealed that 1,875 (38%) of the 4,880 patients with respiratory symptoms or who had been in close contact with COVID-19 patients tested positive for SARS-CoV-2 infection; among those who tested positive, 40.43% were men. In line with these investigations, we also found that men had a higher rate of COVID-19.[31] However, we did not include gender in our statistical analysis of the association between COVID-19 severity and periodontal disease.

Smoking is considered as a risk factor for both COVID-19 and periodontitis.[32,33] We included non-smoking participants in our study. However, a previous study including smokers concluded that smoking does not affect the relationship between periodontal disease and COVID-19.[12] This may be related to the insufficient number of smoker participants in the study, and due to the increasing rate, which may misinterpret the relationship between these two diseases. To overcome this shortcoming, participants who smoke were not included in our study.

Severity of periodontitis was categorized according to NHANES III; and patients were grouped as severe, moderate, mild periodontitis and healthy. In our study, we aimed to compare individuals' periodontitis severity and its correlation with COVID-19 severity within appropriate criteria. In epidemiological studies with high participation conducted by a single physician, as in this study, the NHANES method provides advantages in terms of ease of application, shortening of examination time and precise application of measurements.[34,35]

This study has some limitations. One of these is that cytokine levels were not measured and thus the relationship between COVID-19 and periodontal disease is not addressed biochemically. Another is that periodontal disease evaluation could not be performed during the time of COVID-19 due to the safety requirements.

In conclusion, our study showed that as the severity of periodontitis increases, the severity of COVID-19 increases, regardless of systemic diseases and age in this study. However, further clinical studies are needed to better explain the relationship between these two conditions.

Ethics Committee Approval: The study was approved by Ataturk University School of Medicine's Ethics Committee and carried out in strict compliance with ethical guidelines of World Medical Association's Declaration of Helsinki (B.30.2.ATA.0.01.00/485; 25.11.2021).
Informed Consent: Prior to periodontal examination, each participant was informed about study methodology and written informed consent was obtained.
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Hasta Onamı: Periodontal muayene öncesinde, her katılımcıya çalışma metodolojisi hakkında bilgi verilmiş ve yazılı onam alınmıştır.
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Association between Genetic Polymorphisms in Enamel Formation Genes and Environmental Factors with Caries Experience

Mine Oluşum Genlerindeki Genetik Polimorfizmler ve Çevresel Faktörler ile Çürük Deneyimi Arasındaki İlişki

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ABSTRACT

Objective: The etiology of dental caries involves both environmental and host-related factors. This study aims to evaluate the effect of enamel formation genes (AMELX, ENAM, and AMBN), environmental factors, and their combined influence on caries experience in adults..

Methods: A Case-Control Study was conducted including 114 adults aged 20-65 years. Data regarding oral hygiene habits were obtained through patient declaration. Data regarding caries, plaque and saliva were obtained through clinical examination. Genomic DNA was isolated from saliva. Patients were divided into two groups according to caries experience. Environmental factors (age; amount of plaque; tooth brushing duration; consumption of sugary drinks and foods; use of fluoride toothpaste, fluoride mouthwash and dental floss; Streptococcus mutans and Lactobacillus counts) and host factors (saliva flow rat, pH, buffering capacity and AMELX, ENAM and AMBN) were examined. Real-time PCR was used to evaluate genotype distributions. Data were analyzed using chi-square test and logistic regression with SPSS.

Results: No statistically significant differences were observed in AMELX, ENAM or AMBN Allele and genotype frequencies between groups in selected subjects in Turkey. There was no significant association between the groups with regard to salivary flow rate or consumption of sugary drinks and food. All other factors were found to be associated with high caries experience.

Conclusion: When environmental factors and genotypes were evaluated together using multivariate regression analysis, It was determined that increased age, high plaque levels, and the absence of fluoride mouthwash use increased the likelihood of high caries experience.

Keywords: AMELX, ENAM, AMBN, Genetic Polymorphism, Caries experience

ÖZ

Amaç: Çürüğün etiyolojisi hem çevresel hem de konakçı faktörleri içermektedir. Bu çalışma, mine oluşum genleri (AMELX, ENAM ve AMBN), çevresel faktörler ve bunların birlikte etkisinin yetişkinlerde çürük deneyimi üzerindeki etkisini değerlendirmeyi amaçlamaktadır.

Yöntemler: 20-65 yaş arası 114 yetişkinin dahil edildiği bir vaka kontrol çalışması yürütüldü. Ağız hijyeni alışkanlıklarına ilişkin veriler hasta bildirimi yoluyla elde edildi. Çürük, plak ve tükürük ile ilgili veriler klinik muayene yoluyla elde edildi. Tükürükten genomik DNA izole edildi. Hastalar çürük deneyimine göre iki gruba ayrıldı. Çevresel faktörler (yaş; plak miktarı; diş fırçalama süresi; şekerli içecek ve yiyecek tüketimi; florürlü diş macunu, florürlü gargara ve diş ipi kullanımı; Streptococcus mutans ve Lactobacillus sayıları) ve konakçı faktörler (tükürük akış hızı, pH, tamponlama kapasitesi ve AMELX, ENAM ve AMBN) incelendi. Genotip dağılımlarını değerlendirmek için gerçek zamanlı PCR kullanıldı. Veriler ki-kare testi ve lojistik regresyon kullanılarak SPSS ile analiz edildi.

Bulgular: Türkiye'den seçilen bireylerde AMELX, ENAM veya AMBN alel ve genotip frekansları arasında gruplar arasında istatistiksel olarak anlamlı bir fark gözlenmedi. Gruplar arasında tükürük akış hızı veya şekerli yiyecek ve içecek tüketimi açısından da anlamlı bir ilişki bulunmadı. Diğer tüm faktörlerin yüksek çürük deneyimi ile ilişkili olduğu tespit edildi.

Sonuç: Çevresel faktörler ve genotipler birlikte çok değişkenli regresyon analizi ile değerlendirildiğinde; artan yaş, yüksek plak seviyeleri ve florürlü gargara kullanımının olmamasının yüksek çürük deneyim olasılığını artırdığı belirlendi.

Anahtar Kelimeler: AMELX, ENAM, AMBN, Genetik Polimorfizm, Çürük deneyimi.

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INTRODUCTION

The etiology of caries lesions has been investigated for many years. Environmental factors, such as cariogenic diet, poor oral hygiene, dental plaque, insufficient fluoride exposure, and colonization levels of cariogenic bacteria, and host factors, such as salivary flow rate, salivary buffering capacity, and genetics, play a role in the etiology of caries lesions.[1] Although the pathogenesis of caries lesions has been known for a considerable time, this disease is still an important oral health problem in most countries.[2] While environmental factors have been reported to affect the development of dental caries, susceptibility may vary between individuals exposed to the same environmental factors. It has been suggested that these differences are caused by genetic factors in the etiopathogenesis of dental caries.[3] According to the results of genetic studies, the genes that may affect dental caries susceptibility are classified as those associated with enamel formation, immune response, salivary proteins, and taste receptors.[2] Genetic variation in the genes involved in enamel formation has been reported to cause structural changes in tooth enamel that may lead to mineral losses.[4] The genes associated with mineralization, namely amelogenin (AMELX), enamelin (ENAM), tuftelin (TUFT1), ameloblastin (AMBN), and kallikrein (KLK4), are involved in enamel mineralization, while dentin sialophosphoprotein (DSPP) is involved in dentin mineralization.[5,6] Ninety percent of the organic matrix of enamel is composed of the proline-rich protein amelogenin. It governs the organization and growth of enamel crystals. Ameloblastin is a cell adhesion protein responsible for controlling cell differentiation.[7] Enamelin, one of the proteins effective in mineralization, is found in the mature organic matrix and takes part in enamel formation.[8] Therefore, mutations in the above-mentioned genes may increase the susceptibility of surfaces to caries by causing abnormal protein production. In addition, when the amounts of these proteins are reduced in developing teeth, defective mineralization can occur, which can affect both bacterial adhesion and enamel resistance to acid pH.[9] Identification of the genes that affect susceptibility may contribute to the prevention of dental caries.[2] An early screening test for genetic susceptibility to caries may be developed.[6] In addition, the investigation of genetic factors may facilitate the identification of individuals at high caries risk. It can help in the planning of preventive treatments on a per-person basis.[2,10] According to the results of a meta-analysis published in 2020, three of the studies evaluating the effect of genes on dental caries etiology were performed in adults. Therefore, the aim of the study was to evaluate the impact of genetic and environmental factors and their interactions on caries experience in adults in selected subjects in Turkey. The null hypotheses of the study are as follows: 1) AMELX, ENAM, and AMBN gene polymorphisms are not associated with high caries experience. 2) Environmental factors (age; amount of plaque; tooth brushing duration; use of fluoride toothpaste, fluoride mouthwash, and dental floss; consumption of sugary drinks and food; and *Streptococcus mutans* and *Lactobacillus* counts) and host-related factors (saliva flow rate, saliva pH, and salivary buffering capacity) are not associated with a high caries experience.

METHODS

Subject

The study protocol was approved by the decision of Atatürk University Faculty of Medicine Clinical Research Ethics Committee (Date:13.03.2019 Number: B.30.2.ATA.0.01.00/138) and was supported Ataturk University Scientific Research Projects Foundation (TDH-2019-7313). It was conducted by the Declaration of Helsinki. Informed consent forms prepared accordingly were signed by each person included in the study. Patients within the age range 20 to 65 who presented to the Atatürk University Faculty of Dentistry Restorative Dentistry Clinic for dental treatment and were regarded as adults according to the criteria of the World Health Organization (WHO1998) were selected for the research group[11]. Power analysis was performed using the software package G*Power 3.1. Sample size was calculated from the goodnass of fit tests(contingency tables) with α=0.05 power (1-β)=0.85 and effect size w=0.30, and was determined to be 100 samples to account for possible dropout.[12] Fourteen replacement patients were added to compensate for possible data loss (due to a problem that may occur in genomic DNA extraction). A total of 114 Turkish patients were included in the study.[13]

Inclusion Criteria

Age between 20 and 65 years, regarded as adults according to WHO criteria (1998). Patients presenting to the university's Faculty of Dentistry Restorative Dentistry Clinic for dental treatment.

Exclusion Criteria

Presence of neurological, mental, systemic, or genetic diseases. Severe physical disabilities or acute/chronic upper respiratory tract diseases. Dental fluorosis or use of chlorhexidine-containing preparations within the previous three months. Current orthodontic treatment.

Intraoral and Radiographic Examination

The individuals included in the study were assigned to the groups according to the DMFT scores calculated using the World Health Organization (WHO) criteria.[11] All teeth were examined in detail visually and radiographically by the same dentist. The participants in the study were divided into two extreme groups based on their DMFT (decayed, missing, and filled teeth) scores, as done in other studies, to reduce heterogeneity.[14,15] conducted in adults: a group with high caries experience (DMFT $\geq$ 14, n = 55) and a group with low caries experience (DMFT $\leq$ 5, n = 59). According to the Silness and Löe plaque index, which is accepted worldwide in determining oral hygiene, tooth no. 12, 24, 16, 32, 44 and 36 were evaluated.[16]

Environmental Factors

Environmental factors, such as age, amount of plaque, tooth brushing duration, consumption of sugary drinks and foods, and the use of fluoride toothpaste, fluoride mouthwash, and dental floss, as well as Streptococcus mutans and Lactobacillus counts, were examined.

Host-Related Factor

Host-related factors, such as saliva flow rate, pH, buffering capacity, and the genetic polymorphisms of AMELX, ENAM, and AMBN, were examined.

Salivary and Microbiological Tests

Salivary flow rate, pH, buffering capacity, and the counts of Streptococcus mutans and Lactobacillus were determined using the KariesScreen Test +P (Aurosan GmbH, Essen, Germany).

Salivary Tests

Participants were instructed to chew paraffin (Aurosan GmbH, Essen, Germany) for 5 minutes without swallowing the saliva produced during this period. The saliva was collected into a millimeter-graded container (KariesScreen Test +P, Aurosan GmbH, Essen, Germany). The total volume of saliva was measured and divided by the collection time (5 minutes) to calculate the stimulated salivary flow rate in mL per minute, which was then recorded.

A portion of the collected saliva was applied to the first test strip. The salivary pH was determined by comparing the color change on the strip with the scale provided by the manufacturer. To evaluate buffering capacity, 1 mL of stimulated was mixed with 3 mL of 0.005 N HCl. After 5 minutes, the second test strip was immersed in the

mixture. The value representing the buffering capacity was determined based on the color change observed on the test strip and compared with the scale provided by the manufacturer (pH > 6.0: very good; pH > 5.0: good; pH < 5.0: poor; pH < 4.0: very poor buffering capacity). The values were recorded.

Microbiological Tests

The counts of S. mutans and Lactobacillus were determined using the dip-slide method.[17] For the counts of *S. mutans* and *Lactobacillus*, media containing NaHCO3 tablets were washed with stimulated saliva and incubated at 37°C for 48 hours. The microbial counts were recorded as colony forming units/mL (CFU/mL) using the scale provided by the manufacturer (<10^3 CFU/mL: very low; <10^5 CFU/mL: low; 10^5–10^6 CFU/mL: high; >10^6 CFU/mL: very high).

Genomic DNA Extraction and Analysis of Gene Polymorphisms

Saliva samples were transferred to plastic tubes (Transport Swab, Biologix Group Limited, Shandong, China). The samples were stored at +4 °C until extraction was performed. AMELX, ENAM, and AMBN gene polymorphism analysis was performed by real-time PCR (ABI 7500 Fast Real-Time PCR). The genes and their polymorphisms examined in the study are shown in Table 1.

Table 1. Genes examined in the study and their polymorphisms

Gene	Protein Coded	Reference sequence number
AMELX	Amelogenin	rs17878486
ENAM	Enamelin	rs12640848
AMBN	Ameloblastin	rs17733915

Statistical Analysis

Statistical analysis of the data was performed using SPSS 20 (IBM, Chicago, IL, New York). All genotypes were in Hardy-Weinberg equilibrium. The chi-squared test was used to compare the genotype and allele distributions of the AMELX, ENAM, and AMBN genes between the low and high caries experience groups. The differences between the groups in terms of age, amount of plaque, tooth brushing duration, consumption of sugary drinks and foods, and the use of fluoride toothpaste, fluoride mouthwash, and dental floss, as well as Streptococcus mutans and Lactobacillus counts, were evaluated using logistic regression analysis. Finally, genes and some environmental and host factors were combined in the same model and evaluated using multivariate regression analysis.

RESULTS

The associations between environmental and host factors with caries experience are shown in Table 2. Out of the 114 participants included in the study, 59 were categorized in the low caries experience group (DMFT ≤ 5), while 55 were in the high caries experience group (DMFT ≥ 14). When comparing the groups in terms of gender distribution, no significant difference was observed. The mean age and standard deviation for the high and low caries experience groups were found to be 35 (±12.6) and 27 (±5.7), respectively. The mean age of the high caries experience group was significantly higher than that of the low caries experience group (*P*< .001).

The mean DMFT scores and standard deviations for the high and low caries experience groups were 15.8 (±2.59) and 2.63 (±1.95), respectively. High plaque levels (*P* <.001), high *Lactobacillus* counts (*P*<.05), high *S. mutans* counts (*P* <.001), and a salivary pH of <7 (*P*<.05) were associated with high caries experience. Good salivary buffering capacity was found to be protective against high caries experience (*P*=.001).

No significant differences were found in salivary flow rate, consumption of sugary foods and drinks, and caries experience. However, the absence of fluoridated toothpaste use increased the likelihood of high caries experience by 7,658 times (*P*=.002), the absence of fluoridated mouthwash use by 6,766 times (*P*<.05), the absence of dental floss use by 2,312 times (*P*=.032), and brushing for less than 1 minute increased the likelihood by 11,786 times (*P*= 0.006). The genotype and allele frequency distributions of the AMELX rs17878486, ENAM rs12640848, and AMBN rs17733915 genes are presented in Table 3. No statistically significant differences were found between the low and high caries experience groups in terms of AMELX, ENAM, or AMBN genotypes or allele distributions.

Table 2. Logistic regression analysis for the association between environmental factors, host-related factors, oral health habits and caries experience

	Sample n=114	Low Caries experience Group n=59	High Caries Experience Group n=55	OR(%95 CI)	*P* value
Mean Age	31(10.4)	27 (5.7)	35 (12.6)	1.097 (1.044-1.154)	**<.001***
Gender					
Female	53	31	22	Reference	
Male	61	28	33	1.661 (0.790-3.491)	.181
Plaque Quantity					
Low	83	55	28	Reference	
High	31	4	27	13,259 (4,222-41,641)	**<.001***
S. Mutans count					
Low	26	19	7	Reference	
High	88	40	48	4.703 (2.125-10.410)	**<.001***
Lactobacillus count					
Low	63	43	20	Reference	
High	51	16	35	3.257 (1.244-8.531)	**.016***
Buffering capacity of the saliva					
Good	66	43	23	Reference	
Poor	48	16	32	0.267 (0.122-0.586))	**.001***
Saliva pH					
pH>7	93	54	39	Reference	
pH<7	21	5	16	4.431 (1.497-13.117)	**.007***
Saliva flow rate					
Good	89	47	42	Reference	
Sufficient	14	7	7	1.119 (0.362-3.455)	.845
Insufficient	11	5	6	1.343 (0.382-4.723)	.646
Tooth brushing duration	22	15	7	Reference	
3 minute	57	32	25	1.728 (0.611-4.891)	.303
1-3 minute	22	10	12	2.338 (0.694-7.872)	.170
>1 minute	13	2	11	11.786 (2.041-68.061)	**.006***
<1 minute					
Consumption of sugary drinks and food (daily)	15	9	6	Reference	
None	73	38	35	1.382 (0.446-4.279)	.575
1-2 times	17	8	9	1,687 (0,414-6,878)	.465
3-4 times	9	4	5	1,875 (0,352-9,981)	.461
More than 4					
Use of Fluoride toothpaste	95	56	39	Reference	
Yes	19	3	16	7.658 (2.089-28.075)	**.002***
No					
Use of Fluoride mouthwash	14	12	2	Reference	
Yes	100	47	53	6.766 (1.440-31.801)	**.015***
No					
Use of dental floss	47	30	17	Reference	**.032***
Yes	67	29	38	2.312 (1.074-4.977)	
No					

**P<.05, statistically significant; OR (95% CI)= Odds ratios; 95% confidence intervals; bold forms indicated statistical significance*

Some environmental and host factors that were statistically significant in the univariate regression analysis were selected, and their combined effect with genotypes was evaluated using multivariate regression analysis (Table 4). An increase in age by 1.101 times, high plaque levels by 7.834 times, and the absence of fluoridated mouthwash use by 7.834 times were found to increase the likelihood of high caries experience (*P*<.05).

DISCUSSION

The etiology of dental caries is influenced by the complex relationship between genetic and environmental factors. Very few studies have investigated the effect of genes on caries etiology had been conducted in adults.[18] In a recent meta-analysis, it was emphasized that further studies were needed to increase the level of evidence and support the current conclusions regarding the genetic influence on caries etiology.[19] Based on a literature review, this study was designed to investigate some of the gene polymorphisms previously identified in relation to caries to assess their association with caries experience in Turkish adults.

The first hypothesis, stating that AMELX, ENAM, and AMBN gene polymorphisms are not associated with high caries experience, was accepted. However, as environmental factors such as age, plaque amount, tooth brushing duration, the use of fluoride toothpaste, fluoride mouthwash, and dental floss, as well as Streptococcus mutans and Lactobacillus counts, and host-related factors such as saliva pH and salivary buffering capacity were found to be associated with high caries experience, the second hypothesis was partially rejected.

Gerreth et al.[20] had reported a significant positive association between the AMELX rs17878486 T allele and TT genotype in Polish children and the occurrence of developmental enamel defects in primary dentition. Similarly, Patir et al.[21] had found that the AMELX rs17878486 polymorphism in Turkish children was associated with an increased susceptibility to caries by altering enamel structure. In a study conducted on South Indian children, it was reported that the analyzed polymorphisms of the AMELX gene had not revealed any significant association with early childhood caries (ECC).[22]

In this study, no significant difference was observed in the distribution of AMELX rs17878486 genotypes and alleles between the low and high caries experience groups. This was thought to stem from the fact that this study had focused on adults and permanent teeth, while the other studies had investigated primary dentition in children.[23] Although studies conducted in adults are more limited, one study found no significant association between AMELX polymorphism and susceptibility to dental caries.[13] Therefore, AMELX may have different effects on susceptibility to dental caries in primary and permanent dentition. Enamelin, the largest protein in the enamel matrix during enamel formation, is encoded by the ENAM gene. Mutations in the ENAM gene have been reported to cause autosomal dominant amelogenesis imperfecta.[24] A significant relationship between the ENAM rs12640848 gene and caries risk was identified by Shimizu et al.[25] while Abbasoğlu et al.[26] reported that the ENAM rs12640848 GG genotype was associated with a protective effect against ECC. Additionally, ENAM rs12640848 has been linked to being caries-free.[27] However, in the present study, a caries-free group was not included, which may have contributed to the lack of association observed. Another study was found to support these findings, reporting no significant relationship between the ENAM gene and caries experience.[5] Similarly, in a study investigating the ENAM (rs12640848) genetic polymorphism in Riyadh, Saudi Arabia, no significant association was observed between this polymorphism and either an increased risk or protection against ECC.[28] Furthermore, in a study conducted by Borilova et al.[29] on Czech children, no association was detected between ENAM rs12640848 and dental caries in either primary or permanent dentition. The differences in the results were explained by populations with different genetic backgrounds and varying environmental factors. First, genetic variations among different populations may influence the role of ENAM rs12640848 in caries susceptibility. Second, differences in environmental factors, such as dietary habits, fluoride exposure, and

Table 3. The chi-square test for genotype and allele distribution between caries experience groups

Polymorphism	Genotype frequency			p	Allele Frequency		*P*
AMELXrs17878486	**CC**	**TC**	**TT**		**C**	**T**	
Low Caries experience Group n=59	6 (%10.2)	20 (%33.9)	33 (%55.9)	0.157	32(%27.1)	86 (%72.9)	.444
High Caries Experience Group n=55	1 (%1.8)	23 (%41.8)	31 (%56.4)		25(%22.7)	85 (%77.3)	
ENAM rs12640848	**AA**	**AG**	**GG**		**A**	**G**	
Low Caries experience Group n=59	25(%42.4)	27 (%45.8)	7 (%11.9)	0.705	77(%63.6)	41 (%36.4)	.467
High Caries Experience Group n=55	24(%43.6)	27 (%49.1)	4 (%7.3)		75(%68.2)	35 (%31.8)	
AMBN rs17733915	**CC**	**CT**	**TT**		**C**	**T**	
Low Caries experience Group n=59	22 (%40)	21 (%38.2)	12 (%21.8)	0.144	65(%59.1)	45 (%40.9)	.101
High Caries Experience Group n=55	34(%57.6)	14 (%23.7)	11 (%18.6)		82(%69.5)	36 (%30.5)	

**P <.05, statistically significant.*

Table 4. Multivariate regression analysis combining environmental factors and genotypes

	OR(%95CI)	*P* value
Age	1.101 (1.024-1.185)	**.010***
Plaque Quantity		
Low	Reference	
High	7.834 (1.753-35.002)	**.007***
Use of Fluoride toothpaste		
Yes	Reference	
No	2.022 (0.430-9.517)	.373
Use of Fluoride mouthwash		
Yes	Reference	
No	8.553 (1.062-68.863)	**.044***
S. Mutans count		
Low	Reference	
High	2.325 (0.799-6.761)	.121
Lactobacillus count		
Low	Reference	
High	1.760 (0.525-5.901)	.360
Buffering capacity of the saliva		
Good	Reference	
Poor	1.695 (0.592-4.853)	.325
AMELX		
CC	Reference	
TC	0.078 (0.004-1.720)	.106
TT	0.151 (0.008-2.823)	.206
ENAM		
AA	Reference	
AG	1.484 (0.515-4.283)	.465
GG	0.434 (0.068-2.757)	.376
AMBN		
CC	Reference	
CT	1.957 (0.609-6.287)	.259
TT	0.834 (0.198-3.508)	.804

**P < .05, statistically significant; OR (95% CI)= Odds ratios; 95% confidence intervals; bold forms indicated statistical significance*

oral hygiene practices, could affect the manifestation of genetic predispositions. The ameloblastin protein was found to regulate the elongation of enamel crystals during tooth development.[6] Shaffer et al.[4] ad found a significant association between AMBN rs17733915 and caries experience, where carrying at least one copy of the rare amelogenin marker allele was linked to greater caries experience after adjusting for age.[5] In this study, no statistically significant differences were identified in the distribution of AMBN rs17733915 genotypes or alleles between the groups. It was reported that meta-analyses of AMBN rs17733915 had been thought-provoking. Further studies are needed to understand how variations in genes involved in enamel formation influence the risk of dental caries and how they interact with environmental exposures.[4] The analyzed polymorphisms were shown to have no significant association with caries in the Turkish cohort. However, this did not rule out the presence of a genetic component influencing caries susceptibility.

Based on the fact that gene polymorphisms had been found to remain stable over time, patients aged 20 to 65, who were considered adults according to WHO criteria (1998), were included in the study. To minimize heterogeneity, participants were classified into two distinct groups based on their caries experience: those with a DMFT score below 5 were assigned to the low caries experience group, while those with a DMFT score above 14 were placed in the high caries experience group.[15] Similar grouping strategies based on the DMFT index have been applied in previous studies examining the association between gene polymorphisms and caries.[30] However, it has also been recognized that while the DMFT index provides a standardized method for classification, it offers limited insight into the disease process itself.[25] For this reason, additional comparisons were made between the groups for S. mutans and Lactobacillus counts, saliva flow rate, pH, and buffering capacity. Researchers have suggested that obtaining S. mutans and Lactobacillus counts is a critical step for monitoring the effectiveness of caries prevention strategies in acute periods.[31] In this study, significantly higher S. mutans and Lactobacillus counts were observed in individuals with high caries experience.

It had been reported that factors such as tooth morphology, buffering capacity, salivary flow, dietary habits, oral microbiome, oral hygiene, and a history of dental caries clearly played a significant role in the development of carious lesions.[2] Some researchers had stated that low saliva buffering capacity increased caries susceptibility in adults because it caused the acidic environment to persist in the mouth over a long period, allowing the growth of cariogenic bacteria. Therefore, the groups were compared in terms of salivary flow rate, salivary pH, and salivary buffering capacity. Significantly poor buffering capacity and salivary pH less than seven were detected in individuals in the high caries experience group. Due to the low salivary flow rate and frequent consumption of sugary drinks and foods, the pH level of the dental biofilm was observed to remain low for a long time, giving acid-tolerant oral pathogens an advantage among resident species.[10] Reducing the consumption of sugary foods and drinks and eating these foods with the main meals was identified as an important factor for reducing the risk of caries.[32] In the present study, the amount of sugary food and beverage consumption per day was not associated with caries experience. This discrepancy may be due to differences in dietary habits, variations in the frequency and timing of sugar consumption, or the influence of other protective factors such as fluoride exposure and oral hygiene practices. Even trace amounts of fluoride have been found to be crucial in caries prevention, as an increase in caries development has been reported in patients who discontinued the use of fluoride products.[33] According to our study results, not using fluoride toothpaste and fluoride mouthwash significantly increased the probability of experiencing high caries (P<.05).

Some environmental factors that were found to be statistically significant in the univariate regression analysis were combined with gene polymorphisms in the multivariate regression analysis to evaluate their combined effect. It was determined that increased age, high plaque levels, and the absence of fluoride mouthwash use increased the likelihood of high caries experience. In studies evaluating genetic and environmental factors with logistic regression analysis, increasing age was reported to be a risk factor for caries.[34,35] Taletar et al.[14] had stated that an increase in the amount of plaque was associated with high caries experience. In the study by Wang et al.[35] frequent tooth brushing and the level of fluoride in the water were reported as protective factors for caries. Tannure et al.[34] reported that all individuals in their study used fluoride toothpaste, but no significant difference was found in the use of fluoride mouthwash between individuals with and without caries. In addition it was emphasized that the use of fluorine had not been evaluated together with genetic factors. It had been reported that the impact of environmental exposures varied based on an individual's genome and that other environmental factors, such as antimicrobial or pH-buffering mouthwashes or prescription topical fluorides, might benefit specific subgroups of the population.[4] The presence of certain genotypes was suggested to cause individuals to respond differently to environmental factors such as fluoride or diet. It is believed that the use of fluoride mouthwash should be further investigated in future studies.

The limitation of our study was that information about environmental factors had been obtained from the statements of the participants. Therefore, more objective and reliable data collection methods should be considered in future studies. Future studies may include more closely matched age groups. In addition, although choosing one polymorphism per gene had a limitation, it should be taken into account that analyzing each polymorphism increases the cost. Finally, the presence or absence of white spot lesions and activity classification in individuals in the groups could be evaluated in further studies.

Our study findings indicated that the AMELX rs17878486, ENAM rs12640848, and AMBN rs17733915 gene polymorphisms were not associated with caries experience in Turkish adults. However, high plaque levels, elevated Streptococcus mutans and Lactobacillus counts, a salivary pH lower than 7, brushing duration of less than one minute, and the absence of fluoride toothpaste, fluoride mouthwash, and dental floss use increased the likelihood of high caries experience. In contrast, good salivary buffering capacity was identified as a protective factor against high caries experience.When environmental factors and genotypes were evaluated together, increased age, high plaque levels, and the absence of fluoride mouthwash use were found to increase the likelihood of high caries experience. Further studies are needed to assess the combined effects of different candidate genes, environmental factors, and fluoride mouthwash use on caries experience. Such studies may contribute to the development of individualized preventive treatments, which are essential for public oral health.

Ethics Committee Approval: The study protocol was approved by the decision of the Ataturk University Faculty of Medicine Clinical Research Ethics Committee (Date:13.03.2019 Number: B.30.2.ATA. 0.01.00/138).

Informed Consent: **F**orms were obtained in writing from each participant participating in the study.

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Investigation of the Effect of Preheating Before Polymerization on the Color and Gloss of Composites

Polimerizasyon Öncesi Ön Isıtmanın Kompozitlerin Renk ve Parlaklığı Üzerindeki Etkisinin İncelenmesi

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ABSTRACT

Objective: The aim of this study was to investigate the effects of preheating on the color stability and surface gloss of composite resins before polymerization.

Methods: A total of 90 samples (8 mmx 2 mm) of three composite resins with different particle sizes were prepared. Half of the samples were prepared from composite tubes at room temperature and the other half were prepared from composite tubes preheated to 60°C with the Ease-It™ heating device (n=15). After polymerization and polishing, color measurements were performed using a spectrophotometer and surface gloss was measured with a glossmeter. All samples underwent 10,000 thermal cycles, after which measurements were repeated. The Kolmogorov-Smirnov test was used to assess the normality of data distribution, and Levene's test was applied to evaulate homogeneity of variance (P<.05).

Results: There was no statistically significant difference in the gloss values of preheated and unheated composite resins (P>.05). Heat treatment did not cause a statistically significant difference between the composite types (P>.05), but it caused a statistically significant difference in the mean color change values (P<.001). The average color change values of the preheated groups were significantly lower than the unheated groups (P<.05).

Conclusion: Preheating composite resins prior to polymerization did not significantly affect surface gloss but significantly reduced discoloration.

Keywords: Composite resin, Heating, Polymerization, Staining, Surface gloss.

ÖZ

Amaç: Bu çalışmanın amacı, polimerizasyon öncesi ön ısıtmanın kompozit rezinlerin renk stabilitesi ve yüzey parlaklığı üzerindeki etkilerini araştırmaktır.

Yöntemler : Farklı partikül boyutlarına sahip 3 farklı kompozit rezinden toplam 90 örnek (8mmx 2 mm) hazırlandı. Örneklerin yarısı oda sıcaklığındaki kompozit tüplerden, diğer yarısı ise Ease-ItTM ısıtma cihazı ile 60°C'ye ısıtılmış kompozit tüplerden hazırlandı (n:15). Polimerizasyon ve parlatma işlemlerinden sonra spektrofotometre ile renk ölçümleri ve Glossmetre ile yüzey parlaklığı ölçümleri yapılmıştır. Tüm numuneler 10.000 döngülük bir termal döngüye tabi tutulmuş ve ölçümler tekrarlanmıştır. Sayısal verilerin gruplar arasındaki normal dağılımını değerlendirmek için Kolmogorov-Smirnov testi kullanılırken, varyansın homojenliğini değerlendirmek için Levene testi kullanılmıştır (P<,05).

Bulgular : Önceden ısıtılmış ve ısıtılmamış kompozit rezinlerin parlaklık değerlerinde istatistiksel olarak anlamlı bir fark yoktu (P>,05). Isıl işlem kompozit tipleri arasında istatistiksel olarak anlamlı bir farka neden olmazken (P>,05), ortalama renk değişim değerlerinde istatistiksel olarak anlamlı bir farka neden olmuştur (P<,001). Isıtılan grupların ortalama renk değişim değerleri ısıtılmayan gruplardan anlamlı derecede düşüktü (P<,05).

Sonuç : Ön ısıtma işlemi kompozit rezinlerin parlaklık değerleri üzerinde anlamlı bir fark yaratmamıştır; ancak renk değişimini önemli ölçüde azaltmıştır.

Anahtar Kelimeler: Kompozit rezin, Isıtma, Polimerizasyon, Boyanma, Yüzey parlaklığı.

INTRODUCTION

With the development of aesthetic standards in dentistry, various approaches and techniques have been developed to improve the cosmetic quality of composite resins. One of the current methods introduced during the development of these approaches and techniques is the heating of composites prior to application.

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Heating composite resins before application improves the physical and mechanical properties of these materials by increasing the degree of monomer conversion and provides superior aesthetic results.[1–3] The viscosity and free radical mobility of the material increases with increasing composite body temperature. The polymerization reaction is completed by increasing the spread of polymer chains due to the increase in viscosity. More double bonds develop, leading to a decrease in the amount of unreacted monomer. Preheating of resin-based materials not only increases conversion, but also improves marginal adaptation and reduces polymerization shrinkage.[1,4,5]

The gloss of a resin composite affects its aesthetic qualities, its applications in dentistry and the long-term reliability of the composite restoration. Therefore, gloss is an important factor for visual standards.[6] As the gloss increases, the materials adapt better to the adjacent tooth and the tooth on which it is placed. Even though there is a perfect color match between the tooth and the tissue, differences on surface gloss can be detected.[7]

Surface discoloration is a significant aesthetic problem associated with composite resins. Therefore, long-term color permanence is very important for these materials. Staining; It is affected by the resin formulation, filler particle ratio, and filler particle size. Although there have been many improvements in materials to reduce surface discoloration, discoloration is still a cause of clinical failure for resin composite materials.[8]

The durability of a dental restoration using resin composites may be related to physicochemical and surface properties. Although numerous studies have examined the effect of preheating on various restorative materials, there is insufficient data on surface quality and durability.[1] This study examined how preheating affects the gloss and color stability of composite resins with various filler types and sizes (Harmonize by Kerr, G-aenial Anterior by GC, and Filtek Universal by 3M-ESPE). The first null hypothesis of this study states that there will be no significant difference in gloss between composite resins with and without preheating. The second null hypothesis states that there will be no significant difference in color changes between composite resins with and without preheating treatment.

METHODS

In this study, three different composites were used: Harmonize (nanohybrid, NH), G-aenial Anterior (micro-hybrid, MH), Filtek Universal (nanofilled, (NF)). The composites used for the study are shown in Table 1. The composites were divided into two subgroups due to whether preheating was done or not.

Table 1. Composite materials used in the study and content information

Composite	Filler type	Composition	Brand (Lot number)
G-aenial Anterior	Micro-hybrid	UDMA, dimethacrylate co monomers, Prepolymerized silica, strontium and lanthanoid fluoride-containing fillers 76 wt% Fumed silica	GC Europe, Leuven, Belgium
Harmonize	Nano-hybrid composite	Silica-zirkonia filler Barium glass filler Silica and ytterbium trifluoride İnorganic fillers 75% by weight, 55% by volume Bis-EMA, Bis-GMA, TEGDMA, and/or UDMA	Kerr, Orange, USA
Filtek Universal Restorative	Nano-filled composite	20nm silica filler 4-11nm zirkonya filler Ytterbium trifluoride filler norganic filler 76.5% by weight/ 58.4% by volüme AUDMA, AFM, diurethane-DMA, and 1,12 dodecane-DMA.	3M-ESPE, St. Paul, MN, USA

Group 1 (Preheated Group): For the preparing of Group 1 samples, composite resin tubes were heated to 60°C for 15 minutes using a heating device (Ease-ItTM Heating Unit, Ronvig Dental, Daugaard, Denmark) (Figure 1). The heating device alerts when the temperature of the tubes reaches 60°C. To avoid heat loss when preparing samples from the composite tubes, the tube was quickly removed from the heating device and the required composite material was placed into the Teflon mold with a metal spatula and then quickly placed back into the device. To obtain a flat and smooth surface and to remove residual material, the composite material was placed between transparent polyethylene strips on the top and bottom and sandwiched between two glass coverslips. Composite samples were polymerized for 20 s using a 1000 mW/cm2 LED light device (DTE LUX E, Borkstrasse, Muenster, Germany) in standard mode, keeping the tip in contact with the glass coverslip (n = 15).

Group 2 (Unheated Group): For the preparing of Group 2 samples, the composite resin tubes were kept at room temperature (20-22 °C) until the polymerization stage. The samples were prepared by placing the composite material into teflon molds using a metal spatula. To obtain a flat and smooth surface and to remove residual material, the composite material was placed between transparent polyethylene strips on the top and bottom and sandwiched between two glass coverslips. Composite samples were polymerized for 20 s using a 1000 mW/cm2 LED light device (DTE LUX E, Borkstrasse, Muenster, Germany) in standard mode, keeping the tip in contact with the glass coverslip (n=15).

All specimens were finished and polished using a two-stage spiral polishing system (ClearfilTwist Dia, Kuraray, Japan). Dark blue and light blue spiral tires, respectively, were applied to the specimen surface in circular motions at 10,000 rpm for 30 seconds under water cooling. The spirals were renewed after every five specimens. Specimens were ultrasonically cleaned (Amsco, Reliance Sonic 250, Steris Corp., Mentor, OH, USA) for 10 minutes in deionized water to get rid of polishing residue after the polishing prosedure. Samples were then immersed in distilled water at 37°C in an incubator (FN 500, Nüve, Turkey) for 24 hours. Then initial color and gloss measurements were calculated.

Evaluation Methods

1. Color measurement

A color cabinet was used to maintain uniformity in color measuring. The inside of the color measurement cabinet was covered with a gray background, and measurements were conducted on a white background. The light power inside the color measurement cabinet was adjusted to 6500K (MasterTL-D 90 Graphica 18W/965, Philips, Signify, Poland) following CIE standards.

Color measurements were repeated three times from the center point of each sample using a spectrophotometer (Vita Easyshade V, VITA Zahnfabrik, Germany). Before sample measurements, the spectrophotometer was calibrated with the indicated calibration plate. The average value of the measurements taken was accepted as the final value for each sample. The CIE-L*a*b* color system, defined as a 3D measurement system, was used to evaluate the numerical data obtained: "L" stands for luminance, "a" for the red-green ratio, and "b" for the yellow-blue color ratio. The CIE 2000 formula is the most up-to-date method for the calculation of colour differences and is recommended by CIE due to its superior performance compared to CIELAB and better compatibility with visual assessments. Therefore, calculations were made using the CIE2000 formula over the CIE-Lab values.[9,10]

2. Gloss Measurements

The surface gloss of the samples was measured using a gloss meter (Novo-Curve, Rhopoint Instrumentation, Bexhill on Sea, UK) with a 2 mm × 2 mm area and 60° geometry. Three measurements of each sample were taken at 60° angles of light incidence and reflection.[11] During the measurements, a black opaque plastic cup was placed over the samples to eliminate the effect of ambient light. The obtained gloss values were recorded in GU units.[12] A statistical analysis was performed on the mean values of the measurements

Following the initial color and gloss measurements, all samples were subjected to 10,000 thermal cycles (Mechatronic thermo-cycler, Seelbach, Germany) to simulate the 1-year thermal cycles occurring in the oral cavity. Samples were subjected to 10,000 thermal cycles in a thermal cycler (Thermocycler the 1100, SD Mechatronik, Germany) between 5°C-55°C with a dwell time of 30 seconds and a transition time of 10 seconds.[13] The final color and gloss measurements of all samples were reassessed following the aging process with a thermal cycle.

Statistical Analysis

In this study, the IBM SPSS v25 program (IBM SPSS Corp., Armonk, NY, USA) was used for statistical analysis. Before looking at how important numerical data was for each group, the Kolmogorov-Smirnov test and Levene's Test for Homogeneity of Variance were used to see if the data were normally distributed. A two-way analysis of variance (ANOVA) in repeated measures examined changes in brightness and color values. Bonferroni correction was used for multiple comparisons. The results of the analysis are presented as the mean ± standard deviation.

The sample size required for the study was made for the F test by taking β = 0.80, α = 0.05 and the effect size = 0.35 as a result of the literature research (thesis proposal, article, bibliography) with the G*Power 3.1 programme and a total of 84 samples (n = 28 in each group) should be taken. For this reason, a total of 90 samples, 30 for each group, were planned in the study. Each group was divided into two subgroups as heated and unheated (n=15).[14]

The assumptions of normal distribution (*P*>.05) and homogeneity of variance (*P*>.05) were met. A two-way analysis of variance (ANOVA) with repeated measures was used to look at how the gloss and color of composite resin samples changed depending on whether they were heated or not and after being aged by thermal cycling. The results were evaluated at a 95% confidence interval, with significance at the *P.*<05 level. While interpreting the results, 0.05 was taken as the significance level, and it was stated that there was a difference in the case of *P*<.05 and no significant difference in the case of *P*<.05

RESULTS

In our examination the gloss values of composite groups with and without heat treatment; there is a statistically significant difference between composite types (*P*<.001) (Table 2). However, no statistically significant difference was observed between the gloss values of composite resins with and without heat treatment (Figure 1) (*P*=.344). In addition, the interaction between the different composite types used in the study and heat treatment was statistically significant (*P*<.001). A statistically significant difference was observed in NH and NF composites (*P*<.001). The average gloss value of the heated group was lower than the unheated group in the NH composite, while in the NF composite, the gloss values of the heated group were higher than the unheated group (Figure 1, Table 2).

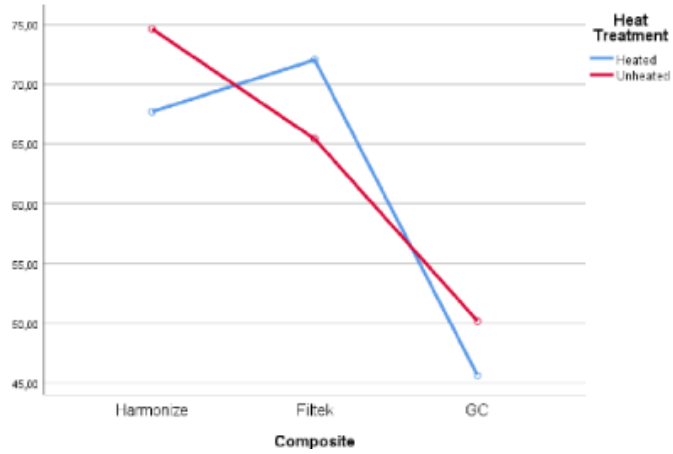

Figure 1. Gloss variation of composite types according to heat treatment or not

When we look at the effect of the aging process on gloss values, the aging process has a statistically significant effect on gloss values (*P*<.001) (Figure 2, Table 2). When we look at the interaction for 'composite' and 'aging', statistically significant differences were obtained (*P*=.042). Also, to find out why this was important, we saw that the gloss values dropped significantly after the aging process for all composite types. The NH composite group had the biggest decrease in gloss values (mean change 11.655±..) (Figure 2, Table 2).

Table 2. Mean gloss values of samples before and after thermal aging

Descriptive Statistics					
Aging	**Composite**	**Heat Treatment**	**Mean**	**Std. Error**	**N**
Before thermal aging	Harmonize	Heated	71,8093	13,01181	15
		Unheated	82,1727	5,81421	15
		Total	76,9910	11,21739	30
	Filtek	Heated	76,4087	9,79443	15
		Unheated	70,6247	9,86910	15
		Total	73,5167	10,09871	30
	GC	Heated	48,5143	7,16240	14
		Unheated	53,0180	6,86071	15
		Total	50,8438	7,25247	29
	Total	Heated	65,9652	15,84226	44
		Unheated	68,6051	14,27216	45
		Total	67,3000	15,04146	89
	Filtek	Heated	67,6693	10,08833	15
		Unheated	60,2127	13,03018	15
		Total	63,9410	12,06142	30
After thermal aging	Harmonize	Heated	63,5780	11,94395	15
		Unheated	67,0933	6,91847	15
		Total	65,3357	9,75565	30
	Filtek	Heated	67,6693	10,08833	15
		Unheated	60,2127	13,03018	15
		Total	63,9410	12,06142	30
	GC	Heated	42,7621	5,42429	14
		Unheated	47,3327	4,83606	15
		Total	45,1262	5,54589	29
	Total	Heated	58,3495	14,40205	44
		Unheated	58,2129	12,05400	45
		Total	58,2804	13,19116	89

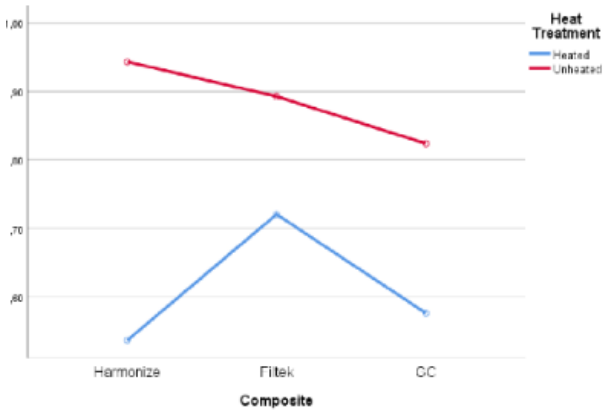

Figure 2. Gloss change in composite types after aging proces

Following analyzing the color changes, no statistically significant difference was found between composite types (P.=47) (Figure 3, Table 3). However, heat treatment has a statistically significant effect on the averagevalues of color change (*P.*<001). In addition, when we look at the interaction between the different composite types used in the study and heat treatment applications, there is no statistically significant difference (*P*=.388). The color change values of the heat-treated groups were found to be statistically significantly lower than the unheated group (Figure 3, Table 3). There was a statistically significant change between the color values of the heated groups and the unheated groups (*P*<.001) (mean change 0.275) (Table 3).

Tablo 3. Mean values of color change

Descriptive Statistics				
Composite	Heat Treatment	Mean	Std. error	N
Harmonize	Heated	0,54	0,16	15
	Unheated	0,94	0,39	15
	Total	0,74	0,36	30
Filtek	Heated	0,72	0,21	15
	Unheated	0,89	0,40	15
	Total	0,81	0,32	30
GC	Heated	0,58	0,23	14
	Unheated	0,82	0,49	15
	Total	0,70	0,40	29
Total	Heated	0,61	0,21	44
	Unheated	0,89	0,42	45
	Total	0,75	0,36	89

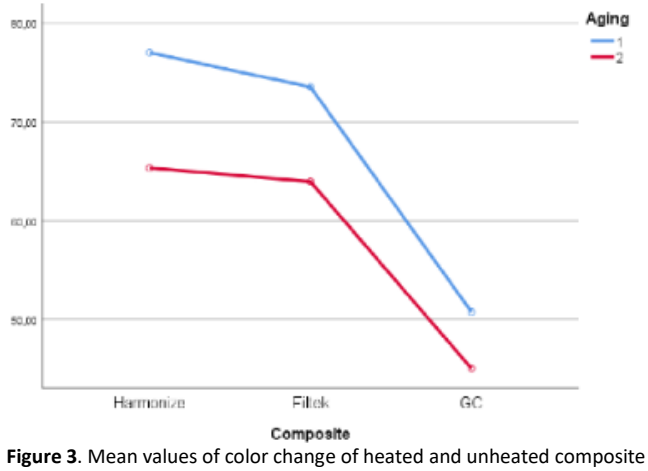

Figure 3. Mean values of color change of heated and unheated composite types

DISCUSSION

Gloss is a very important factor for an aesthetic and natural appearance on composite resin restorations.[15] The greater amount of light reflected directly onto any surface, the higher gloss value recorded for that surface. A glossy surface is also an indicator of a smooth surface.[16,17] In addition to improving the aesthetic appearance, increased surface gloss also prevents plaque retention and the formation of colored biofilms over time.[18]

In this study, no significant difference was found in the gloss of the composite resins as a result of the preheating process applied to the resin samples. The fact that the resin composite depends on the filler material rather than the resin matrix, which is the part of the resin composite sensitive to preheating, may explain the lack of a significant effect of preheating on gloss.[19] Therefore, the first null hypothesis of the study was accepted.

Although no significant difference was found on gloss, when the data obtained were evaluated; the gloss of MH resin composite was lower than NF and NH resin composites at all time points, although not significantly. The inorganic filler size of MH resin composite is larger than NF and NH composites. Gloss is affected by several factors such as filler size distribution, filler refractive index and viscosity of resin matrix components.[20] When the filler size increases, light can be reflected more diffusely. Therefore, gloss values may have been recorded lower.[6,21] Also as another result; preheating decreased the gloss of NH and MH resin composites, while it increased the gloss of NF resin composites. Composite resins with different organic matrix and particle sizes exhibit different properties.[22,23] This is thought to be due to the smaller particle size of the NF composite. However, further research on this subject is required to reach definitive conclusions.

While gloss values can be obtained using various measurement methods, they also vary depending on experimental conditions such as the spectral distribution of light and viewing angle. Gloss measurements at 60◦ angle are considered more reliable clinically as they are closer to the angle at which an average person would observe any surface, and therefore in this study, surface gloss measurements were performed at 60◦ angle.[21]

Surface quality and gloss of resin composites are known to be related to material type, polishing procedure,[24] light source and aging procedure.[25,26] The polishing system applied with a polishing kit containing diamond particles was applied as the standard protocol in this study due to their ability to produce smooth and shiny surfaces on various resin composites.[21,27,28] A single operator polished all of the specimens, and the same light source cured them all to ensure consistency in the experimental procedure.

The thermal cycle is a current and widely used method to simulate the aging effect of daily thermal changes in the oral environment on resin materials. With this method, it is possible to understand the long-term performance of composite materials. According to studies, exposing the samples to 10,000 thermal cycles at 5-55 C° simulates 1-year thermal aging under clinical conditions. In this study, the color and gloss changes of resin samples after 1 year of thermal aging were examined, so the samples were exposed to 10,000 cycles of thermal cycling.[28] Studies on this subject have shown that thermal change and water absorption on resin samples over time can cause changes in the microstructure, leading to an increase in coloration and gloss loss. In other words, thermal cycling can affect the surface morphology of the composite material, causing light to scatter in different ways on the

surface.[28] In this study, as shown in previous studies,[28,29] all composite samples lost gloss at the end of thermal aging. The highest gloss loss after thermal cycling was obtained in the NH composite group (mean change 11.655). Unlike other composite groups, NH composite resin contains Bis-GMA, Bis-EMA, and TEG-DMA hydrophilic monomers that increase the water absorption capacity of resin molecules. There is also no UDMA monomer, which is less hydrophilic compared to other monomers. Therefore, NH composite may have shown higher water absorption and consequently more gloss loss than other composite resins when exposed to thermal cycling.[30,31]

The temperature of preheating range for composite resins has been accepted as 54-68ºC as it does not cause pulpal irritation.[1] Therefore, in this study, preheating of composite samples was performed at 60ºC. Although preheating times have been reported in different studies ranging from 40 seconds to 24 hours, it has been reported that 15 minutes is the most appropriate time in the clinical setting.[1] Therefore, in the present study, preheating was performed for 15 minutes.

In this study; significant differences were found between composite resins with and without preheating treatment in terms of color changes and the second null hypothesis of the study was rejected. However, as a striking result, it was found that the heated groups were significantly less colored than the unheated groups in all composite types. It was thought that this may be due to the improved physical and chemical properties due to the increase in the degree of monomer conversion as a result of preheating.[2,3] The CIELAB color space is a three-dimensional color measurement system consisting of a lightness scale (L*) and a contrasting color axis for both redness-greenness (a*) and yellowness-blueness (b*).[32] The differences between the set of L*a*b* values (ΔL*, Δa*, Δb*) can be used to calculate the color difference (E*). Importantly, relating b findings to clinical significance depends on how the human eye is able to perceive color differences. Paravina et al.[9] reported a color perceptibility threshold (ΔE_{00}) of 0.8 and an acceptability threshold of 1.8. Perceptibility and acceptability tolerance thresholds need to be discussed to determine which differences in color or surface gloss can significantly affect the aesthetic outcome of a dental restoration.[9,33] Considering the results of this study, the coloration of all specimens was below the acceptable threshold. This result is thought to be due to the fact that the samples were not exposed to a coloring solution. However, another striking result was obtained when analyzed in terms of the perceptible threshold value. All of the heated resin samples were colored below the detectable threshold value (ΔE_{00}: 0.8), regardless of the composite type, while all of the unheated samples showed coloration above the detectable threshold value. This result is in agreement with previous studies reporting that the degree of polymerization increases the resistance to discoloration.[33–35] Sousa et al.[33] put heated composites in cola and fruit juice for 7 days and found that the heated (68°C) groups lost less color. Mundim et al.,[36] on the other hand, didn't see a big difference in the color stability of composites heated at 60°C compared to those heated at 8 and 25°C. The results found by different studies may vary depending on the preheating method, temperature, type of solution stored, duration, or aging method.

Within the limitations of the present study, controlling the temperature is a major challenge and a major limitation of this study. Another important limitation of this study is that the effect of mechanical modification of these materials during tooth brushing was not considered. If the surface roughness of the samples had been measured, it would have allowed us to obtain more detailed information on whether the loss of gloss was due to the surface properties of the samples or to water absorption. The conditions of this study were in vitro. The presence of other aging agents should also be evaluated to further adapt the results to clinical conditions. Despite these limitations, this study proved that preheating has positive effects on the surface coloration of selected composite materials. The effect of preheating on the gloss of the composites was also simultaneously investigated. By selecting composites with different filler types in the study, it was also possible to evaluate materials with different indications. From all these perspectives, this study is one of the rare studies examining the effects of preheating on clinical practice.

CONCLUSION

Although preheating did not have a significant effect on the gloss of the composites, it reduced surface discolouration. Therefore, the inclusion of heating in addition to the techniques applied in the fight against external discolouration of composite resin materials will provide more clinically successful results.

Ethics Committee Approval: Since this study did not involve any living subjects and was conducted entirely in vitro, ethical approval was not required.
Informed Consent: There are no participants in the study.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept – B.E., N.A.; Design - S.K., İ.K.Ç.; Supervision - B.E., N.A., S.K.; Resources - B.E., N.A., S.K.; Materials- B.E., N.A., S.K.; Data Total and/or Processing - N.A., S.K., E.A.O.; Analysis and/or Interpretation - N.A., S.K., E.A.O.; Literature Search - B.E., N.A., S.K.; Writing - B.E., İ.K.Ç., S.K.; Critical Review - B.E., S.K.
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Etik Komite Onayı: Bu çalışmada canlı materyal kullanılmadığından ve incelemeler tamamen in vitro ortamda gerçekleştirildiğinden etik onay gerekmemiştir.
Hasta Onamı: Çalışmada herhangi bir katılımcı bulunmamaktadır.
Hakem Değerlendirmesi: Dış bağımsız.
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ATATÜRK ÜNİVERSİTESİ YAYINLARI
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Determination of the İncidence and Types of Tooth Development Anomalies Using Cone Beam Computed Tomography

Diş Gelişim Bozukluklarının Görülme Sıklığının ve Tiplerinin Konik Işınlı Bilgisayarlı Tomografi Kullanarak Belirlenmesi

ABSTRACT

Objective: The aim of this retrospective study is to evaluate the presence of dental anomalies and the prevalence of anomaly types by examining cone beam computed tomography (CBCT) images obtained from patients presenting to the Faculty of Dentistry at Fırat University.
Methods: CBCT images of 1180 patients referred to the Department of Oral, Dental, and Maxillofacial Radiology were utilized. Images of patients with intact teeth, devoid of prosthetic or endodontic treatments, lacking periapical lesions, and with completed root development were included. Basic descriptive statistics such as frequency analysis were employed for data analysis, while comparisons were made using the chi-square test and Fisher's exact test.
Results: Anomalies were detected in 125 out of 1180 patients (80 females, 45 males), totaling 176 anomalous teeth. The prevalence of dental anomalies was determined to be 10.6%. Distribution by gender revealed 12% in females and 8.72% in males. Through radiographic examination, the most commonly identified anomalies were taurodontism (4.1%), radix molaris (3.3%), dilaceration (3%), dens invaginatus (0.17%), and fusion (0.08%). No cases of gemination were encountered.
Conclusion: Dentists should possess adequate knowledge regarding the prevalence of dental anomalies and their types, especially during root canal treatments. Misdiagnosis and improper treatment of a tooth with a planned root canal therapy due to dental anomalies can lead to treatment failure. The presence of dental anomalies can be successfully detected using CBCT, aiding in the assessment of root and canal structures of teeth.
Keywords: Dental Anomaly, Endodontics, CBCT

ÖZ

Amaç: Bu retrospektif çalışmanın amacı, Fırat Üniversitesi Diş Hekimliği Fakültesi'ne başvuran hastalardan alınan konik ışınlı bilgisayarlı tomografi (CBCT) görüntülerini inceleyerek dental anomalilerin varlığını ve anomali tiplerinin yaygınlığını değerlendirmektir.
Yöntemler: Ağız, Diş ve Çene Radyolojisi Anabilim Dalı'na sevk edilen 1180 hastanın CBCT görüntüleri kullanıldı. Sağlam dişleri olan, protez veya endodontik tedavi görmemiş, periapikal lezyonları olmayan ve kök gelişimi tamamlanmış hastaların görüntüleri dahil edildi. Veri analizi için frekans analizi gibi temel tanımlayıcı istatistikler kullanılırken, karşılaştırmalar ki-kare testi ve Fisher'ın kesin testi kullanılarak yapıldı.
Bulgular: 1180 hastanın 125'inde (80 kadın, 45 erkek) anomaliler tespit edildi ve toplam 176 anomalili diş bulundu. Dental anomalilerin yaygınlığı %10,6 olarak belirlendi. Cinsiyete göre dağılım kadınlarda %12, erkeklerde ise %8,72 olarak ortaya çıkmıştır. Radyografik inceleme ile en sık tespit edilen anomaliler taurodontizm (%4,1), radix molaris (%3,3), dilaserasyon (%3), dens invaginatus (%0,17) ve füzyon (%0,08) olmuştur. Geminasyon vakalarına rastlanmamıştır.
Sonuç: Diş hekimleri, özellikle kanal tedavileri sırasında, dental anomalilerin yaygınlığı ve tipleri hakkında yeterli bilgiye sahip olmalıdır. Dental anomaliler nedeniyle planlanan kanal tedavisi olan bir dişin yanlış teşhisi ve uygunsuz tedavisi, tedavi başarısızlığına yol açabilir. Dental anomalilerin varlığı, CBCT kullanılarak başarıyla tespit edilebilir ve dişlerin kök ve kanal yapılarının değerlendirilmesine yardımcı olur.
Anahtar Kelimeler: Diş Anomalisi, Endodonti, CBCT

INTRODUCTION

Developmental dental anomalies refer to irregularities observed in the number, size, shape, and eruption status of teeth during their formation process. Anomalies in tooth count, shape, and position arise from disruptions occurring during the morphodifferentiation stage of tooth development.[1]

Anomalies present in the crown and root regions pose clinical challenges that endanger the impermeable sealing of the root canal system, which is the primary goal of endodontic treatment's biomechanical and chemical preparation. Therefore, anatomical variations in the crown and root canal system, along with characteristic differences among races, should be identified before or during endodontic treatment.[2]

The prevalence and patterns of developmental dental anomalies provide significant insights into phylogenetic and genetic studies and aid in understanding differences among races. There are studies reporting the types and prevalence of dental anomalies in different populations.[3,4]

Various imaging methods are employed to determine tooth morphology and the prevalence of various anomalies. The internal anatomy of tooth roots and canals has been evaluated using canal staining, plastic resin injection, conventional radiographs, digital and contrast-enhanced radiographic techniques, in vitro macroscopic examination, scanning electron microscopy, computed tomography (CT), micro-CT, and cone beam computed tomography (CBCT).[5]

CBCT, one of these imaging methods, has emerged as a practical tool for non-invasive and three-dimensional (3D) reconstruction imaging, potentially aiding clinicians in endodontic treatments and root canal morphology analysis. It allows observation of an area in three different planes: axial, sagittal, and vertical. root canal morphology, the number of root canals, and their position and course relative to each other can be visualized in three dimensions.[6]

Some anomalies such as dilaceration, dens invaginatus, taurodontism, and radix molaris cannot be detected without radiographic examination. Therefore, findings from clinical-anatomical studies using CBCT can assist clinicians in preventing potential complications during endodontic treatments and significantly enhance treatment success.[7]

The aim of this retrospective study is to evaluate the presence of dental anomalies and the prevalence of anomaly types among the patient population presenting to our clinic within specific dates using CBCT.

METHODS

This study was conducted retrospectively using cone beam computed tomography (CBCT) images obtained from the archive of the Faculty of Dentistry at Fırat University between January 2016 and February 2021. Ethical approval for the study was obtained from the Non-Interventional Research Ethics Committee of Fırat University (Date: April 22, 2021 Number:2021/06-05).

Initially, CBCT images of a total of 1750 patients, both male and female, were evaluated. However, only patients over the age of 16 with fully erupted and completed root development healthy teeth were included in the evaluation. However, patients older than 16 years of age with fully erupted and healthy teeth with complete root development were included in the evaluation. Patients with unclear or corrupted CBCT images, those with pathological problems such as tooth loss (excluding third molars at age 20), posts, crowns, root canal treatments, and root resorption, were excluded from the study. Based on these criteria, CBCT images of a total of 1180 patients, comprising 516 males and 664 females, were utilized for the study.

For CBCT imaging, the Planmeca ProMax 3D Mid (Helsinki, FINLAND) with a voxel size of 0.4 mm and slice thickness of 0.2 mm was used. Three-dimensional images were generated using the Planmeca Romexis 4.4.3.R software according to the manufacturer's instructions.

The examination and measurements of the transferred axial, coronal, and sagittal two-dimensional slice images were conducted by an endodontist on a computer screen with a resolution of 1280 x 1024 pixels and a 15.6-inch display, adjusting contrast and brightness using the image processing toolbar in the Planmeca Romexis 4.4.3.R software to obtain the best image quality. In cases where a clear decision could not be reached, another endodontist was consulted to reach a consensus.

Criteria for Evaluating Dental Anomalies

- Radix Molaris: The presence of an additional root in mandibular molars was classified as radix entomolaris (RE) if located lingually (distolingually) and radix paramolaris (RP) if located facially (mesiobuccally).[8]
- Dens Invaginatus: Evaluated based on Hulsmann[9] classification, which categorizes invaginations into four groups: Type I, Type II, and Type III (A and B).
- Taurodontism: It was classified as hypotaurodont, mesotaurodont, hypertaurodont and pyramidal according to the taurodont index used by Bürklein et al.[10]
- Dilaceration: Defined as a deviation of more than 90 degrees of the apical part of the root from the normal axis of the tooth.[11]
- Fusion: Classified into four types: Type I - Bifid crown - Single root, Type II - Broad crown - Broad root, Type III - Compound two crowns - Broad root with a conical shape, Type IV - Compound two crowns - Two fused roots.[12]
- Gemination: Defined as teeth containing a large and dysmorphic crown. Separation may occur only in the crown or may be partially or completely separated into two crown shapes on a single root.[13]

Statistical Analysis

Power analysis for the chi-square test conducted using the G Power program (G*Power 3.1 software; Heinrich Heine University, Düsseldorf, Germany) revealed a minimum sample size of 59 to achieve a power of 0.95 with an alpha (error rate) of 0.05 and a minimum effect size (W) of 0.40, based on previous studies indicating anomalies in approximately 5% of the evaluated CBCT images. Therefore, 1180 CBCT evaluations were deemed appropriate to meet the minimum anomaly sample required. Basic descriptive statistics, such as frequency analysis, and comparisons using the chi-square test and Fisher's exact test were employed for data analysis. All analyses were conducted with a 95% confidence interval.

RESULTS

CBCT images of a total of 1180 patients (664 females and 516 males), aged between 16 and 64, who presented to the Faculty of Dentistry, Fırat University, were screened for the presence of dental anomalies. CBCT images were evaluated and anomalies were detected in 125 of 1180 patients (total rate 10.6%), including 80 of 664 females (gender ratio 12%) and 45 of 516 male (gender ratio 8.72%). Multiple anomaly types were observed in 3 patients, while anomalies were found in multiple teeth of 25 patients. Upon image examination, radix molaris was identified in 39 patients (3.3%), with 23 females (3.46%) and 16 males (3.1%). Taurodontism was detected in 48 patients (4.1%), with 28 females (4.21%) and 20 males (3.87%). Dilaceration was found in 35 patients (3%), with 28 females (4.21%) and 7 males (1.35%). Dens invaginatus was observed in 2 patients (0.17%), with 1 female (0.15%) and 1 male (0.19%). Fusion was detected in 1 male patient (0.19%), while gemination was not encountered. Statistical analysis revealed no significant difference in the types of anomalies between genders (P=.834) (Table 1).

Table 1. Prevalence distribution of anomaly types according to gender

Anomalies	Female	Male	Total
Radix molaris	23 (3.46%)	16 (3.1%)	39 (3.3%)
Taurodontism	28 (4.21%)	20 (3.87%)	48 (4.1%)
Dilaceration	28 (4.21%)	7 (1.35%)	35 (3%)
Dens invaginatus	1 (0.15%)	1 (0.19%)	2 (0.17%)
Fusion	_	1 (0.19%)	1 (0.08%)
Gemination	_	_	0
Total	80 (12%)	45 (8.72%)	125 (10.6%)

Radix molaris was classified as radix entomolaris and radix paramolaris. Among these patients, radix entomolaris was observed in 30 (76.9%), and radix paramolaris was observed in 9 (23.1%). Evaluation indicated a statistically significant difference between radix entomolaris and radix paramolaris (*P*=.001) (Table 2). Statistical analysis did not reveal a significant difference between genders (*P*=.863).

Table 2. Demographic data of Radix Molaris samples.

	Number of Patients with Radix Molaris (n=39)	*P*
Female	23	.863
Male	16	
Radix Entemolaris	30	.001
Radix Paramolaris	9	

Taurodontism was subclassified into hypotaurodontism, mesotaurodontism, hypertaurodontism, and pyramidal types. Among the 48 patients with taurodontism, 20 (41.6%) were hypotaurodont, 3 (6.25%) were mesotaurodont, 3 (6.25%) were hypertaurodont, and 22 (45.8%) were pyramidal types. Statistical analysis revealed a significant difference among the subclasses of taurodontism (*P*=.001). Of these, 30 (62.5%) were observed in the maxilla, and 18 (37.5%) were in the mandible. A statistically significant difference was found between the distribution of taurodontism in the maxilla and mandible (*P*=.001) (Table 3). Statistical analysis did not reveal a significant difference between genders in patients with taurodontism (*P*=.469).

Dilaceration was classified according to the tooth groups where it was detected (incisors, premolars, and molars). Among the 35 patients, dilaceration was detected in 17 (48.6%) incisors, 14 (40%) premolars, and 4 (11.4%) molars. Dilaceration was statistically more prevalent in incisors and premolars compared to molars (*P*=.001) (Table 4). No statistically significant difference was found between the genders of patients with dilaceration (*P*=.498).

Table 3. Demographic data of Taurodontism samples.

	Number of Patients with Taurondontism (n=48)	*P*
Female	28	.469
Male	20	
Hypotaurodont	20	.001
Mesotaurodont	3	
Hypertaurodont	3	
Pyramidal	22	
Mandible	18	.001
Maxilla	30	

Table 4. Demographic data of Dilaceration samples.

	Number of Patients with Dilaceration (n=35)	*P*
Female	28	.498
Male	7	
Incisor	17	.001
Premolar	14	
Molar	4	

Among the 2 patients with dens invaginatus, it was observed in the left upper canine (type 3(A)) in the male patient and in the right upper central incisor (type 3(B)) in the female patient. All observed anomalies were in the maxilla; dens invaginatus was not observed in the mandible. No statistically significant difference was found between genders (*P*=.674). Fusion was detected in only 1 (0.08%) patient, who was a male, observed in the left lower lateral incisor (type 2 = wide crown wide root). Gemination was not detected in the examined patient images. CBCT images of all dental anomalies are shown in Figure 1.

Figure 1. CBCT images of dental anomalies. **A:** Taurodontism, **B:** Dilaceration, **C:** Fusion, **D:** Dens Invaginatus, **E:** Radix Molaris

DISCUSSION

Dental anomalies have an important place in the diagnosis, treatment and prognosis of the tooth. Especially, shape anomalies that significantly affect root-crown morphology have a considerable impact on the difficulty and success rate of endodontic treatment. This study aimed to investigate shape anomalies that can be radiologically detected and affect crown-root morphology. In the literature, there are studies evaluating patients within a similar age range whose tooth development has been completed.[14] In our study, as in other studies, CBCT images of permanent dentition were evaluated. The age range of the examined CBCT images was found to be between 16 and 64 years.

When similar studies are examined, Keleş et al.[5] used panoramic radiographs in a study conducted on the Turkish subpopulation. Taşsöker et al.[15], on the other hand, used CBCT in their study. Karabulut et al.[16] used panoramic and periapical radiographs in their study on the population of Northern Cyprus. Different techniques have been used in recent studies to examine the prevalence of anatomical variations. Among these techniques, panoramic radiographs, periapical radiographs, and CBCT are the most commonly used imaging methods. These methods have advantages and disadvantages compared to each other. CBCT has high resolution and accuracy compared to other radiographic techniques. Examining images in three different sections reduces the margin of error in the evaluation.[15] Due to these advantages, CBCT was used in our study.

Similar to the study by Alavi et al.[17] our study included patients without any tooth loss (excluding third molars), without prosthetic restorations, and without undergoing endodontic treatment. Images with any restorations, having undergone surgical operations, or having undergone endodontic treatment were not included in the study as they could cause changes in tooth crown-root morphology and also create artifacts radiographically, hindering accurate evaluation.

In studies conducted in different populations, the prevalence of dental anomalies ranges widely from 2.5% to 48.6%.[4,14,18] In our study, the prevalence of dental anomalies was found to be 10.6%. Different prevalence values in the literature can be attributed to differences in races, diagnostic methods used (radiographic techniques), and criteria for evaluating anomalies.

While some researchers report statistically significant differences between genders in the prevalence of dental anomalies, others have reported no significant difference.[16,19] In our study, although the frequency of dental anomalies was higher in females compared to males, there was no statistically significant difference.

In studies using periapical radiography, the frequency of radix molaris in the Turkish population has been reported as 1% [20], 21.09% in the Taiwanese population.[21] In studies evaluating CBCT images, the frequency has been reported as 29.7% in the Chinese population [22] and 2.06% in the Turkish population.[23] In this study, the prevalence of radix molaris was found to be 3.3%. When evaluated according to radix molaris types, Demirbuğa et al.[23] stated that the prevalence of radix entomolaris was higher than radix paramolaris. In our study, it was also determined that radix entomolaris was significantly more prevalent than radix paramolaris.

In the literature, although the prevalence of taurodontism varies widely from 2.5% to 48.6% in different populations, in the Turkish population, this rate has been reported as 1%-17.1%.[5,18,24,25] In this study, the rate of taurodontism was found to be 4.1%. The variability in the frequency of taurodontism may be attributed to differences among races. When evaluated in terms of taurodontism types, Umar et al.[26] reported in their study on the Turkish population that hyper-taurodontism was the most common and hypo-taurodontism was the least common. In our study, hypo-taurodontism and pyramidal type were the most common, while hyper-taurodontism was the least common. The variation in the prevalence of taurodontism types is thought to be due to the imaging techniques used. It is anticipated that CBCT, compared to periapical and panoramic radiographs, will provide more accurate measurements and evaluations on three-dimensional images. Additionally, similar to the findings of this study, two studies conducted on the Turkish population have also found that taurodontism is more common in females compared to males.[26,27]

In some prevalence studies conducted on dilaceration, the prevalence value has been reported to range from 3.2% to 17%.[3,4,11] Karabulut et al.[16] reported a dilaceration prevalence of 20.6% in their study examining panoramic radiographs in the population of Northern Cyprus. Uslu et al.[24], in their study on the Turkish population using panoramic and periapical radiographs, found a dilaceration prevalence of 3.2%. In our study, the prevalence value of dilaceration was 3%. The finding of widespread prevalence values in studies may be attributed to trauma during tooth development.

Although the etiology of dens invaginatus is not fully reported, there are views regarding its association with environmental and genetic factors.[28-30] The frequency of occurrence of dens invaginatus in studies conducted on the Turkish population has varied between 0.5% and 2.54%.[5,14,29] In this study, the frequency of dens invaginatus was found to be 0.17%. These three studies used panoramic and periapical radiographs as imaging methods. Therefore, the values may differ from this study. It has been reported that dens invaginatus is most commonly seen in the maxilla and rarely in the mandible. Studies conducted on the Turkish population have also reported that it is most commonly seen in the maxilla.[5,14,29] In our study, all teeth with dens invaginatus were detected in the maxilla. When evaluated according to the types of this anomaly, according to the Hulsmann classification, type 3 dens invaginatus was the most common. Keleş et al.[5] did not classify dens invaginatus in their studies. Gündüz et al.[29] and Çakıcı et al.[14], in their studies examining panoramic and periapical radiographs in the Turkish population, reported that type 1 dens invaginatus was the most common. It is believed that using three-dimensional imaging methods will provide more accurate results when classifying dens invaginatus.

In prevalence studies conducted, fusion and gemination have been evaluated together as twin teeth. Data from the literature indicate that twin teeth are seen in 0.0% to 0.8% of the general population without gender distinction.[31,32] Şekerci et al.[33] reported a prevalence of 0.29% for twin teeth (fusion and gemination) in their study examining panoramic radiographs in the Cappadocia region. Karabulut et al.[16] reported a fusion frequency of 0.1% in their study examining panoramic radiographs in the population of Northern Cyprus. In this study, this value was found to be 0.08%. The results obtained are in line with the literature. Çelenk et al.[34], in their studies, reported that fusion is more common in the mandible than in the maxilla. Şekerci et al.[33] reported that the prevalence of fusion was higher in the maxilla in their study. In our study, fused teeth were detected in the mandible.

CONCLUSION

In general, it is believed that conducting prevalence studies in different racial populations, differences in diagnostic criteria, and variations in the use of radiographic techniques contribute to different results in the literature. It is thought that the use of more reliable radiographic techniques will increase the accuracy of the studies conducted.

Knowledge of both normal and abnormal anatomy of teeth determines the procedures for root canal treatment and can directly affect the likelihood of treatment success. It is recommended to detect these anomalies using appropriate radiographic methods before or during canal treatment.

Ethics Committee Approval: Ethical approval for the study was obtained from the Non-Interventional Research Ethics Committee of Fırat University (Date: April 22, 2021 Number:2021/06-05.

Informed Consent: Informed consent was not obtained due to the retrospective nature of this study.

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Effects of Two Appliances Used in the Correction of Class II Malocclusion on Mandibular Symphysis Morphology: Biblock and Activator

Sınıf II Maloklüzyonun Düzeltilmesinde Kullanılan İki Apareyin Mandibular Simfiz Morfolojisi Üzerindeki Etkileri: Biblok ve Aktivatör

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ABSTRACT

Objective: This study aimed to evaluate and compare the effects of two functional appliances, the Activator (AA) and the Biblock (BA), on mandibular symphysis (MS) morphology in patients with Class II malocclusion.

Methods: Records of patients treated at the Department of Orthodontics, Ankara University, between 2011 and 2021 were retrospectively analyzed. Inclusion criteria were mandibular deficiency, optimal growth pattern, and CS2–CS3 cervical maturation stage. Twenty patients treated with the Biblock (10 females, 10 males; mean age: 13.52±1.36 years) were identified. An age- and sex-matched control group of 20 patients treated with the Activator (mean age: 13.78±1.18 years) was selected. Lateral cephalometric radiographs taken before and after treatment were evaluated for angular and linear changes. Intragroup comparisons were performed with paired t-tests, and intergroup comparisons with independent t-tests.

Results: The mean treatment duration was 17.39±5.94 months in the BA group and 17.03±4.37 months in the AA group. Both appliances significantly improved Class II malocclusion. Significant anterior MS changes were observed in both groups, except for B-Ida-Idp. Upper MS width remained unchanged, while significant increases occurred in the middle (AA: 0.4±0.5 mm; BA: 0.4±0.5 mm) and lower widths (AA: 0.9±0.2 mm; BA: 0.8 ± 0.7 mm) (P<.005). Intergroup comparisons showed greater reduction in Pg-B-Ida and greater increase in Pg-B in the BA group (P<.05).

Conclusion: Functional treatment flattened MS curvature, promoted forward growth of B and Pg points, and altered anterior MS morphology. The effects of AA and BA on MS morphology were largely similar.

Keywords: Class II malocclusion, Functional appliance, Biblock, Activator, Mandibular symphysis morphology

ÖZ

Amaç: Amaç: Bu çalışmanın amacı, Sınıf II maloklüzyonları tedavi etmek için kullanılan iki fonksiyonel aparey olan Aktivatör (AA) ve Biblok'un (BA) mandibular simfiz (MS) morfolojisi üzerindeki etkilerini araştırmak ve karşılaştırmaktır.

Yöntemler: Mandibular retrognati ve optimum büyüme paterninden kaynaklanan iskeletsel Sınıf 2 maloklüzyonu olan 40 hasta bu retrospektif çalışmaya dahil edildi. BA grubunun seçilmesinin ardından (n=20, 10 erkek ve 10 kız, ortalama yaş=13,52±1,36), cinsiyet ve gelişim açısından bu grupla tam olarak eşleşen AA ile tedavi edilen aktif kontrol grubu (n=20, 10 erkek ve 10 kız, ortalama yaş=13,78±1,18) seçildi. Tüm hastalar T0'da CS2-CS3 servikal matürasyon aşamasındaydı. İskeletsel, dental ve MS ile ilgili sefalometrik değişkenler ölçüldü. Bulgular: Tedavi süresi BA ile 17,39±5,94 ay, AA ile 17,03±4,37 ay olarak bulundu. Sınıf II maloklüzyon her iki grupta da anlamlı olarak düzeldi. Her iki grupta da B-Ida-Idp hariç, MS'in anterior ölçümlerinde anlamlı değişiklikler vardı. Her iki grupta da üst MS genişliğinde değişiklik olmazken, orta genişlikte (AA:0,4±0,5 mm; BA:0,4±0,5 mm) ve alt genişlikte (AA:0,9±0,2 mm; BA:0,8±0,7 mm) anlamlı artış gözlendi (P<,005). Gruplar karşılaştırıldığında, Pg-B-Ida'daki azalma ve Pg-B'deki artış BA grubunda anlamlı olarak daha fazlaydı (P<,05).

Sonuç: Fonksiyonel tedavi ile MS eğriliği düzleşti, B ve Pg noktaları öne doğru büyüdü ve MS'nin ön morfolojisi değişti. AA ve BA'nın MS morfolojisi üzerindeki etkileri benzerdir.

Anahtar Kelimeler: Sınıf II maloklüzyon, Fonksiyonel aparey, Biblock, Aktivatör, Mandibular simfiz morfolojisi

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INTRODUCTION

Functional treatment improves jaw relations and profile by changing facial proportions in individuals who continue to grow. Functional treatment of Class II malocclusion is possible with various appliances.[1-5] Activator (AA) and Biblock (BA) are some of these appliances. AA has been the subject of many studies[1-3,5] until today, while BA was designed by the second author (MR) and has been used for over ten years. Many studies reported that functional appliances inhibit maxillary development and stimulate mandibular growth.[2-5]

Through stimulation of mandibular growth, the mandible is repositioned sagittally-vertically and the morphology of the mandibular symphysis (MS) reshapes. MS morphology is an important anatomical structure for orthodontic treatment planning and ideal facial aesthetics. Many factors, such as genetic factors, functional adaptation of muscles, changes in vertical face dimensions, and position of lower incisors are responsible for the formation of MS morphology.[6-8] The lower incisor orthodontic movement amount is closely related to the MS dimension, an anatomical limitation. According to Al-Khateeb et al.[9], the inclination of the lower incisors may indirectly affect the MS morphology during the growth period because positional changes of the teeth are a dentoalveolar compensation against deviations in the anteroposterior direction in jaw relations. The low labiolingual thickness of the alveolar bone in MS causes the lower incisors to have thin bone support. One of the undesirable effects of functional treatments is lower incisor protrusion.[4] Therefore, clinicians need to carefully evaluate the amount of protrusion that occurs with functional treatment and the limitations of MS. In addition, uncontrolled protrusion of the lower incisors may cause the gingival margin to slide downwards and bone dehiscence. [10,11]

The literature focuses on the structure of MS[8,9,12,13] rather than the changes that occur with the treatment effect. No study has investigated the changes in MS morphology with the effect of functional treatment. Therefore, this study aimed to investigate and compare the effects of AA and BA on MS morphology.

METHODS

The study received ethical approval from the Ethics Committee of Gazi University (October 3,2023, Approval Number: 2023-1174). This retrospective study included 40 skeletal Class II malocclusion patients treated with AA or BA in Gazi University.

Individuals with Angle Class II malocclusion, Class 2 relationship originating from the mandible (ANB>4°, SNB≤78°, SNA≤84°; according to Steiner analysis,[14] optimum angle (26°≤SN\MP≤38°; according to Steiner analysis,[14] and CS2-CS3 skeletal maturation phase (according to the cervical vertebra maturation (CVM) index of Baccetti[15] and demonstrates good cooperation according to clinical record information were included in the study. Individuals with a history of orthodontic treatment, systemic disease, or syndrome were excluded from the study.

The sample size was calculated based on a previous study with GPower software (GPower Version 3.1.9.7, Heinrich-Heine University, Düsseldorf, Germany) using two independent samples t-tests. Based on an alpha significance level of 0.05 and a beta of 0.1 to achieve 90% power, to detect a minimum difference of 1,36 mm overjet elimination with a 1.85 mm of estimated standard deviation,[16] 16 subjects per group was required.

The BA group comprised of 20 cases treated by the second author (MR) between 2011 and 2021. The mean age was 13.52 years (SD=1.36) (Table 1), 10 girls and 10 boys. The AA group consisted of 20 Class II cases, 10 girls and 10 boys, with a mean age of 13.78 (SD = 1.18) (Table 1), and their sex and developmental stage were matched with the BA group.

Table 1. Chronological Age (year) and Treatment duration (month) of the groups

	AA			BA			*P*
	$\bar{X}$± SD	Min	Maks	$\bar{X}$± SD	Min	Maks	
Chronological age (T0)	13.78±1.18	11.57	15.5	13.52±1.36	10.83	16	.520
Treatment duration	17.03±4.37	10.92	26.04	17.39±5.94	10.92	27	.826

Both groups had records collected before treatment (T0) and after functional appliance application (T1). Patients were evaluated with the CVM index[15] before treatment (T0). They were in the CS2-CS3 period in terms of skeletal maturity.

Bite record was taken with a one-step advancement (maximum jumping) in both appliance groups and involved adding 3 mm vertically to the freeway space and using sagittal activation with mandibular advancement up to 7 mm. All appliances were made by the same orthodontic technician using 0.7 mm stainless steel wire and heat-curing acrylic.

Treatment Protocol

BA consisted of two parts - the maxillary and mandibular plates. Both plates included Adams clasps, ball clasps, and a labial bow (Figure 1A). A midline expansion screw was also incorporated into both plates. Patients were instructed to activate the maxillary expansion screw (Leone S.P.A., Standard 11 mm, Floransa, Italy) once every five days and the mandibular screw (Leone S.P.A., Universal 10 mm, Floransa, Italy) once a week until the desired expansion was achieved. The expansion protocol lasted an average of 6 months. When protrusion was required, pitpoints (protrusion points), small acrylic pieces, were added to the lingual of the upper anterior teeth (Figure 1B). Pitpoints were constructed as an alternative to labiolingual springs. To move the mandible anteriorly easily, spontaneously, and effectively, a curve of spee was designed between two plates in the BA (Figure 1C). In addition, anterior movement of the mandible was supported by using 200 grams of intraoral elastics on each side (400 grams in total). This elastic was applied between the U-bend of the maxillary labial bow and the mandibular Adams clasps (Figure 1D). Intra and extraoral images of an individual treated with BA are shown in Figure 2.

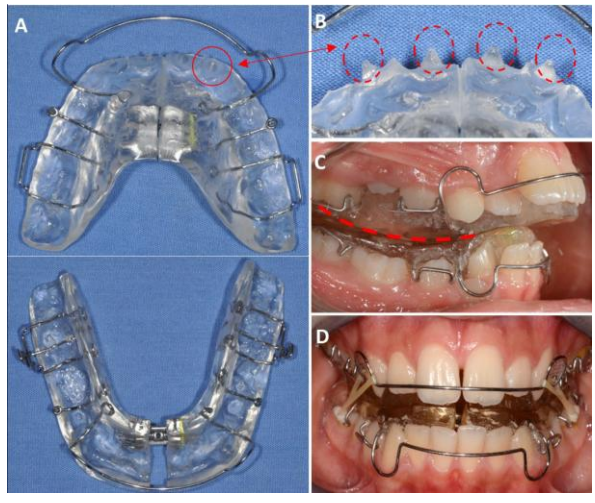

Figure 1. BA introduction. A: BA design; B: Pitpoint application; C: Curve of spee application; D: Class II elastic application

Figure 2. Progression of the patient in the BA group from start to finish. A: Class II malocclusion, increased overbite and convex profile; B: 8 months progress; C: Treatment finished at 12 months with Class I molar relationship, decreased overbite and improved profile.

AA design included a labial bow, Adams and ball clasps in the upper dental arch, and an acrylic cap splint in the lower anterior teeth. A labiolingual spring was applied when the upper anterior teeth protrusion was necessary. Trimming from acrylic was performed for posterior interdigitation after the Class I relationship was achieved, especially in Class II div 2 cases. Intra and extraoral images of an individual treated with AA are shown in Figure 3.

Figure 3. Progression of the patient in the AA group from start to finish. A: Class II malocclusion, increased overjet and convex profile; B: 10 months progress; C: Treatment finished at 16 months with Class I molar relationship, decreased overjet and improved profile.

Both groups were advised to use their appliances full time, excluding meals, and were invited for examination every four weeks. Both groups received treatment until the mandible no longer moved backward and achieved maximum interdigitation of posterior teeth.

Cephalometric Analysis

For standardization, all radiographs were obtained using the same X-ray device (Sirona Orthophos XG5, Sirona Dental Company, New York, USA). Changes on lateral cephalometric radiographs in T0 and T1 were analyzed by PORDIOS (Purpose on Request Digitizer Input Output System for Windows). The same examiner (BÇ) performed all radiographic measurements and analyses. Figures 4 and 5 show the cephalometric variables used in the analysis.

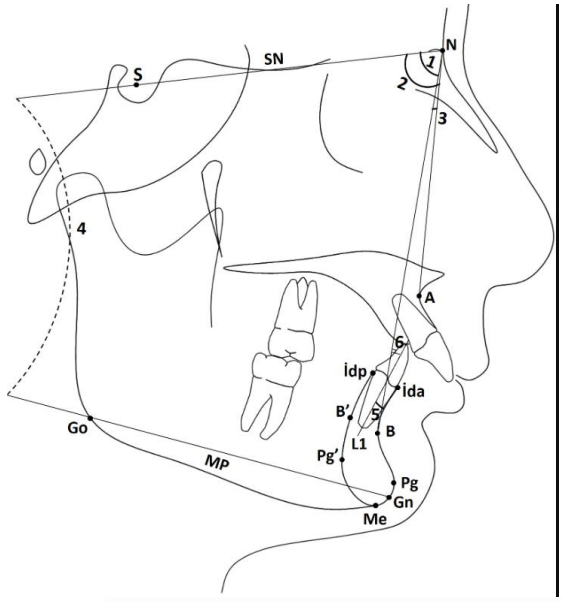

Figure 4. The landmarks, reference planes, and dentoskeletal parameters definition. *Landmarks:* S (Sella); N (Nasion); A (Point A); B (Point B); Pg (Pogonion); Gn (Gnathion); Me (Menton); Go (Gonion); Pg' (Pg point projection in the posterior symphysis); B' (B point projection in the posterior symphysis); Ida (Infradentale anterior; the highest anterior point of alveolar bone; Idp (Infradentale posterior; the highest posterior point of alveolar bone). *Reference planes:* SN (Nasion-Sella line); MP (Mandibular plane); L1 (long axis of the most labial mandibular incisor). *Dentoskeletal parameters:* 1. SNA(°): Nasion-centered angle between S, N, and A; 2. SNB(°): Nasion-centered angle between S, N, and B; 3. ANB(°): Nasion-centered angle between A, N, and B; 4. SN/MP(°): Angle of SN with MP; 5. L1/NB(°): Angle between the L1 and NB; 6. L1⊥NB(mm): The perpendicular distance L1 and NB.

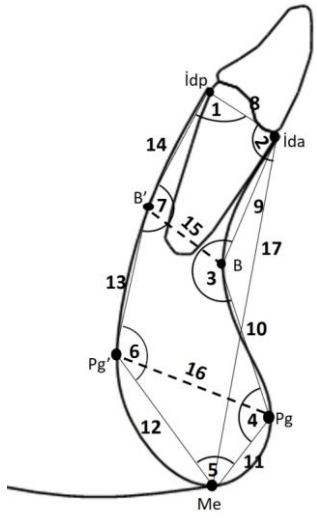

Figure 5. Symphysis parameters definition. 1. Ida-Idp-B'(°): Symphysis posterior-superior surface angle; 2. B-Ida-Idp(°): Symphysis anterior superior surface angle; 3. Pg-B-Ida(°): Symphysis anterior-middle surface angle; 4. Me-Pg-B(°): Symphysis anterior-lower surface angle; 5. Pg'-Me-Pg(°): Mental angle; 6. B'-Pg'-Me(°): Symphysis posterior-lower surface angle; 7. Idp-B'-Pg'(°): Symphysis posterior-middle surface angle; 8. Ida-Idp(mm): Upper MS width; 9. B-Ida(mm): Anterior-superior symphysis length; 10. Pg-B(mm): Anterior-middle MS length; 11.Me-Pg(mm): Anterior-inferior MS length; 12. Pg'-Me(mm): Posterior-inferior MS length, 13. B'-Pg'(mm): Posterior-middle MS length; 14. Idp-B'(mm): Posterior-superior MS length; 15. B-B'(mm): Middle MS width; 16. Pg-Pg'(mm): Lower MS width; 17. Ida-Me(mm): Anterior height of MS.

Method error analysis

Measurement error was made on lateral cephalograms of 20 randomly selected patients. The intraclass correlation coefficient (ICC) between the two measurements was used to calculate R. R showed high reliability, with reliability values ranging from 0.805 to 0.976 (Table 2).

Table 2. Error measurements

Measure	R	Measure	R	Measure	R	Measure	R
SNA	0.932	İda-Me	0.825	B'-Pg'-Me	0.901	İdp-B'	0.975
SNB	0.906	Me-Pg-B	0.976	Pg'-Me-Pg	0.968	B'-Pg'	0.88
ANB	0.91	Pg-B-İda	0.922	Me-Pg	0.87	B-B'	0.88
SN/MP	0.9	B-İda-İdp	0.864	Pg-B	0.886	Pg-Pg'	0.95
L1⊥NB	0.939	İda-İdp-B'	0.805	B-İda	0.932	Pg'-Me	0.874
L1/NB	0.914	İdp-B'-Pg'	0.912	İda-İdp	0.966		

Statistical Analysis

SPSS Statistics software (version 22.0. IBM SPSS Corp., Armonk, NY, USA) was used to analyze the data. The normality of the data was tested with Shapiro-Wilk's test. The data were analyzed based on means, standard deviations, and differences. Differences within and between groups between the two time points were tested for significance using paired-sample t-tests and independent-sample t-tests. *P* <.05 was considered statistically significant

RESULTS

The mean treatment duration was 17.03±4.37 months for the AA group and 17.39±5.94 months for the BA group; the mean age was 13.78±1.18 years for the AA group and 13.52±1.36 years for the BA group, with no statistically significant differences between groups in these two parameters (Table 1). Initial analysis of the group's dentoskeletal and MS characteristics at T0 showed no statistically significant differences between the two groups, except for B-B' (Table 3).

Dentoskeletal Changes

Both groups had significant skeletal measurements and changes in SNA, SNB, ANB, and SN/MP angles. In the AA group, SNA (0.5±0.4°) and ANB (2.6±0.8°) decreased; SNB (2.1±0.9°), SN/MP (1.3±1.1°) increased (*P*<.001). In the BA group, SNA (0.3±0.5°) and ANB (2.1±0.8°) decreased; SNB (2±0.7°) and SN/MP (1±1.2°) increased (*P*<.05). Significant lower incisor protrusion was observed in both groups. The AA group showed an increase of 3.1±2.1° in the L1/NB angle and 1.1±0.8 mm in the L1⊥NB, while the BA group demonstrated an increase of 3.1°±2.7 in the L1/NB angle and 1.2±0.9 mm in the L1⊥NB (*P*<.001). No significant differences exist between groups on dentoskeletal measurements (*P*>.05) (Table 4).

MS Changes

Changes in anterior surface measurements of the MS were significant in both groups, except for B-Ida-Idp. In the AA group, B-Ida (0.6±0.6 mm), Pg-B (0.7±0.7 mm), Me-Pg (0.7±0.7 mm) increased significantly, while Pg-B-Ida (3.4 ±2 mm) and Me-Pg-B (2±1.3°) decreased significantly. In the BA group, B-Ida (0.7±1.2 mm), Pg-B (1.5± 1.1 mm), Me-Pg (0.7±1 mm) increased significantly, while Pg-B-Ida (5±1.6 mm) and Me-Pg-B (2.3±1.8°) decreased significantly (*P*<.05). In the AA group, 0.4±0.5 mm in the B-B' dimension, 0.9±0.2 mm in the Pg-Pg' dimension, and 1.4±1.2 mm in the Ida-Me dimension the increase was statistically significant (*P*<.005). Similarly, in the BA group, the B-B' dimension increased by 0.4±0.5 mm, the Pg-Pg' dimension increased by 0.8±0.7 mm, and the Ida-Me dimension increased by 1.8±1.9 mm (*P*<.005). No statistically significant differences existed between the groups in the MS measurements, except for Pg-B-Ida and Pg-B (Table 4).

Table 3. Descriptive statistics and comparison of T0 values within groups

	AA						BA						
	T0			T1			T0			T1			
	Min	Max	X̄± SD	Min	Max	X̄± SD	Min	Max	X̄± SD	Min	Max	X̄± SD	T0-T0 Pa
SNA	78.9	84	81.6±1.4	78.4	83.7	81.1±1.5	76.9	83.5	81.2±2	76.8	83.3	80.9±1.9	0.463
SNB	73	78	76±1.4	75.2	80.2	78.1±1.2	70.4	78.8	75.4±2.2	71.2	80.9	77.4±2.2	0.323
ANB	4.1	8.3	5.7±1.2	1.1	5.1	3±1.2	4.3	8.4	5.8±1.1	1.7	5.9	3.7±1.2	0.718
SN/MP	26.3	35.6	31.3±3	27	37.7	32.6±3	27.4	37.7	32.8±2.9	28.4	39	33.9±2.9	0.112
L1⊥NB	1.3	11.5	5.3±2.2	1.4	13.2	6.3±2.7	2.7	9.6	5.4±2.1	3.6	11.2	6.6±2.2	0.89
L1/NB	18.7	35.3	28.5±4.7	21.3	39	31.6±4.5	18.4	40.3	28.2±5	22.5	40.8	31.3±5	0.842
İda-Me	20.9	34.2	29.6±3.3	21.7	36.7	31±4.1	23.5	37.1	29.6±3.5	24.8	39.5	31.4±3.7	0.993
Me-Pg-B	120.2	143.1	135.2±6.4	116.4	141.1	133.1±6.4	122.7	147	135.9±6.2	119.1	144	133.6±6.4	0.721
Pg-B-İda	203.5	220.2	211.7±5.2	201.9	216.3	208.3±4.5	201.3	228.9	216.5±7	197.4	221.9	211.5±6.5	0.078
B-İda-İdp	89.7	109.7	99±5.2	86.7	111	98.7±6.4	81	112.8	101.1±6.4	89.5	113.6	100.9±5.1	0.262
İda-İdp-B'	80.2	105.9	94.8±6.9	82.5	107.1	94.9±6.9	74.1	117.1	92.9±12.2	80.7	116	94.8±9.6	0.546
İdp-B'-Pg'	145.8	178.6	169.1±8	157.8	178.5	168.2±5.7	154.4	178.2	165.5±8	150.4	176.6	165.3±7.8	0.169
B'-Pg'-Me	108.7	141.7	127.3±9.3	111.2	141.9	127.4±7.9	99.4	144.4	123.8±12.6	101.6	150.4	124.1±12.9	0.323
Pg'-Me-Pg	53	80.9	73.3±7	54.4	81.8	77.2±6.6	54	90.5	75.2±8.8	60.3	90.9	80.8±7.1	0.128
Me-Pg	4.9	12.9	9.4±1.7	4.7	14.9	10.2±2.1	6.5	11.8	8.9±1.5	7	12	9.7±1.3	0.305
Pg-B	8.7	14.6	12.1±1.7	9.8	16.2	12.8±1.8	8.4	15.2	11.6±1.8	9.8	17.2	13.1±2.1	0.357
B-İda	5.4	11.8	9.2±2	6.6	12.4	9.8±2	7	15.5	10.6±2.3	7.3	16.2	11.3±2.5	0.057
İda-İdp	5.2	9.9	7.5±1.2	5.4	10.4	7.5±1.3	5.7	8.2	7±0.6	5.6	8.1	6.9±0.6	0.093
İdp-B'	4.6	13.7	10.1±2.4	5.4	13.5	10.1±2.4	8.1	14.6	10.8±2	8	14.5	10.8±1.9	0.292
B'-Pg'	7.6	14.3	10.5±1.9	8.2	13.7	10.7±1.6	9.7	17.2	11.5±1.8	9.4	15.9	11.8±1.7	0.089
B-B'	7.1	9.9	8.5±0.9	7.3	10.8	8.9±1	7.3	10.2	9.2±0.7	8.4	11.3	9.6±0.8	0.008*
Pg-Pg'	7.2	11	9.8±1	7.9	12.3	10.7±1.1	7.9	11	9.8±1	8.6	12.1	10.6±1.2	0.995
Pg'-Me	11.2	19.4	16.2±2.3	10.3	19.8	16.2±2.5	10.1	18	14.9±2.3	9.7	18.2	15±2.8	0.073

*Independent sample t test, X̄:Mean, SD:Standard deviation, Min:Minimum, Max:Maximum, *P<.05, Pa:T0-T0.*

Table 4. Comparison of T0 and T1 values within groups and comparison of differences between groups with treatment

	AA				BA				
	T0 X̄± SD	T1 X̄± SD	T1-T0 X̄± SD	Pb	T0 X̄± SD	T1 X̄± SD	T1-T0 X̄± SD	Pb	Pc
SNA	81.6±1.4	81.1±1.5	-0.5±0.4	<.000*	81.2±2	80.9±1.9	-0.3±0.5	0.016*	0.263
SNB	76±1.4	78.1±1.2	2.1±0.9	<.000*	75.4±2.2	77.4±2.2	2±0.7	<.000*	0.617
ANB	5.7±1.2	3±1.2	-2.6±0.8	<.000*	5.8±1.1	3.7±1.2	-2.1±0.8	<.000*	0.057
SN/MP	31.3±3	32.6±3	1.3±1.1	<.000*	32.8±2.9	33.9±2.9	1±1.2	0.001*	0.444
L1⊥NB	5.3±2.2	6.3±2.7	1.1±0.8	<.000*	5.4±2.1	6.6±2.2	1.2±0.9	<.000*	0.673
L1/NB	28.5±4.7	31.6±4.5	3.1±2.1	<.000*	28.2±5	31.3±5	3.1±2.7	<.000*	0.992
İda-Me	29.6±3.3	31±4.1	1.4±1.2	<.000*	29.6±3.5	31.4±3.7	1.8±1.9	<.000*	0.418
Me-Pg-B	135.2±6.4	133.1±6.4	-2±1.3	<.000*	135.9±6.2	133.6±6.4	-2.3±1.8	<.000*	0.656
Pg-B-İda	211.7±5.2	208.3±4.5	-3.4±2	<.000*	216.5±7	211.5±6.5	-5±1.6	<.000*	0.01*
B-İda-İdp	99±5.2	98.7±6.4	-0.3±5	0.815	101.1±6.4	100.9±5.1	-0.2±4	0.862	0.94
İda-İdp-B’	94.8±6.9	94.9±6.9	0.1±5.7	0.933	92.9±12.2	94.8±9.6	2±8	0.278	0.396
İdp-B'-Pg'	169.1±8	168.2±5.7	-0.9±5.7	0.498	165.5±8	165.3±7.8	-0.3±3.6	0.757	0.68
B'-Pg'-Me	127.3±9.3	127.4±7.9	0±4.3	0.974	123.8±12.6	124.1±12.9	0.3±5.6	0.828	0.879
Pg'-Me-Pg	73.3±7	77.2±6.6	3.9±2.5	<.000*	75.2±8.8	80.8±7.1	5.6±6	0.001*	0.261
Me-Pg	9.4±1.7	10.2±2.1	0.7±0.7	<.000*	8.9±1.5	9.7±1.3	0.7±1	0.004*	0.983
Pg-B	12.1±1.7	12.8±1.8	0.7±0.7	<.000*	11.6±1.8	13.1±2.1	1.5±1.1	<.000*	0.009*
B-İda	9.2±2	9.8±2	0.6±0.6	<.000*	10.6±2.3	11.3±2.5	0.7±1.2	0.017*	0.652
İda-İdp	7.5±1.2	7.5±1.3	0±0.5	0.668	7±0.6	6.9±0.6	-0.1±0.3	0.35	0.401
İdp-B'	10.1±2.4	10.1±2.4	0±0.6	0.758	10.8±2	10.8±1.9	-0.1±0.3	0.26	0.868
B’-Pg’	10.5±1.9	10.7±1.6	0.2±1.3	0.602	11.7±1.8	11.8±1.7	0±1.3	0.918	0.76
B-B’	8.5±0.9	8.9±1	0.4±0.5	0.002*	9.2±0.7	9.6±0.8	0.4±0.5	0.001*	0.795
Pg-Pg’	9.8±1	10.7±1.1	0.9±0.2	<.000*	9.8±1	10.6±1.2	0.8±0.7	<.000*	0.619
Pg'-Me	16.2±2.3	16.2±2.5	0±1	0.996	14.9±2.3	15±2.8	0.1±1.5	0.745	0.786

*X̄: Mean, SD: Standard deviation, Min: Minimum, Max: Maximum, *P<.05, Pb:T1-T0/Dependent sample t test Pc:T1-T0-T1-T0/Independent sample t test*

DISCUSSION

In this study, the effects of two functional appliances, AA and newly designed BA, on MS morphology in Class II malocclusion were investigated and their effects were compared. The present results showed, with the effect of functional treatment in Class II malocclusions, positive changes occurred especially on the anterior surface of the MS, and the effect of AA and BA appliances on MS morphology was similar.

Although the present study used a retrospective design, it followed a rigorous classification of patients with skeletal Class II malocclusion, and only growing patients (CS2-CS3) were included in the selected study. Following the selection of the BA group, the AA group was selected, which matched this group's sex and development. Many studies have reported that growth patterns and malocclusion characteristics affect MS morphology.[8,9,12,13,17] Therefore, to ensure standardization between groups, skeletal Class II patients with optimum growth pattern resulting from mandibular retrognathia according to Steiner analysis[14] were included in the study. Additionally, the fact that the T0 values of the groups were statistically similar (Tables 1 and 3) showed that the groups were homogenous.

As in many studies, the decrease in SNA angle in this study was due to functional appliances' maxillary inhibition effect (headgear effect).[18,19] Similar to previous studies,[18-20] the increase in SNB in both groups was due to stimulation of mandibular growth. The combination of mandibular stimulation and maxillary inhibition resulted in a decrease in the ANB angle. The SN/MP angle increase in both groups showed that posterior rotation of the mandible occurred. A recent meta-analysis of 16 studies by Xie et al.[21] reported that the SN/MP angle was statistically significantly increased in AA treatment. However, studies reporting no significant changes in SN/MP with AA treatment suggest an individuality factor for both patient and physician.[18,20,22]

In this study, a cap splint was placed on the AA and an expansion screw was placed on the mandibular plate of the BA in order to prevent lower incisor protrusion, which is an undesirable effect of functional appliances.[19-25] Despite these precautions, similar levels of protrusion of 3.1° occurred in AA and BA. It was thought that the low Cl II elastic force applied between the two plates in the BA group was effective in lower incisor tooth protrusion. This effect could be eliminated by increasing the elastic strength (translation of force from orthodontic effect to orthopedic effect). However, the mandibular plate may jump out of the mouth as the elastic force increases.

Controversial whether there is a change in MS morphology with growth. Evaluating the growth of MS in childhood and adolescence, Buschang et al.[26] reported vertical spontaneous growth on the upper anterior surface of MS, especially during adolescence. Contrary to previous findings, Ruiz et al.[27] found no spontaneous changes in MS morphology during adolescent growth.

MS morphology changes with the effect of orthodontic treatment. Wehrbein et al.[10] reconstructed the tooth movements for 19 months by removing the jawbone of an individual receiving orthodontic treatment during autopsy and reported that as a result, the alveolar bone width decreased due to the lower incisor protrusion. However, no significant correlation was noted in our study between the protrusion movement made by treatment in the lower incisor and the change in the bone at the crest. Although studies[7,8] evaluating morphological differences rather than changes occurring with treatment examine MS dimensions using three-dimensional tomographies, they only provide cross-sectional information. Therefore, conflicts regarding radiation dose and ethical considerations continue to make two-dimensional lateral cephalometric films advantageous when determining changes occurring with treatment.

Pitpoint was applied to every BA group patient except those with excessively protrusive maxillary incisors. The aim here was to prevent the retrusion that occurs in the maxillary incisors due to functional

treatment from overshadowing the forward movement of the mandible. MS anterior middle surface angle and length changes were statistically significantly greater in the BA group than in the AA group (BA: 5°, 1.5 mm; AA: 3.4°, 0.7 mm). This improvement with BA treatment compared to AA may be due to more effective advancement of the mandible with pitpoint application and spee design.

The increase in the mental angle and the decrease in the MS anterior lower surface angle were due to the Pg point growing anterior due to the treatment effect. The fact of the increase in the width of the lower MS also reflects this. The decrease in the MS anterior middle surface angle was associated with the anterior growth of point B. The fact of the increase in the width of the lower MS also reflects this. The decrease in the MS anterior lower surface angle, the increase in the anterior and middle length, and the increase in the middle width indicate that the MS curvature is flattened with functional treatment.

Limitations

Although the study's retrospectively is a limitation, all individuals who met the inclusion criteria during the archive search were included. The results of this study are limited to a short-term evaluation. Long-term studies are needed to evaluate relapse after a patient's puberty ends. An untreated control group would be useful in determining changes in MS with treatment, but a control group could not be included in the study for ethical reasons.

CONCLUSION

1. The width of the middle and lower MS increased with the treatment effect.
2. MS curvature was flattened with functional treatment.
3. With functional treatment, B and Pg points grew anteriorly and the anterior morphology of the MS changed.
4. The results of this study indicated that the effects of AA and BA on MS morphology were similar.
5. Further studies with long-term prospective design, including an untreated control group, are needed to determine the changes in MS morphology due to the effect of functional treatment

Ethics Committee Approval: This retrospective study was approved by Gazi University's Ethical Committee (October 3, 2023/17; protocol number 2023-1174) in accordance with the Declaration of Helsinki.

Informed Consent: Since the study is a retrospectively designed archival study, informed consent is not required.

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Assessment of Oral Disease Burden Using a Cluster Analysis in Dentistry

Diş Hekimliğinde Kümeleme Analizi Kullanılarak Ağız Hastalıkları Yükünün Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to identify homogeneous groups for Oral Disease Burden (ODB) with age through the K-Means cluster analysis in dentistry.

Methods: In this retrospective study, 465 adult patients and 276 elderly patients treated at integrated students' clinics in a public dental school (F/M:381/360; 18-91 years) were included. The ODB score (0-5 points) was calculated through the presence of periodontal problems, dental caries, pulpitis, need of prosthetic treatment, and need of tooth extraction. Homogeneous groups for ODB severity were identified within the dataset by K-Means cluster analysis.

Results: The highest ratios of oral health problems were periodontal problems (94.0%) in young adult patients (cluster-1; n=201; 18-36 years; ODB: 2.92±1.14) and (87.4%) in adult patients (cluster-2; n=199; 37-55 years, ODB: 3.28±1.34) and need for prosthetic treatment (90.7%) in older adult patients (cluster-3; n=193; 56-70 years, ODB: 3.19±1.41) and (87.2%) in elderly patients (cluster-4; n=148; 71-91 years, ODB: 2.33±1.30). The highest ODB score was found in adult patients among four clusters. Older adult patients had elevated ODB score compared to that in elderly patients (*P*=.000).

Conclusion: Needs of different treatment protocols or complex treatments were determined according to ODB with age groups defined by K-Means cluster analysis. These results may provide clues for developing patient empowerment strategies to improve oral health status as well as work force planning in integrated student clinics in dental schools.

Keywords: Oral disease burden, treatment needs, cluster analysis, patient empowerment

ÖZ

Amaç: Bu çalışmanın amacı, diş hekimliğinde Ağız Hastalıkları Yükü (AHY) için yaşa bağlı homojen grupları K-Ortalamalar kümeleme analizi ile belirlemektir.

Yöntemler: Bu retrospektif çalışmaya, bir kamu diş hekimliği fakültesinin entegre öğrenci kliniklerinde tedavi edilen 465 yetişkin hasta ve 276 yaşlı hasta (K/E: 381/360; 18-91 yaş) dahil edilmiştir. AHY skoru (0-5 puan); periodontal problemler, diş çürüğü, pulpitis, protetik tedavi ihtiyacı ve diş çekim ihtiyacı aracılığıyla hesaplanmıştır. AHY şiddeti için homojen gruplar veri seti içinde K-Ortalamalar kümeleme analizi ile belirlenmiştir.

Bulgular: Periodontal problemlerin en yüksek oranda genç yetişkin hastalarda (%94.0) (küme-1; n=201; 18-36 yaş; AHY: 2,92±1,14) ve yetişkin hastalarda (%87,4) (küme-2; n=199; 37-55 yaş, AHY: 3,28±1,34) görüldüğü, ileri yetişkin hastalarda (%90,7) (küme-3; n=193; 56-70 yaş, AHY: 3,19±1,41) ve yaşlı hastalarda (%87,2) (küme-4; n=148; 71-91 yaş, AHY: 2,33±1,30) ise protetik tedavi ihtiyacının ön planda olduğu belirlenmiştir. Dört küme arasında en yüksek AHY puanı yetişkin hastalarda görülmüştür. İleri yetişkin hastaların AHY puanı yaşlı hastalara göre daha yüksek bulunmuştur (*P*=,000).

Sonuç: K-Ortalamalar kümeleme analizi ile tanımlanan yaş gruplarıyla birlikte AHY'ye göre farklı tedavi protokolleri veya kompleks tedavilere ihtiyaçların olduğu belirlenmiştir. Bu sonuçlar, ağız sağlığı düzeyinin iyileştirilmesine yönelik hasta güçlendirme stratejisinin geliştirilmesine ve entegre öğrenci kliniklerindeki iş gücünün planlanmasına yönelik ipuçları sağlayabilmektedir.

Anahtar Kelimeler: Ağız hastalıkları yükü, tedavi ihtiyaçları, kümeleme analizi, hasta güçlendirme

INTRODUCTION

Since oral diseases are a global public health problem for health policies owing to their high frequencies, progressive nature and serious effects on general health status,[1,2] evidence-based oral health data[3,4] are needed to develop oral health policies,[5-8] define risk groups, improve clinical outcomes[3,4,7,9,10] and establish patient empowerment strategies.[11] In addition to public and private dental healthcare providers, dental schools are other elements to deliver dental healthcare and also dental education by providing real professional experience simulation for students.[12]

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The ODB is an outcome measure which reflects the treatment needs of patients and the intensities of oral diseases. The ODB consists of four components regarding presence of periodontal problems, dental caries, need of prosthetic treatment, and need of tooth extraction.[1] Although age is accepted as the predisposing factor for poor oral health as well as unmet treatment needs, different age groups are defined as risk groups according to cut-off values in different studies.[2,5,13] Therefore, it is necessary to identify patient groups with similar clinical characteristics or define associations among clinical manifestations.[12] These are essential while planning dental healthcare delivery and dental education.[1,2]

At this point, a K-Means cluster analysis as a data mining application and an unsupervised learning algorithm could help us to understand the complex relations. It divides large datasets with "n" data points into "k" clusters.[14] For example, this analysis could be a good option to predict diabetes patients[15] as well as to identify patients with high periodontitis risk[16] and nutrition-related pattern in adults with morbid obesity[17] in health science studies.

Finally, the detailed information regarding both ODB and age groups is also needed for developing patient empowerment strategies and for designing clinical practice. Yet, the limited information is available about the ODB in the literature.[1] Therefore, this study aimed to identify homogeneous groups for ODB with age through the K-Means cluster analysis in dentistry.

METHODS

This retrospective study was performed by using anonymous data from Hospital Information Management System (HIMS) in a public dental school, Marmara University, Istanbul, Turkey. Data of 465 adult patients and 276 elderly patients (F/M:381/360; mean age: 51.72±18.78 years; 18-91 years) treated in students' integrated clinics between September 2018 and March 2020 were included in the study. While determining the sample size complete count method was used. Patients with missing data were not included in the study. Data regarding socio-demographic profile (age, gender, marital status), place of residence and treatment history of patients were used. The study was approved by the Ethics Committees of the Medical School of Marmara University (Date: January 8, 2021, Number: 09.2021.17).

Age and ODB were important two main variables in the study. Age is accepted a main predisposing factor for oral health[1,2] in both prevalence and clinical studies.[1,18,19] The study group covered a wide range of age groups (18-64 yeas and ≥65 years old). In literature, individuals between the ages of 18-64 years are considered as adults, and those aged ≥65 years as elderly.[20,21] The health status of these two age groups differs markedly in terms of lifestyle, diet, general health risks, visits to the dentist, and access to services.[19] This classification can reveal clinically important differences in the planning and implementation of dental services, as the groups may have different treatment needs.

In the calculation of ODB, the presence of periodontal problems, dental caries, need of prosthetic treatment, and need of tooth extraction were scored as "1" for each clinical manifestation whereas absence of the condition was scored as "0". Each clinical condition was given one point to calculate the total ODB score.[1] However, a patient's dental treatment process goes through many different pathways. A tooth with pulpitis is a serious condition that causes particularly severe toothache, cause to tooth extraction if left untreated. This leads to an increased oral disease burden.[22] Therefore, incorporating pulpitis into the assessment may allow a more comprehensive and accurate reflection of the total disease burden on oral health. In the present study, pulpitis was also included as a variable to assess the ODB for comprehensively evaluating the oral health. Finally, the total ODB score could vary between 1 and 5, with the highest score indicating the worst possible clinical situation.

Statistical Analysis

Data were analyzed by using SPSS 28.0 statistic program (IBM SPSS Corp., Armonk, NY, USA). Data were presented as "mean" and "standard deviation" whereas the categorical data were presented as "n" and "%". The Chi-square test was used to compare categorical variables. Kruskal Wallis test, one of the non-parametric analysis tests, was used to compare the adult and elderly groups. Statistical significance was accepted as $P<.05$. In addition, both K-Means cluster analysis and Receiver Operating Characteristic (ROC) curve analysis were performed to identify ODB-related age groups. K-Means cluster analysis as a common machine learning approach was used to evaluate complex relationships and determine relationships between variables.[23] This analysis helped to identify homogeneous groups together with ODB and age and minimized the intra-cluster differences in the study. The optimal number of clusters was determined using the Elbow Method and expert opinion was also consulted to validate the number of clusters. Finally, four clusters were developed according to the K-Means cluster Analysis.

ROC curve analysis provides a systematic way to predict cut-off values.[24] The area under the ROC curve can be considered as a measure of potentially predictive accuracy.[25] ROC curve analysis was performed to determine the cut-off value for age in edentulous patients.

RESULTS

In the study, 62.8% (n=465; 18-64 years; 39.83±12.94 years) were adult patients and 37.2% were elderly patients (n=276; ≥65 years; 71.75±5.04 years). The distribution of ODB components were presented according to age groups in Table 1 (P=.000). The ODB score was 3.11±1.29 in the adult patients and 2.72±1.40 in elderly patients. The highest ratios of oral health related problems were periodontal problems in the adult group (88.6%) and prosthetic treatment need (89.9%) in the elderly group. Dental caries (72.0%) and pulpitis (50.8%) were commonly seen in adult patients. Besides, half of patients needed of tooth extraction (adult: 51.0% and elderly: 56.5%) in both age groups.

Table 1. The profile of the study group

	Adult Patients (18-64 years) (n=465)		Elderly Patients (≥65 years) (n=276)	
	Mean	**SD**	**Mean**	**SD**
Age (Years)	39.83	12.94	71.75	5.04
Oral Diseases Burden Score	3.11	1.29	2.72	1.40
	n	**%**	**n**	**%**
Gender				
Female	247	53.1	134	48.6
Male	218	46.9	142	51.4
Total	465	100	276	100
Marital Status				
Married	313	67.3	208	75.4
Single	125	26.9	62	22.5
Unknown	27	5.8	6	2.2
Total	465	100	276	100
Oral Diseases Burden				
Periodontal Problems	412	88.6	154	55.8
Dental Caries	335	72.0	85	30.8
Pulpitis	236	50.8	110	39.9
Need of Prosthetic Treatment	230	49.5	248	89.9
Need of Tooth Extraction	237	51.0	156	56.5

K-Means Cluster Analysis

Since close associations were available between ODB score and age, the K-Means cluster analysis was performed to classify patient's oral health profile according to age groups. Four clusters were identified by the K-Means analysis (*P*<.05), without gender differences (*P*=.881) (Table 2).

The highest ODB score was observed in adult patients (cluster-2; n=199; 37-55 years) whereas elderly patients (cluster-4; n=148; 71-91 years) had the lowest ODB score. Young adult patients had lower the ODB score (2.92±1.14) than those in adult patients (3.28±1.34) (*P*=.000) (Table 2) (Figure 1).

Table 2. The profile of patients according to K-Means cluster analysis

	Cluster-1 Young Adult Patients (n=201)	Cluster-2 Adult Patients (n=199)	Cluster-3 Older Adult Patients (n=193)	Cluster-4 Elderly Patients (n=148)	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*P*
Age (years; range)	27.25±5.46 (18-36)	46.04±5.59 (37-55)	65.08±4.28 (56-70)	75.16±4.52 (71-91)	**.000***
Oral Diseases Burden Score	2.92±1.14	3.28±1.34	3.19±1.41	2.33±1.30	**.000***
	n (%)	n (%)	n (%)	n (%)	
Gender					
Female	106 (52.7)	100 (50.3)	102 (52.8)	73 (49.3)	.881
Male	95 (47.3)	99 (49.7)	91 (47.2)	75 (50.7)	
Oral Diseases Burden					
Periodontal Problems	189 (94.0)	174 (87.4)	135 (69.9)	68 (45.9)	**.000****
Dental Caries	166 (82.6)	142 (71.4)	78 (40.4)	34 (23.0)	**.000****
Pulpitis	91 (45.3)	110 (55.3)	104 (53.9)	41 (27.7)	**.000****
Need of Prosthetic Treatment	50 (24.9)	124 (62.3)	175 (90.7)	129 (87.2)	**.000****
Need of Tooth Extraction	91 (45.3)	104 (52.3)	125 (64.8)	73 (49.3)	**.001****

*Kruskal-Wallis test, **Chi-square test

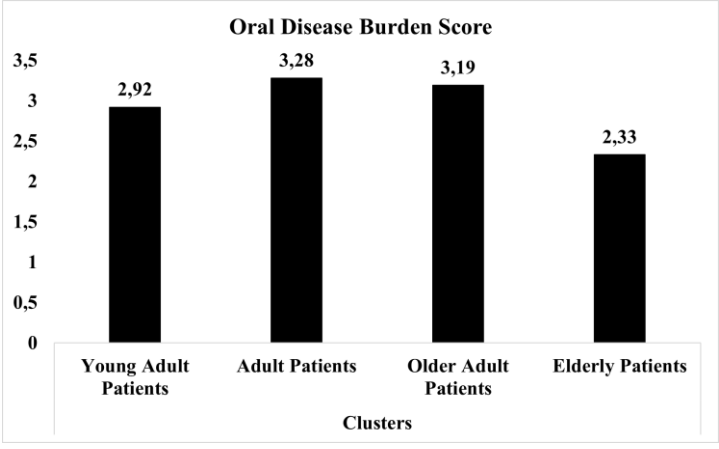

Figure 1. The distribution of ODB scores in clusters defined by ODB and age

The highest ratios of oral health related problems were periodontal problems (94.0%; 87.4%, respectively) and dental caries (82.6%; 71.4%) in both young adult patients (cluster-1; n=201; 18-36 years) and adult patients comparing to those in other clusters (periodontal problems: 69.9% and 45.9% and dental caries: 40.4% and 23.0%) (*P*=.000; *P*=.000). However, the need for prosthetic treatment was significantly lower in young adult patients (24.9%) than adult patients (62.3%) (*P*=.000) (Table 2). The need for scaling and root planning (the initial step of periodontal treatment), and tooth filling were highest in young adult patients (93.0%; 83.6%) followed by adult patients (85.4%; 75.9%) (*P*=.000; *P*=.000).

Older adult patients (cluster-3; n=193; 56-70 years) had elevated ODB score (3.19±1.41 vs. 2.33±1.30) compared to that in elderly patients (cluster-4; n=148; 71-91 years) (*P*=.000). In elderly patients, the ratios of dental caries (23.0%) and pulpitis (27.7%) were around one fourth of the group whereas need of tooth extraction and periodontal problems were seen almost half of the group (49.3%, 45.9%). The ratios of all these clinical manifestations were higher in older adult patients (40.4%; 53.9%; 64.8%; 69.9% respectively) than elderly patients (*P*=.000; *P*=.000; *P*=.000 and *P*=.001 respectively) (Table 2).

Majority of patients in need for prosthetic treatment are both older adult patients (90.7%) and elderly patients (87.2%) (Table 2). The need for crowns (57.5%), partial dentures (45.6%) and temporomandibular joint treatments (50.8%) was higher in older adult patients compared to elderly patients (30.4%; 27.7%; 41.2%) (*P*=.000; *P*=.001; *P*=.000). On contrary, complete dentures were the most needed prosthetic treatment in elderly patients (57.4%) compared to older adult patients (38.9%) (*P*=.001).

ROC Curve Analysis

Patients who were edentulous and in need for complete dentures were older (72.9±5.08 years) than the other patients who had naturel dentition (69.34±3.89 years) (*P*=.000). When the ROC curve analysis was performed, the cut-off value of age in elderly patients for edentulism was 69.5 years according to ROC curve analysis (AUC: 0.728) (Figure 2).

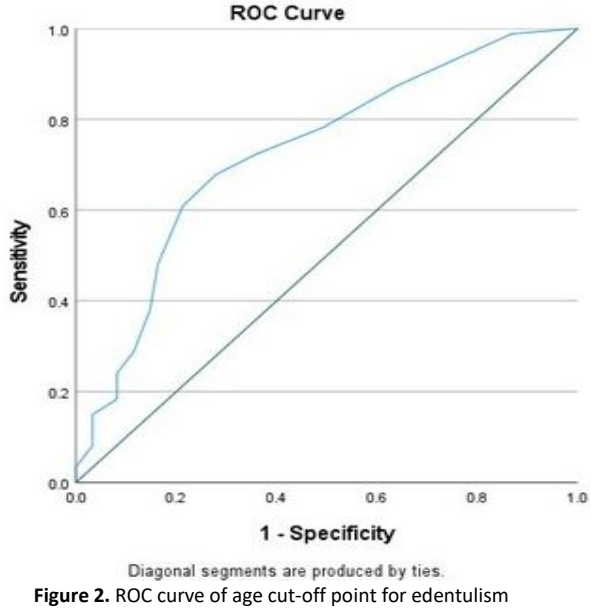

Figure 2. ROC curve of age cut-off point for edentulism

DISCUSSION

Nowadays, patient-centred care focusing on patients' needs and preferences could be though as essential points in dentistry.[26] Since dental schools give dental care service to population through dental students, they have important roles in improving oral health, giving oral health education and developing patient empowerment strategies in a population. Patient empowerment strategies for the sustainability of oral health are changing oral hygiene habits positively and understanding of unmet dental needs of patients.[13] In addition, the assessment of evidence-based clinical data in HIMS of a public dental school provides information for planning of dental care delivery[3] and clinical training curriculum of dental students.[12] In the present study, ODB reflecting treatment needs was examined in a

database extracted from HIMS from a public dental school. Age[1,2] and ODB were main components of the K-Means cluster analysis for the study.

It was performed to define the homogeneous ODB profile with age in the study. The K-Means cluster analysis is popular in many fields such as medicine and business to define homogeneous groups.[16] In this study, four clusters were developed through this analysis.

In this study, periodontal problems and dental caries are similarly the most common oral health problems in young adult patients (cluster-1). As predicted, untreated dental problems in the early stages of life continued to adulthood (cluster-2). Similarly, dental caries and periodontal problems are the most significant oral health burden[27] in studies performed in different countries.[2,19,24,28-30]

Since they can be prevented by oral hygiene education and regular dental visits,[2,29] patients should be educated to establish oral hygiene habits from childhood.[13,26] Moreover, routine dental checkups are another important issue for both improving oral health and increasing oral health literacy.[11]

The need of tooth extraction was observed over the half of older adult patients (cluster-3; 64,8%) and adult patients (cluster-2; 52,3%). A tooth with incurable dental and periodontal problems is extracted. Then, prosthodontic treatment need is inevitable to protect oral functions. Therefore, elevated oral disease burden could be predicted in these age groups

Majority of patients in older adult patients (cluster-3) and elderly groups (cluster-4) need prosthodontic treatments. If patient empowerment strategies and preventive applications are not performed in the early ages, improving oral functions and solving aesthetic problems could only be achieved by prosthodontic treatments.[22]

In elderly patients aged ≥65 years in France, almost half of them (53%) needed dental prosthesis (53%) and tooth extractions (45.1%).[6] Similarly, prosthetic treatment (62%) was the most needed treatment option in patient aged ≥70 years in Holland.[31]

In the study, the ODB score was found to be low in elderly patients as expected because 70 years of age was found to be cut-off value for being edentulous according to ROC curve analysis. Elderly are a vulnerable group who are socio-economically disadvantaged, have different systemic diseases and problems in accessing services due to decreases in their mobilities.[5,32,33] Therefore, dentists should work in collaboration with other professional groups that are experts on aging.[34,35] Additionally, the curriculum of dental schools should be updated to include gerodontology education.

The study gave some clues to develop patient empowerment strategies for patients treated in a public dental school located in Istanbul that has unique position to reflect the population. However, the main limitation of the study was that patient records were obtained from HIMS in a limited period.

CONCLUSION

Different treatment protocols or complex treatment needs were identified based on the ODB within the age groups defined by K-Means cluster analysis. These results may provide clues for developing patient empowerment strategies to improve oral health status as well as work force planning in integrated student clinics in dental schools.

Ethics Committee Approval: Approval was obtained from Marmara University Faculty of Medicine Ethics Committee (Date: January 8, 2021, Number: 09.2021.17)

Informed Consent: Since the study uses secondary data containing anonymized patient data, informed consent is not required.

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Comparison of the Effectiveness of Bleaching Treatment with Activation Using Polywave LED Light, Diode Laser and Er, Cr:YSGG Laser: An *in Vitro* Study

Polywave LED Işığı, Diyot Lazer ve Er, Cr:YSGG Lazer Kullanılarak Aktivasyon ile Ağartma Tedavisinin Etkinliğinin Karşılaştırılması: Bir *İn Vitro* Çalışma

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ABSTRACT

Objective: This study aimed to evaluate the efficacy of non-vital bleaching treatments activated by laser systems and polywave LED light.

Methods: Forty-eight extracted maxillary incisors were artificially stained using sheep blood and then the initial colour values of the stained teeth were measured and recorded with a spectrophotometer. The teeth were randomly assigned to four groups (n = 12 per group): Group 1 (control) received 30-minute non-vital bleaching treatments with 35% hydrogen peroxide (H_2O_2); Group 2 received 35% H_2O_2 with a diode laser (940 nm); Group 3 received 35% H_2O_2 with an Er,Cr YSGG laser (2780 nm); and Group 4 received 35% H_2O_2 with polywave LED light (480 nm). Spectrophotometric evaluation of color change (ΔE_{00}) was performed on days 1, 3, and 7 post-treatment.

Results: Intergroup comparisons showed significantly lower ΔE_{00} values in Group 1 (control) and Group 4 (H_2O_2 + polywave LED light) compared to Groups 2 (H_2O_2 + diode laser) and 3 (H_2O_2 + Er,CrYSGG laser) across all time points ($P < .05$). No significant difference in ΔE_{00} was observed between Groups 2 and 3 at any time point ($P > .05$).

Conclusion: Both the diode laser and Er,CrYSGG laser significantly enhanced the efficacy of non-vital bleaching. Although polywave LED light achieved adequate bleaching results, its activation was less effective than that of laser systems.

Keywords: Color change, laser, LED, spectrophotometric analysis, teeth bleaching, nonvital

ÖZ

Amaç: Bu in vitro çalışmanın amacı, lazer ve polywave LED ışığı ile aktive edilen non-vital beyazlatma tedavilerinin etkinliğini değerlendirmektir.

Yöntemler: Kırk sekiz adet çekilmiş maksiller kesici diş koyun kanı kullanılarak yapay olarak renklendirilmiş ve daha sonra renklenen dişlerin ilk renk değerleri spektrofotometre ile ölçülmüş ve kaydedilmiştir. Dişler rastgele dört gruba ayrılmıştır (n = 12): Grup 1'e (kontrol) %35 hidrojen peroksit (H_2O_2) ile 30 dakikalık vital olmayan beyazlatma uygulanmıştır; Grup 2'ye diyot lazer (940 nm) ile %35 H_2O_2 uygulanmıştır; Grup 3'e Er,CrYSGG lazer (2780 nm) ile %35 H_2O_2; ve Grup 4'e polywave LED ışık (480 nm) ile %35 H_2O_2 verilmiştir. Renk değişiminin (ΔE_{00}) spektrofotometrik değerlendirmesi tedavi sonrası 1, 3 ve 7. günlerde yapılmıştır.

Bulgular: Gruplar arası karşılaştırmalar, tüm zaman noktalarında Grup 2 (H_2O_2 + diyot lazer) ve Grup 3 (H_2O_2 + Er,CrYSGG lazer) ile karşılaştırıldığında Grup 1 (kontrol) ve Grup 4'te (H_2O_2 + polidalga LED) önemli ölçüde daha düşük ΔE_{00} değerleri göstermiştir ($P <,05$). Grup 2 ve 3 arasında hiçbir zaman noktasında ΔE_{00} açısından anlamlı bir fark gözlenmemiştir ($P > ,05$).

Sonuç: Hem diyot lazer hem de Er,CrYSGG lazer non-vital beyazlatmanın etkinliğini önemli ölçüde artırmıştır. Polywave LED ışığı yeterli ağartma sonuçları elde etmesine rağmen, aktivasyonu lazer tedavilerinden daha az etkili olmuştur.

Anahtar Kelimeler: Renk değişimi, lazer, LED, spektrofotometrik analiz, diş beyazlatma, nonvital

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INTRODUCTION

Intrinsic discoloration is often observed in non-vital teeth for various reasons following endodontic treatment. Contributing factors include pulp damage, infection within the pulp cavity, residual pulp tissue, and materials used in root canal fillings.[1] These internal discolorations can cause significant aesthetic concerns for patients.[2] Non-vital bleaching, a minimally invasive and low-cost treatment, is commonly used to eliminate the discoloration in non-vital teeth.[3] While bleaching treatments for vital teeth can be performed either in-office by a dentist or at home by the patient, non-vital bleaching treatments must be administered by a dentist.[4]

In-office bleaching procedures for vital teeth typically involve the use of high-concentration bleaching agents, such as 25–40% H_2O_2 or 35–38% carbamide peroxide, due to their short-term application. In contrast, low-concentration bleaching agents are preferred for at-home treatments that require longer durations.[5] For non-vital, root canal-treated teeth, H_2O_2 or sodium perborate is commonly utilized. These agents may be applied either individually or in combination.[6] Research indicates that increasing the concentration of the bleaching agent, as well as extending the application time, enhances the bleaching effect and leads to more noticeable color changes. Furthermore, the energy from a light source absorbed by the bleaching gel is converted into heat, which accelerates the decomposition of H_2O_2, thereby amplifying the bleaching effect.[7]

To enhance the efficacy of bleaching agents through the application of heat, dental bleaching treatments have increasingly incorporated light-curing and laser systems.[8] Theoretically, the activation of H_2O_2 by light or laser systems a significant benefit: the heat produced during activation promotes the formation of oxygen free radicals and accelerates the decomposition of oxygen. However, excessive heat application can damage the structural integrity of the tooth and increase tooth sensitivity.[9] As a result, light or laser-activated H_2O_2 is suggested to improve the overall bleaching effectiveness.[10,11] Additionally, this method provides a substantial advantage by achieving desired outcomes more quickly, thus reducing the amount of time the patient spends in the dental office.[12]

The application of lasers in clinical practices, such as tooth bleaching, can lead to more efficient and comfortable treatments.[13] Historically, lasers have been utilized in various dental procedures and are now also incorporated into bleaching protocols.[14] Dental lasers emit a monochromatic wavelength, which results in less thermal stress on the pulp compared to other light sources.[10] The effectiveness of these lasers in tooth whitening varies based on specific parameters, including wavelength and power.[15] Diode lasers operating within the 810-980 nm wavelength range exhibit significant absorption in pigmented tissues, whereas their absorption in water and minerals is comparatively low. Elevated temperatures can potentially harm the dental pulp.[9] Among the available laser types, Erbium lasers are characterized by their high absorption coefficients for water.[16] Specifically, the Er,Cr:YSGG laser demonstrates superior water absorption, albeit with limited penetration depth in dental hard tissues, thereby mitigating the risk of pulp damage. This feature is particularly significant when tooth hard tissues interact with aqueous bleaching gels during treatment.[17]

Light-emitting diode (LED) light curing devices generate lower energy output compared to laser systemss, and they are generally more cost-effective.[18] The LED systems utilized in bleaching procedures fall under the category of broadband light sources, emitting light primarily in the blue spectrum, without extending significantly into the infrared (IR) range, unlike quartz-tungsten-hybrid (QTH) or plasma arc systems.[10]

This in vitro study aims to assess the effectiveness of non-vital bleaching treatments for endodontically treated teeth using two distinct laser systems (Er,Cr:YSGG and Diode laser) alongside a Polywave LED light. The uniqueness of this research is the evaluation of the efficacy of a Polywave LED light for intracoronal bleaching of non-vital teeth in comparison with two different laser systems, a subject that, to the best of knowledge, has not been previously explored. Prior to conducting the study, a null hypothesis (H01) was established, postulating that the bleaching efficacy of the Polywave LED light curing would not significantly differ from that of the laser systems.

METHODS

The study was approved by the Research Ethics committee at Afyonkarahisar University (2021/221, date: April 2, 2021). The sample size for the study was determined using the G*Power software (Version 3.0.10, Franz Faul, Germany) with 80% statistical power, a 0.05 significance level, and an effect size of 0.5 (medium).[19]

Preparation of Samples

Forty-eight adult incisors from the maxilla that had been collected within six months. Informed consent was obtained in writing from patients over the age of 18. Teeth were checked under a stereo microscope with a rise of 4× (Euromex, New York, USA). Decayed, deformed and broken teeth were identified and replaced with new ones. The selected teeth were disinfected with chloramine-T solution and then kept in distilled water. Normal oval coronal access to the root canals was achieved and the thickness of the buccal wall of the teeth was reduced with a carbide bur. Buccal wall thickness was standardized to 2.6±0.3. Endodontic access cavities were prepared in the teeth. After the root canals of the sample teeth were expanded with rotary instruments, coronal expansion was also performed. During the expansion of the root canals, the root canals were washed with sodium hypochlorite (2 ml 2.5 percent). Finally, the root canals were washed using distilled water and dried using paper dots. The apical openings were closed using a packable dental composite resin.

Tooth Staining

The artificial staining of the specimens was performed using a modified method based on the procedure described by Freccia and Peters[20] in line with protocols from previous studies.[21,22] Initially, the specimens were placed in Eppendorf tubes containing sheep blood and centrifuged at 3,400 rpm for 20 minutes at 37 °C (Micro 220R; Hettich, Germany). After centrifugation, the plasma (supernatant) was separated from the sediment, and the plasma was discarded. The tubes underwent a second centrifugation under the same conditions for another 20 minutes, followed by a series of centrifugations performed twice daily for three consecutive days. After each centrifugation, the specimens were rinsed with distilled water, returned to the tubes, and stored at 37°C in 100% humidity. On the fourth day, the teeth were removed from the original blood-containing tubes and transferred to clean tubes. To promote hemolysis of the erythrocytes, 0.5 mL of distilled water was added to the original tubes, followed by another 20-minute centrifugation. This process resulted in a membranous precipitate and hemolysate, with the supernatant discarded. The teeth were then returned to these tubes and centrifuged once daily for three days, with each session lasting 20 minutes. After completing the artificial staining procedure, the specimens were washed under running water and dried with an air spray. No root canal filling with sealer or gutta-percha was applied to the teeth. Instead, 2 mm-thick

glass ionomer cement (Equia Forte HT, GC, Japan) was placed 1 mm apical to the cementoenamel junction (CEJ).

Basic Color Calculation

The color of the teeth was measured in a circular area of 5 mm in diameter on the buccal, coronal part of the teeth each time. Basic color measurement was carried out on a white background using a spectrophotometer (Vita Easyshade, VITA Zahnfabrik, Germany). Samples were equally distributed into four groups based on their initial color measurements. A one-way ANOVA was conducted to assess whether there were statistically significant differences in initial colors among the groups. The results of the analysis indicated that there were no significant differences in initial color values across the groups (*P*=.633).

Bleaching Procedure

The bleaching activation systems employed in this research are outlined in Table 1. A devital bleaching protocol was applied to samples divided into four groups (n = 12), as described below:

Group 1: A 35% H_2O_2 (pH: 5.0, Opalescence Endo; Ultradent, USA) was introduced into the pulp chambers of the specimens. The solution was mixed using a bonding brush for one minute and left to act for 10 minutes, with the procedure repeated three times, totaling 30 minutes. After each session, the pulp chambers were thoroughly cleaned of residual bleaching agent and dried. Temporary restorations were placed, and the specimens were stored in artificial saliva.

Table 1. Characteristics of the bleaching activation systems used in the study

Device	Type	Wave length	Intensity	Manufacturer
Er,Cr:YSGG laser	Laser	2780 nm	2,5 W	Waterlase MD turbo, BIOLASE, Irvine, CA, USA
Diyod laser	Laser	940 nm	2 W	Epic, iLase by BIOLASE, Irvine, CA, USA
LED light curing unit	Polywave LED	395-480 nm	1 W	Ultradent Products Inc, South Jordan, UT, USA

Group 2: Following the initial placement of the bleaching gel as described in Group 1, it was left for two minutes before laser activation. A diode laser (940 nm, Epic, iLase, BIOLASE, USA) operating at 2 W was applied without direct contact with the bleaching agent for one minute (40 Hz frequency, 50 mJ energy). This protocol was repeated three times. Upon completion, the bleaching agent was removed, and temporary restorations were placed.

Group 3: The bleaching procedure followed the same initial steps as Group 1. After two minutes of exposure to the bleaching gel, Er,CrYSGG laser activation (2780 nm, Waterlase MD Turbo, BIOLASE, USA) was applied for one minute. This process was repeated three times. The laser system was equipped with a Z-type glass tip (MZ8) with an 800 µm diameter and 6 mm length. The laser parameters included 2.5 W output power, 700 µs pulse duration (S-mode), 10 Hz pulse frequency, and an energy density of 0.9 J/cm², without water or air cooling, based on the protocol described by Dionysopoulos et al.[6]

Group 4: As in Group 1, the bleaching agent was applied to the pulp chamber and allowed to act for two minutes. Subsequently, a Polywave LED light (Valo LED, Ultradent, USA) was used at an intensity of 1000 mW/cm² in standard mode for 15-second intervals. This light activation was repeated with five-minute breaks, totaling two minutes of cumulative exposure.[23]

All bleaching procedures were conducted by a single operator to ensure consistency throughout the experiment.

Evaluation of Color Change

Color measurements were performed on day 1, day 3, and day 7 following the bleaching procedures. Immediate post-bleaching measurements were avoided, as the dehydration of dental tissues during treatment could temporarily alter the color perception. Instead, sufficient time was allowed for the tooth color to stabilize. To maintain consistency across all groups, the temporary filling materials placed in the specimens were left intact throughout the evaluation period.

Baseline color measurements were performed using a spectrophotometer (Vita Easyshade). A white background (L* = 92.3, a*=1.4, b* = -5.6) was used when determining the CIELAB coordinates of each sample. Each sample was measured in triplicate for mean L, a*, and b* values. The color coordinates of the samples treated with bleaching were measured one day after the bleaching treatment (T0-T1), three days after the bleaching treatment (T0-T2), and seven days after the bleaching treatment (T0-T3). The color differences (ΔE_{00}) of the specimens were quantified in accordance with the CIEDE2000 formula, as outlined below [24]:

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L} \right)^2 + \left(\frac{\Delta C'}{K_C S_C} \right)^2 + \left(\frac{\Delta H'}{K_H S_H} \right)^2 + R_T \left(\frac{\Delta C'}{K_C S_C} \right) \left(\frac{\Delta H'}{K_H S_H} \right) \right]^{1/2}$$

where lightness, chroma, and hue differences between color measurements are expressed by ΔL', ΔC', and ΔH'. K_L, K_C, and K_H were used as parametric factors for the effect of imaging and lighting conditions, and these were accepted as 1 in this study. The weighting functions for the color difference adjustment that take into account the position change of the L*, a* and b* coordinates are indicated by S_L, S_C and S_H.[24,25] The parametric factors of the CIEDE2000 color difference formula were determined as 1.[26]

Statistical Analysis

The obtained data were analyzed using SPSS 20 (IBM SPSS Corp., Armonk, NY, USA) software. Firstly, Kolmogorov-Smirnov test and Levene test were used to control the distribution of data and homogeneity of variances. Repeated Measures ANOVA and Bonferroni tests were used to compare the ΔE_{00} values obtained with the measurement periods of each group and analysed using Huynh-Feldt corrections, if required. One-Way ANOVA and Tukey post-hoc tests were used to compare the ΔE_{00} values of the groups at each measurement period. *P*-values <.05 were considered statistically significant.

RESULTS

The results of the Repeated Measures ANOVA test performed to compare the ΔE_{00} values obtained for three separate measurement periods for each group are given in Table 2 and Figure 1. According to test findings, no significant difference was found between all time periods measured in all groups except the Er,Cr:YSGG group (*P* >.05). In the Er,Cr:YSGG group, a significant increase was observed in the T0-T2 period, but no significant difference was found in the T0-T3 period. This shows that the bleaching effect continues after seven days. In addition, significant differences were found between the groups according to the One-Way ANOVA test results used to compare the bleaching groups for each time period (*P*<.05). In all three time periods, ΔE_{00} values of the control group and Polywave LED light group were significantly lower than Er,Cr:YSGG and Diode laser groups (*P*<.05). However, no significant difference was found between these binary groups (*P*>.05). In this study, Er,Cr:YSGG and Diode Laser applications resulted in significantly better bleaching outcomes compared to the other groups.

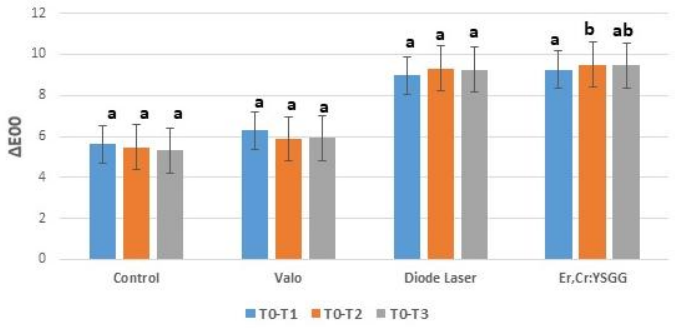

Figure 1. Graph of color changes (ΔE_{00}) after 1 (T0-T1), 3 (T0-T2) and 7 (T0-T3) days of different bleaching procedures. Different letters indicate a significant difference between groups at a 5% significance level.

Table 2. Results Of Repeated Measures Anova

Groups	Source	SS	df	MS	F	*P*
Diode Laser	Huynh-Feldt	0.855	2	0.428	1.254	.305
Er,Cr:YSGG	Huynh-Feldt	0.482	1.804	0.267	8.350	.003*
Led light curing unit	Huynh-Feldt	1.292	1.771	0.729	2.257	.136
Control	Huynh-Feldt	0.614	1.305	0.470	0.938	.375

SS: Sum of squares, df: degrees of freedom, MS: mean of squares, F: F distribution, *P<.05 significant

Table 3. Mean and standard deviation of ΔL, Δa, Δb colour indices and ΔE values for subgroups of the bleaching different activator systems

GROUPS			N	Mean ± Std. Deviation
Diod Laser	T1-T0	ΔL	12	7.08 ± 1.73
		Δa	12	-1.96 ± 0.66
		Δb	12	-11.49 ± 1.69
		ΔE_{00}	12	8.97 ± 1.36
	T2-T0	ΔL	12	7.20 ± 1.88
		Δa	12	-2.21 ± 0.46
		Δb	12	-12.07 ± 1.34
		ΔE_{00}	12	9.32 ± 1.29
	T3-T0	ΔL	12	7.18 ± 1.67
		Δa	12	-2.25 ± 0.39
		Δb	12	-12.04 ± 1.60
		ΔE_{00}	12	9.26 ± 1.45
Er,Cr:YSGG	T1-T0	ΔL	12	7.48 ± 1.10
		Δa	12	-1.94 ± 0.91
		Δb	12	-11.48 ± 1.54
		ΔE_{00}	12	9.24 ± 0.87
	T2-T0	ΔL	12	7.48 ± 1.07
		Δa	12	-2.27 ± 0.85
		Δb	12	-12.02 ± 1.61
		ΔE_{00}	12	9.50 ± 0.71
	T3-T0	ΔL	12	7.42 ± 1.12
		Δa	12	-2.28 ± 0.80
		Δb	12	-12.02 ± 1.68
		ΔE_{00}	12	9.46 ± 0.72
Led light curing unit	T1-T0	ΔL	12	4.66 ± 1.70
		Δa	12	-3.07 ± 0.51
		Δb	12	-7.79 ± 1.36
		ΔE_{00}	12	6.30 ± 1.13
	T2-T0	ΔL	12	3.73 ± 2.18
		Δa	12	-3.06 ± 0.59
		Δb	12	-7.71 ± 1.41
		ΔE_{00}	12	5.88 ± 1.31
	T3-T0	ΔL	12	3.45 ± 2.14
		Δa	12	-3.03 ± 0.63
		Δb	12	-8.05 ± 1.82
		ΔE_{00}	12	5.92 ± 1.42
Control	T1-T0	ΔL	12	4.37 ± 1.37
		Δa	12	-1.81 ± 0.71
		Δb	12	-7.29 ± 1.34
		ΔE_{00}	12	5.62 ± 1.09
	T2-T0	ΔL	12	4.14 ± 1.46
		Δa	12	-2.16 ± 0.63
		Δb	12	-7.03 ± 1.63
		ΔE_{00}	12	5.48 ± 1.32
	T3-T0	ΔL	12	3.46 ± 1.42
		Δa	12	-2.58 ± 0.64
		Δb	12	-7.27 ± 1.54
		ΔE_{00}	12	5.30 ± 1.24

DISCUSSION

Based on the findings of this study, the null hypothesis (H_{01}) was rejected. The hypothesis proposed that the use of a Polywave LED light, intended to enhance the effectiveness of bleaching treatment in non-vital teeth, would show no significant difference compared to laser-assisted bleaching treatments. However, at all evaluated time points following the bleaching procedures, the Er,Cr:YSGG laser exhibited the most substantial color change. Furthermore, the ΔE_{00} values in both the control group and the Polywave LED light group were significantly lower compared to the Er,Cr:YSGG and Diode laser groups across all three time intervals These results indicate that Polywave LED light is less effective than laser-assisted bleaching treatments for non-vital teeth.

In this study, clinical discoloration was simulated by staining the teeth with sheep blood. The samples were incubated in Eppendorf tubes containing sheep blood to ensure thorough staining. After centrifugation, the hemoglobin-rich precipitate was separated, and the plasma was carefully removed from the tubes.[20]

The present study has been found to have similar results with studies investigating the effectiveness of Er,Cr:YSGG laser and Diode laser activation in non-vital bleaching treatments.[6,27] The laser radiation absorbed by the bleaching gel enables the bleaching process to be accelerated and a more effective bleaching process by converting the light energy into heat energy.[10] The wavelength of the laser and the composition of the bleaching gel significantly influence the gel's absorption of laser radiation. Notably, when the Er,Cr:YSGG laser is operated at a wavelength of 2780 nm, it demonstrates superior absorption in water-based bleaching gels due to its high affinity for water molecules. This interaction leads to an increase in the gel's temperature during the procedure.[28] A study evaluating the bleaching efficiency of the Er,Cr:YSGG laser at varying power settings reported a significant increase in color change when the laser was used at 2.5 W.[6] The effectiveness of the Er,Cr:YSGG laser in office bleaching has also been examined, with findings indicating superior outcomes compared to conventional methods.[28] However, research on the use of the Er,Cr:YSGG laser for bleaching non-vital teeth remains scarce in the literature.

Diode lasers are commonly employed in bleaching treatments with wavelengths typically ranging from 790 nm to 980 nm.[29] Primary advantages of diode lasers include compact size, portability, and the use of flexible optic fibers.[30] In a study comparing the bleaching efficiency of a 1.6 W diode laser (808 nm) and LED light (470 nm), the diode laser demonstrated superior performance over both LED light and bleaching gel alone.[29] Similarly, in research evaluating diode lasers at 810, 940, and 980 nm wavelengths, no statistically significant difference in bleaching efficiency was observed among the different wavelengths (P >.05). However, it was noted that the diode laser operating at 940 nm exhibited the highest bleaching efficiency.[27]

A study comparing polywave LED light, halogen, and diode lasers for the activation of in-office bleaching reported no significant difference between the light-activated and non-activated groups at the end of the treatment.[31] In the clinical study by Gürgan et al.[32], spectrophotometric evaluation of office bleaching revealed that the ΔE_{00} value in the diode laser group was statistically higher than in the non-activated group and the LED light group. However, in the present study, a direct comparison could not be made, as no prior research was found in the literature comparing polywave LED light with various laser systems in the bleaching of non-vital teeth.

Laser systems are not always available in clinics for bleaching treatments; however, LED used for the polymerization of composite resins are more commonly accessible. These light-curing units provide a more cost-effective and practical alternative to laser systems.[33] In a study investigating the activation efficiency with polywave LED light in office bleaching, it was observed that the colour change increased.[33] Ragain et al. [34] investigated the impact of polywave LED light on the proliferation of gingival epithelial cells, reporting no adverse effects on cell proliferation within 24 hours following an 18-second exposure. In the present study, the polywave LED light (Valo LED) was applied for 15 seconds, followed by a 5-minute interval, and then reapplied for another 15 seconds, resulting in a total exposure time of 2 minutes. This protocol was implemented to minimize any potential negative effects on epithelial cells. Additionally, the use of a rubber dam during clinical bleaching procedures serves to protect the gingival tissue from heat exposure.

Investigating the results of the study, it was found that the colour change of the samples in all groups far exceeded the thresholds of detectability (ΔE_{00}>1.2) and acceptability (ΔE_{00}>2.7). The detectability threshold refers to the smallest color difference that can be perceived by an observer. The acceptability threshold denotes the color change that is deemed acceptable to an observer in comparison to a reference color.[26] Optical thresholds are very important in evaluating treatments and comparing different treatments in dentistry.[35] Therefore, the effectiveness of the teeth bleaching treatments tested in this study was confirmed.

The results from this in vitro study indicated that polywave LED light exhibited less activation strength compared to the Er,Cr:YSGG laser and Diode laser in non-vital bleaching. Nonetheless, a satisfactory color change was noted in teeth treated with polywave LED light. Furthermore, no significant differences were observed between the activation effects of the Er,Cr:YSGG laser and the Diode laser in non-vital bleaching treatments.

Ethics Committee Approval: Ethics approval for the study incl. usage of retrospective anonymized data was obtained through the Ethics Committee of Afyonkarahisar University of Health Sciences (No: 2021/221, date: April 2, 2021).

Informed Consent: Consent was obtained from the patients for the use of their extracted teeth.

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Knowledge Mapping of Polyetheretherketone in Dentistry: A Bibliometric Analysis

Diş Hekimliğinde Polietereterketonun Bilgi Haritalaması: Bibliyometrik Analiz

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ABSTRACT

Objective: To assess advancements in polyetheretherketone (PEEK) research within the field of dentistry.

Methods: The search was conducted within the Web of Science database and included publications related to PEEK through 2023. The search string utilized comprised the primary focus term "PEEK" and additional keywords such as "dent*", "oral, restorat*", "prosthodont*", and "tooth, teeth, and implant*". Metadata encompassing titles, abstracts, keywords, authors, affiliations, countries, and references were extracted. Bibliometric metrics, including documents, authors, journals, and keywords, were subjected to descriptive statistical analysis.

Results: Between 1997 and 2022, 607 publications on PEEK were found to have 12,115 citations. Scholars in China (138 articles, 22.7%), Germany (118 articles, 19.4%), and the United States (71 articles, 11.8%) actively participated in the research on PEEK, and publications from Germany (3594 citations, 29.7%) had the most citations. Dental Materials (45 articles, 7.4%) and the Journal of Prosthetic Dentistry (42 articles, 6.9%) were the major contributing journals. Among 1433 keywords, application-, material- and manufacturing-related keywords yielded highly normalized citations. The term map revealed that the major application of PEEK was related to dental implants.

Conclusion: This analysis yielded crucial insights into the overall number of publications and citations related to PEEK and its diverse applications. China has emerged as the predominant contributor in this field, publishing the greatest number of articles. Notably, the University of Zurich from Switzerland stood out as the foremost institute in terms of both productivity and impact.

Keywords: Bibliometrics, polyetheretherketone, research

ÖZ

Amaç: Diş hekimliği alanında polietereterketon (PEEK) araştırmalarındaki gelişmeleri değerlendirmektir.

Yöntemler: Tarama, Web of Science veritabanında gerçekleştirildi ve 2023 yılına kadar PEEK ile ilgili yayınlar dahil edildi. Kullanılan arama dizisi, birincil odak terimi olan "PEEK" ile "dent*", "oral, restorat*", "prosthodont*", "tooth, teeth, and implant*" gibi ek anahtar sözcüklerden oluşuyordu. Başlıkları, özetleri, anahtar kelimeleri, yazarları, bağlantıları, ülkeleri ve referansları kapsayan meta veriler elde edildi. Belgeler, yazarlar, dergiler ve anahtar kelimeler de dahil olmak üzere bibliyometrik ölçümler tanımlayıcı istatistiksel analize tabi tutuldu.

Bulgular: 1997-2022 yılları arasında PEEK ile ilgili 607 yayına 12.115 atıf yapıldığı tespit edildi. PEEK araştırmalarına Çin (138 makale, %22,7), Almanya (118 makale, %19,4) ve Amerika Birleşik Devletleri'nden (71 makale, %11,8) akademisyenler aktif olarak katıldı ve Almanya'daki yayınlar (3594 atıf, %29,7) en çok atıfı aldı. Dental Materials (45 makale, %7,4) ve Journal of Prosthetic Dentistry (42 makale, %6,9) katkıda bulunan başlıca dergiler oldu. 1433 anahtar kelime arasında uygulama, materyal ve üretimle ilgili anahtar kelimeler yüksek oranda normalleştirilmiş atıfa sahipti. Terim haritası, PEEK'in temel uygulamasının dental implantlar ile ilgili olduğunu ortaya çıkardı.

Sonuç: Bu analiz, PEEK ve onun çeşitli uygulamalarıyla ilgili yayın ve atıf sayısına ilişkin önemli bilgiler sağlamaktadır. Çin, bu alanda en fazla makale yayınlayan en önemli katılımcı olarak ortaya çıkmıştır. Özellikle İsviçre'deki Zürih Üniversitesi hem üretkenlik hem de etki açısından bu alanda önde gelen kurum olarak öne çıkmıştır.

Anahtar Kelimeler: Bibliyometrik, polietereterketon, araştırma

INTRODUCTION

Polyetherether-ketone (PEEK) is a thermoplastic semicrystalline linear polycyclic aromatic polymer that was introduced as an alternative dental implant material due to its biological adaptation capacity and advantageous mechanical properties.[1] Polyetherether-ketone (PEEK) is a thermoplastic semicrystalline linear polycyclic aromatic polymer that was introduced as an alternative dental implant material due to

This study involved an oral presentation at the 27th International Dental Congress of the TDA.

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its biological adaptation capacity and advantageous mechanical properties.[1] When reinforced with carbon fibers, PEEK has a high flexural strength (140–170 MPa) and elastic modulus (18 GPa).[2] In comparison to titanium, which has an elastic modulus of 110 GPa, PEEK has an elastic modulus of 3-4 GPa that closely aligns with that of bone.[3,4] Due to any parafunctional habit hypersensitivity, PEEK abutments or screws have been chosen over titanium because PEEK can sustain the same amount of force.[5]

PEEK has been applied as a restorative material for crowns, fixed partial dentures, and conventional prostheses. In the case of removable partial prostheses, PEEK can be used for clasps instead of Co-Cr alloys.[4] A variant of PEEK, PEKK, demonstrates superior shock absorption properties with a lower stress level of 49 MPa, while PEEK exhibits a higher stress concentration at 58 MPa.[1] A decrease in stress concentration corresponds to a reduced risk of fractures in the denture base. In root canal irrigation, PEEK polymer tips are recommended due to their chemical resistance and nonreactivity with chemical endodontic irritants.[6] To address this concern, PEEK, especially when combined with digital technology, has emerged as a more viable material, serving as an option for conventional self-curing and heat-curing space maintainers and providing a favorable and aesthetically pleasing option for patients.[7]

PEEK material received approval for dental use in 1988, with its introduction into the field of implantology starting in 2011. Its effectiveness in applications such as temporary and permanent implant abutments and healing screws has quickly been demonstrated.[8] In the field of dentistry, preliminary research and application have demonstrated the extensive utilization of PEEK and PEKK as dental implants, implant abutments, clasps for removable prostheses, obturators, and other materials due to their exceptional handling and mechanical, biological, and aesthetic features.[9]

The dentistry market currently offers more than 1300 dental implant systems of varying sizes, shapes, and surface features.[10] In recent years, dentists and researchers have focused on developing alternatives to traditional dental implants, implant abutments, and restorations. PEEK and zirconium dioxide are among the materials that have been explored.[11] However, zirconium dioxide is limited by its low-temperature degradation and high Young's modulus.[12] There is no difference between PEEK osseointegration and that of zirconia or titanium dental implant materials.[2]

The mechanical properties of PEEK are highly regarded, yet its aesthetic limitations pose a challenge. To enhance its visual appeal, veneering is needed. However, the inert surface of PEEK presents a significant obstacle to adhesion, thereby limiting its potential for common use in restorations. Various surface characterization techniques have been explored to improve the adhesion of PEEK.[13]

In the last decade, there has been a significant surge in the interest of researchers in PEEK.[14,15] It is imperative that the studies carried out in this field be assessed to gauge the effect of PEEK-based studies on applied clinical research. Bibliometric analysis is a commonly employed scientific domain for determining the same phenomenon, as it offers both qualitative and quantitative analysis of the impact of scientific data.[16] Comprehensive analysis of the current state of research results distribution is achieved through the utilization of expertise and methodologies such as mathematics, statistics, and literature.[17]

The aim of this study was to employ a bibliometric approach, a potent tool,[18,19] to assess the research advancements of PEEK in the field of dentistry. This method enables the identification of key contributors, organizations, countries, and journals. Furthermore, it facilitates an analysis of impactful keywords and research trends

METHODS

Since the data used in this study were provided by a public platform, ethics committee approval was not needed. This study was conducted in accordance with the principles of the Declaration of Helsinki. The author selected the Clarivate-owned Web of Science (WoS) database to obtain PEEK data. The data were extracted on April 18, 2023, at Bursa Uludag University, Bursa, Türkiye. This process was independently completed by one researcher to reduce errors.

Keywords and Data Retrieval

The search for data related to PEEK was conducted on WoS's advanced search interface by entering the terms "Article Title, Abstract, Author Keywords" in the search topic field. The finalized search string consisted of the primary focus (PEEK) and keywords that included dent*, oral, restorat*, prosthodont*, tooth, teeth, and implant*.

Inclusion and Exclusion Criteria

Articles published before 2023 were included in the search. Articles, reviews, proceedings papers and early accesses were selected from the document types summarized in the WoS database. After applying document types and title/abstract scanning, articles whose title, abstract and keywords were not in English were excluded. The date limitation criterion was applied, and the publication date of the studies ranged from 1997 to 2022.

Data Analysis

The specific tools and methods chosen depend on the research objectives, the complexity of the dataset, and the preferences of the researchers conducting the bibliometric analysis.

Bibliographic database (WoS): The Web of Science (WoS) was used to retrieve relevant documents and scientific publications related to PEEK.

The bibliometric software program (VOS viewer v1.6.14; Center for Science and Technology Studies, Leiden University):[20] The search results from WoS were exported as tab-delimited files and subsequently processed using a specific software program. This program analyzed various components, including titles, abstracts, keywords, authors, affiliations, countries, and references. Visualization of collaboration and citation networks was accomplished through network diagrams or overlaying with VOSviewer. In these visualizations, the size of each node reflects the volume of publications, the proximity between nodes indicates the strength of their relationship, and the color of each node conveys specific information through various representations. Coauthor relationships were depicted through network visualizations, where nodes of the same color formed clusters, signifying significant collaborative efforts in the research, as explained in the figure captions.

The citation score for each publication was calculated by dividing its number of citations by the average citation count across all publications on PEEK during the respective period. A publication was deemed to have received more citations than the average if its score surpassed one. The normalized citation score for each specific term within the published documents was determined by computing the mean of all normalized citation scores for documents containing that particular term. This process was conducted automatically through the use of the VOSviewer software program's term map visualizations.

For the data cleaning and preprocessing steps, Microsoft Excel (version 16.0) was used to ensure data quality and consistency. The data acquired through WoS functions, namely, "analyze results" and "citation reports," were collected and subsequently summarized. After extraction, the data were processed using a spreadsheet. The analysis involved identifying the most cited articles and examining

the publication years of these articles. In the context of collaborative work, equal weights were assigned to each collaborator.

The statistical software SciMAT, a dedicated bibliometric analysis software, was used to process and statistically analyze the retrieved data.

A supplemental search was conducted on the included publications regarding the application of PEEK in dentistry.

RESULTS

A total of 607 studies were identified in searches conducted within the WoS database, spanning the period from 1997 to 2022. Notably, there was a discernible upward trajectory in the yearly publication of articles, with a particularly pronounced increase observed after 2017 (Figure 1).

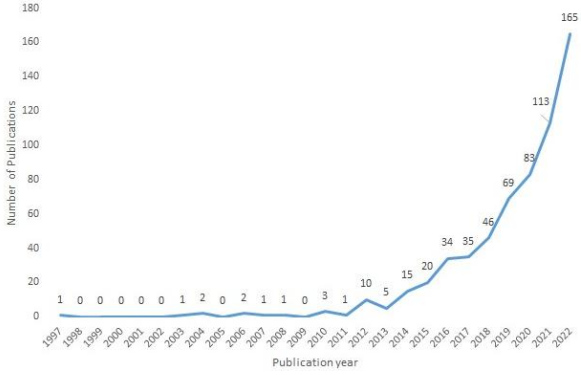

Figure 1. Annual number of published articles related to PEEK from 1997 to 2022.

Of the 607 publications, 282 articles (46.5%) were categorized under "Dentistry, Oral Surgery, and Medicine (DOSM)" in the Journal Citation Reports. The remaining 325 articles (53.5%) fell into the non-DOSM category and encompassed fields such as materials science, polymer science, engineering, and chemistry. Publications on PEEK received 12 115 citations in total. Each article received 19.96 citations on average. Twenty-five publications were cited more than 100 times. The majority of the most cited articles are in vitro studies. Table 1 displays the 20 most cited articles, of which the majority were laboratory studies in the DOSM category.

In the included articles, a collective effort of 2466 authors was observed, averaging 4.06 authors per article. Table 2 highlights the top 10 most productive and most cited authors. The collaborative network predominantly revolved around highly productive authors, as depicted in Figure 2A. Prof. Stawarczyk B emerged as the most productive author, with her studies receiving the greatest number of citations. Notably, the top 10 most productive authors also ranked among the top 10 most cited authors.

The research on PEEK involved 774 organizations from 59 countries. Notably, the University of Zurich emerged as the most productive and highly cited institution, contributing 42 publications with a total of 383 citations (Table 3). The majority of the studies were conducted within university settings. Jilin University and the University of Sheffield exhibited a high number of citations per article, indicating the impactful nature of their research on PEEK. Collaborative institutions focused on the University of Zurich, Ludwig Maximilians University Munich, Munich University, Federal University of Santa Catarina, Chinese Academy of Sciences, King Saud University, University of Florida, and Bern University also contributed significantly to this field (Figure 2B).

The distribution of highly productive countries closely mirrored that of institutions. China contributed the most articles (138, 22.7%), followed by Germany (118, 19.4%) and the United States (71, 11.8%). According to Table 3, the number of studies from several countries in Asia (Türkiye, India, Saudi Arabia and Egypt) is increasing. Two countries, Germany and China, received notably high citation counts, each surpassing 3000 citations (Germany with 3594 citations and China with 3106 citations). This level of citations was significantly greater than that of other countries. Figure 2C shows that international collaboration was centered mainly on China, the United States, Germany, Switzerland and Türkiye.

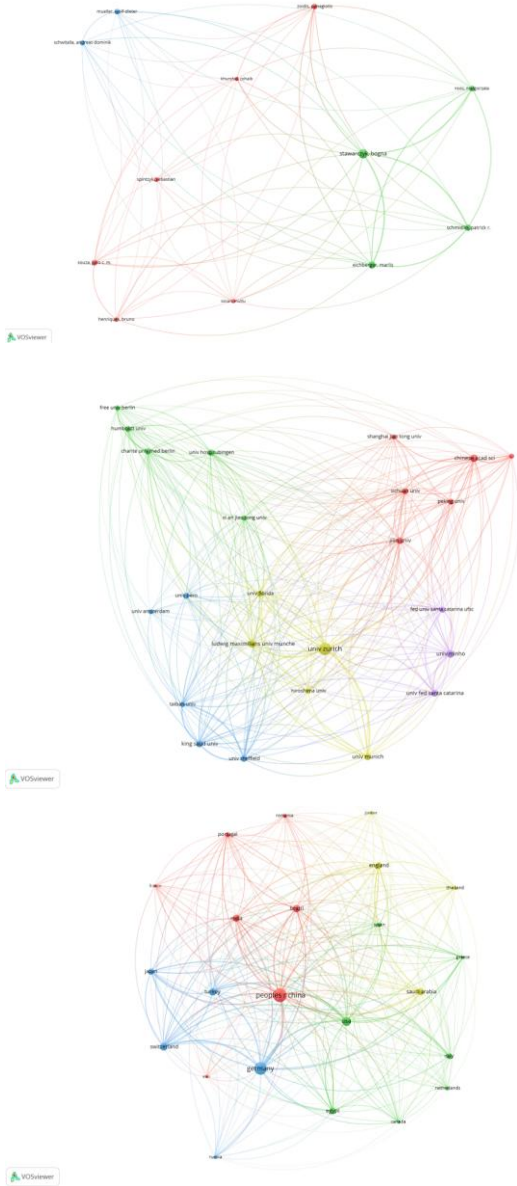

Figure 2. Network of collaboration. A. Collaboration among authors with more than 5 publications. B. Collaboration among institutions with more than 10 publications. C. Collaboration among countries with more than 10 publications. The size of each bubble represents the number of documents. The link length represents the degree of collaboration closeness.

Table 1. Characteristics of the 20 most cited articles in the PEEK research area.

Title	Authors	Journals	Publication year	Total Citations	Average per year	Type of study	Research areas
Applications of polyetheretherketone (PEEK) in oral implantology and prosthodontics	Najeeb, Shariq; Zafar, Muhammad S.; Khurshid, Zohaib; Siddiqui, Fahad	*Journal of Prosthodontic Research*	2016	403	50.38	Systematic review	Dentistry, Oral Surgery & Medicine
Surface characteristics of dental implants: A review	Rupp, F.; Liang, L.; Geis-Gerstorfer, J.; Scheideler, L.; Huettig, F.	*Dental Materials*	2018	285	47.5	Narrative review	Dentistry, Oral Surgery & Medicine
Polyetheretherketone (PEEK) for medical applications	Panayotov, Ivan Vladislavov; Orti, Valerie; Cuisinier, Frederic; Yachouh, Jacques	*Journal of Materials Science-Materials in Medicine*	2016	262	32.75	Narrative review	Engineering/Materials Science
Polyetheretherketone/ nanofluorohydroxyapatite composite with antimicrobial activity and osseointegration properties	Wang, Lixin; He, Shu; Wu, Xiaomian; Liang, Shanshan; Mu, Zhonglin; Wei, Jie; Deng, Feng; Deng, Yi; Wei, Shicheng	*Biomaterials*	2014	213	21.3	Laboratory study	Engineering/Materials Science
Application of Intra-Oral Dental Scanners in the Digital Workflow of Implantology	van der Meer, Wicher J.; Andriessen, Frank S.; Wismeijer, Daniel; Ren, Yijin	*Plos One*	2012	177	14.75	Laboratory study	Science & Technology - Other Topics
Enhanced osteointegration on tantalum-implanted polyetheretherketone surface with bone-like elastic modulus	Lu, Tao; Wen, Jin; Qian, Shi; Cao, Huiliang; Ning, Congqin; Pan, Xiaoxia; Jiang, Xinquan; Liu, Xuanyong; Chu, Paul K.	*Biomaterials*	2015	176	19.56	Laboratory study and animal study	Engineering/Materials Science
PEEK Dental Implants: A Review of the Literature	Schwitalla, Andreas; Mueller, Wolf-Dieter	*Journal of Oral Implantology*	2013	171	15.55	Systematic review	Dentistry, Oral Surgery & Medicine
Polyetheretherketone-A suitable material for fixed dental prostheses?	Stawarczyk, Bogna; Beuer, Florian; Wimmer, Timea; Jahn, Dirk; Sener, Beatrice; Roos, Malgorzata; Schmidlin, Patrick R.	*Journal of Biomedical Materials Research Part B-Applied Biomaterials*	2013	164	14.91	Laboratory study	Engineering/Materials Science
Influence of sulfur content on bone formation and antibacterial ability of sulfonated PEEK	Ouyang, Liping; Zhao, Yaochao; Jin, Guodong; Lu, Tao; Li, Jinhua; Qiao, Yuqin; Ning, Congqin; Zhang, Xianlong; Chu, Paul K.; Liu, Xuanyong	*Biomaterials*	2016	148	18.5	Laboratory study and animal study	Engineering/Materials Science
Carbon Fiber Reinforced PEEK Composites Based on 3D-Printing Technology for Orthopedic and Dental Applications	Han, Xingting; Yang, Dong; Yang, Chuncheng; Spintzyk, Sebastian; Scheideler, Lutz; Li, Ping; Li, Dichen; Geis-Gerstorfer, Juergen; Rupp, Frank	*Journal of Clinical Medicine*	2019	147	29.4	Laboratory study	General & Internal Medicine
Effect of different surface pretreatments and luting materials on shear bond strength to PEEK	Schmidlin, Patrick R.; Stawarczyk, Bogna; Wieland, Marco; Attin, Thomas; Haemmerle, Christoph H. F.; Fischer, Jens	*Dental Materials*	2010	147	10.5	Laboratory study	Dentistry, Oral Surgery & Medicine and Materials Science
Accuracy of digital impressions of multiple dental implants: an in vitro study	Vandeweghe, Stefan; Vervack, Valentin; Dierens, Melissa; De Bruyn, Hugo	*Clinical Oral Implants Research*	2017	129	18.43	Laboratory study	Dentistry, Oral Surgery & Medicine and Engineering
Effect of surface roughness on osteogenesis in vitro and osseointegration in vivo of carbon fiber-reinforced polyetheretherketone-nanohydroxyapatite composite	Deng, Yi; Liu, Xiaochen; Xu, Anxiu; Wang, Lixin; Luo, Zuyuan; Zheng, Yunfei; Deng, Feng; Wei, Jie; Tang, Zhihui; Wei, Shicheng	*International Journal of Nanomedicine*	2015	125	13.89	Laboratory study and animal study and theoretical study	Science & Technology - Other Topics and Pharmacology & Pharmacy
Stress shielding and fatigue limits of poly-ether-ether-ketone dental implants	Lee, Woo-Taek; Koak, Jai-Young; Lim, Young-Jun; Kim, Seong-Kyun; Kwon, Ho-Beom; Kim, Myung-Joo	*Journal of Biomedical Materials Research Part B-Applied Biomaterials*	2012	121	10.08	Laboratory study and finite element study	Engineering/Materials Science
Biofilm formation on the surface of modern implant abutment materials	Hahnel, Sebastian; Wieser, Angela; Lang, Reinhold; Rosentritt, Martin	*Clinical Oral Implants Research*	2015	119	13.22	Laboratory study	Dentistry, Oral Surgery & Medicine and Engineering
Nano-TiO2/PEEK bioactive composite as a bone substitute material: in vitro and in vivo studies	Wu, Xiaomian; Liu, Xiaochen; Wei, Jie; Ma, Jian; Deng, Feng; Wei, Shicheng	*International Journal of Nanomedicine*	2012	118	9.83	Laboratory study and animal study	Science & Technology - Other Topics and Pharmacology & Pharmacy
Influence of surface conditioning on bonding to polyetheretherketon (PEEK)	Kern, Matthias; Lehmann, Frank	*Dental Materials*	2012	117	9.75	Laboratory study	Dentistry, Oral Surgery & Medicine and Materials Science
The improvement of adhesive properties of PEEK through different pretreatments	Hallmann, Lubica; Mehl, Albert; Sereno, Nuno; Haemmerle, Christoph H. F.	*Applied Surface Science*	2012	114	9.5	Laboratory study	Chemistry and Materials Science and Physics
The Use of a Modified Poly-Ether-Ether-Ketone (PEEK) as an Alternative Framework Material for Removable Dental Prostheses. A Clinical Report	Zoidis, Panagiotis; Papathanasiou, Ioannis; Polyzois, Gregory	*Journal of Prosthodontics-Implant Esthetic and Reconstructive Dentistry*	2016	113	14.13	Clinical study	Dentistry, Oral Surgery & Medicine
Plasma-sprayed titanium coating to polyetheretherketone improves the bone-implant interface	Walsh, William R.; Bertollo, Nicky; Christou, Chrisopher; Schaffner, Dominik; Mobbs, Ralph J.	*Spine Journal*	2015	113	12.56	Laboratory study	Neurosciences & Neurology and Orthopedics

Table 2. List of the top 10 most productive authors and the top 10 most cited authors

Top 10 productive authors				Top 10 cited authors			
Author	Institution	Country or Region	Number of Articles	Author	Institution	Country or Region	Number of Citations
Stawarczyk, Bogna	Ludwig-Maximilians University Munich	Germany	36	Stawarczyk , Bogna	Ludwig-Maximilians University Munich	Germany	1366
Eichberger, Marlis	Ludwig-Maximilians University Munich	Germany	24	Schmidlin, Patrick R.	University of Zurich	Switzerland	1219
Schmidlin, Patrick R.	University of Zurich	Switzerland	20	Roos, Malgorzata	University of Zurich	Switzerland	818
Roos, Malgorzata	University of Zurich	Switzerland	16	Eichberger, Marlis	Ludwig-Maximilians University Munich	Germany	648
Zoidis, Panagiotis	University of Florida	Florida	16	Mueller, Wolf-Dieter	Charité-University Medicine Berlin	Germany	473
Mueller, Wolf-Dieter	Charité-University Medicine Berlin	Germany	15	Zoidis, Panagiotis	University of Florida	Florida	430
Souza, Julio C. M.	University of Minho, University Institute of Health Sciences	Portugal	15	Schwitalla, Andreas Dominik	Charité - Universitätsmedizin Berlin	Germany	302
Schwitalla, Andreas Dominik	Charité - Universitätsmedizin Berlin	Germany	14	Spintzyk, Sebastian	University Hospital Tübingen, Carinthia University of Applied Sciences	Germany, Austria	299
Spintzyk, Sebastian	University Hospital Tübingen, Carinthia University of Applied Sciences	Germany, Austria	12	Souza, Julio C. M.	University of Minho, University Institute of Health Sciences	Portugal	243
Henriques, Bruno	Federal University of Santa Catarina, University of Minho	Brazil, Portugal	11	Henriques, Bruno	Federal University of Santa Catarina, University of Minho	Brazil, Portugal	178

Table 3. List of the top 10 contributing countries and institutions

Top 10 contributing countries				Top 10 contributing instutions				
Country	Number of articles	Number of citations	Citations per article	Institution	Country	Number of articles	Number of citations	Citations per article
China	138	3106	22.50	University of Zurich	Switzerland	42	383	9.12
Germany	118	3594	30.46	Ludwig Maximilian University of Munich	Germany	20	310	15.50
Usa	71	1192	16.79	Chinese Academy of Sciences	China	19	240	12.63
Switzerland	55	1912	34.76	University of Minho	Portugal	19	342	18.00
Brazil	51	724	14.20	Jilin University	China	16	784	49.00
Turkey	50	246	4.92	King Saud University	Saudi Arabia	16	308	19.25
India	46	434	9.43	Federal University of Santa Catarina	Brazil	15	245	16.33
Saudi Arabia	45	1351	30.02	Ludwig Maximilian University of Munich	Germany	15	258	17.20
England	42	1860	44.29	University Hospital Tübingen	Germany	14	328	23.43
Egypt	41	211	5.15	University of Sheffield	UK	14	575	41.07

Of the 217 journals that featured publications related to PEEK, only two journals were responsible for more than 40 publications: Dental Materials (45, 7.4%) and the Journal of Prosthetic Dentistry (42, 6.9%). These were followed by polymers (36, 5.9%) and materials (33, 5.4%). Dental Materials was the most productive and the most cited journal (Figure 3). The top ten journals produced 263 publications (43.3%) and 5669 citations (46.8%). Three journals from ELSEVIER, two from MDPI, one from WILEY, one from QUINTESSENCE PUBLISHING, one from SPRINGER HEIDELBERG, one from JAPAN PROSTHODONTIC SOC and one from JAPANESE SOC DENTAL MATERIALS DEVICES are on this list. According to the Journal Citation Reports of 2022, five journals from this list were in the 1st quartile (Q1), one was in the 2nd quartile (Q2), and four were in the 3rd quartile (Q3).

Fourteen keywords were regarded as high-frequency keywords (those with more than 20 occurrences) among 1433 keywords in the studies on PEEK (Table 4). The term map indicated that the primary

application of PEEK in the field of dentistry was closely associated with dental implants. (Figure 4). The application-related keywords “dental implant”, “prosthodontics” and “osseointegration” are material-related keywords (“PEKK”), and the manufacturing-related keyword “3D printing” was highly related to the number of citations.

The terms “PEEK” and “polyetheretherketone” were excluded from the summary because they are universal focuses of PEEK-related research. According to the average publication year of the highly recurring keywords, the oldest keyword was “bioactivity” (2017.88), and the newest keyword was “dental materials” (2020.95).

The supplementary searches revealed researchers' focus on diverse applications of PEEK restorations, indicating a broad interest in the subject (Figure 5). Dental implants were most commonly investigated, with 211 published articles. The next most common complications were a fixed prosthesis framework and an implant abutment (131 and 90 articles, respectively).

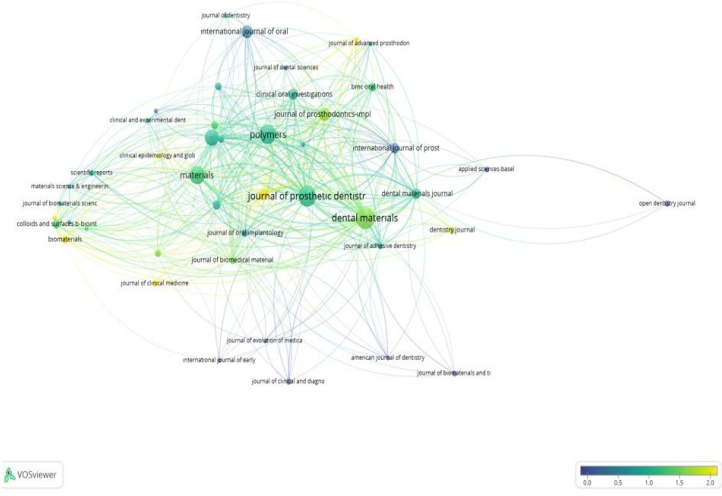

Figure 3. Citation map of journals whose total link strength was greater than 10. The size of each bubble represents the number of documents. The color of the bubble represents the relative citation count. Yellow bubbles represent higher average normalized citations.

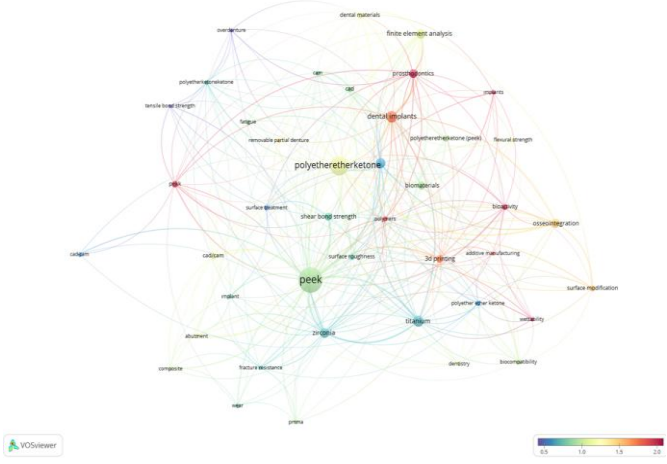

Figure 4. Term map of keywords whose total link strength was greater than 10. The size of each bubble represents the frequency of the keyword. The color of the bubble represents the average normalized citations. The red bubbles represent more broadly normalized citations.

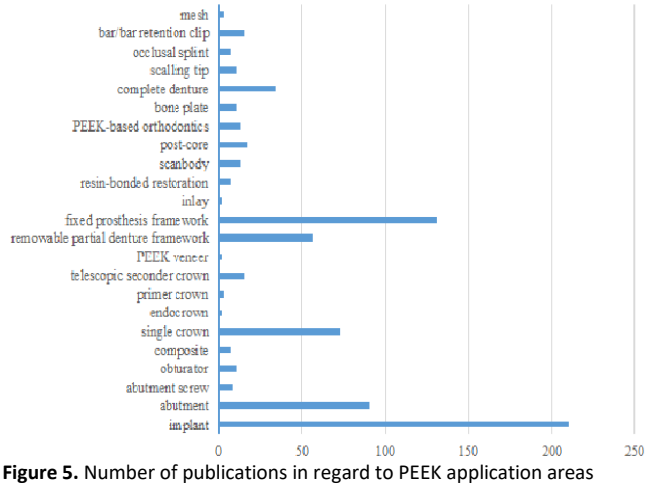

Figure 5. Number of publications in regard to PEEK application areas

Table 4. List of high-impact keywords (>20 occurrences) in descending order of their occurrence frequency

Keywords	Occurrences	Average normalized citations
prosthodontics	35	2.22
PEKK	22	1.89
dental implants	55	1.72
3D printing	28	1.67
osseointegration	39	1.45
polyetheretherketone	126	1.2
finite element analysis	41	1.12
polyetheretherketone (PEEK)	21	0.99
PEEK	203	0.96
biomaterials	32	0.96
shear bond strength	28	0.79
zirconia	42	0.74
titanium	50	0.73
dental implant	43	0.68

DISCUSSION

This bibliometric analysis was conducted using the WoS database from 1997 to 2023. In accordance with bibliometric terminology, the distribution of the quantity of scientific articles published serves as a valuable indicator of the advancements observed in a specific field.[21] The number of publications pertaining to time-changed PEEK tubes has significantly increased. It was determined that 515 of a total of 607 publications were cited at least once, and a total of 12115 citations were identified. The number of citations of PEEK-related publications has tended to increase over the years. With a total of 3546 citations, it received the most citations in 2022. It can be predicted that the increasing trend of citations will continue in the coming years. It is anticipated that more publications will emerge in the future as global research clusters continue to emerge, fostering collaboration among diverse authors and organizations.

Researchers utilize keywords when scouring search engines for articles related to a specific topic.[22] The frequency and average normalized citation value of keywords can reflect the researcher's current research focus. In this study, high-impact keywords were broadly grouped into three categories: application areas, material types, and manufacturing techniques. PEKK, a popular dental polymer, frequently appears to have great influence as a keyword in PEEK-related research and is often used as a comparable material for high-performance polymers. Compared with PEEK, PEKK has advantages in digital manufacturing because it provides a greater variety of printable

polymers. The placement of ketone bonds in the aromatic ring is paramount for maintaining the melting temperature and crystallization rate. The results are aesthetically more pleasing and offer enhanced wear and friction characteristics. Furthermore, PEEK cannot match the antibacterial performance of PEKK because it results in greater bacterial adhesion on its surface.[23,24] Despite the fact that no restorative material can be regarded as optimal for all clinical considerations, attempts have been made to maximize the characteristics of materials through strategies such as the modification of microstructure, optimization of fabrication processes, or synthesis of hybrid materials due to the clinical search for superior materials.[25]

In a clinical trial, both zirconia and PEEK abutments demonstrated equivalent survival rates and similar biological and esthetic outcomes during the 5-year evaluation. The combination of PEEK abutments with ceramic crowns, such as zirconia abutments, exhibited a 100% survival rate without any instances of fracture or restoration loss.[26] This finding suggested that titanium-supported PEEK abutments, which serve as an economically viable alternative to zirconia abutments, may not present clinical issues in the short term.

Implant-supported fixed dental prostheses can cause up to 33.6% of dental prostheses to fail after a 5-year period.[27] In a recent systematic review, it was determined that prostheses utilizing PEEK as a framework exhibited an impressive cumulative survival rate of 97.3%.[28] Notably, this survival rate was found to be equivalent to that of other framework materials commonly employed in complete-arch implant-supported fixed dental prostheses, including titanium, cobalt-chromium, zirconia, and monolithic zirconia.

Previous bibliometric analysis studies have shown that the rates of bias in in vitro studies are greater than those in other studies.[29,30] The findings of these existing studies were similar to our findings, and a greater proportion of the included articles utilized in vitro study designs. This situation can be explained by ethical concerns, complications and risks of harm to patients, especially when new materials are used in clinical trials. However, additional clinical studies are needed to better analyze dental materials.

Numerous bibliometric analyses conducted in the past have revealed a consistent trend wherein a significant number of scientific papers have been published in journals with high impact factors, aligning with the principles of Bradford's law.[31,32] Two preeminent journals, Dental Materials (IF2022=5) and the Journal of Prosthetic Dentistry (IF2022=4.6), have emerged as the primary sources of research on PEEK. These journals have been recognized for their significant impact on prosthodontic research in other bibliometric analyses.[19,33] In conjunction with Polymers and Materials, these journals have contributed to more than half of all publications on the subject. Bradford's law has identified these journals as core publications in the field of PEEK research.[34,35]

Publishing a large number of articles to solidify the foundation of the topic and/or having influential publications with high citations are important indicators of author influence. In this study, Stawarczyk B both affected the field of PEEK research. Notably, there was significant overlap between the top 10 most productive and highly cited lists for institutes and countries, referred to as the contributing lists. Countries such as China, Germany, Switzerland and England stood out for their substantial volume of articles and citation counts exceeding 1800, highlighting their impact. The significant representation of multiple Asian countries in the lists of the top 10 contributors can be ascribed to widespread academic exchanges and collaborations involving distinguished researchers.[33]

This study employs bibliometric methods to trace the evolution of PEEK in dentistry; however, it is important to acknowledge several limitations. First, the use of only one database (WoS) may have resulted in the exclusion of relevant articles on PEEK. Data accuracy would increase across different databases. Second, the study focused on articles published in high-impact factor journals and those published in English, potentially overlooking impactful papers published in other languages. Third, the absence of self-citation correction by journals or authors could have led to inflated self-citations, potentially affecting the analysis. While authors may need to reference their prior work in a study series, it is essential to avoid improper self-referencing solely for citation count manipulation. Finally, while this study incorporates citation analysis, a combination of quality assessments would provide a more comprehensive evaluation of high-quality articles. Unlike systematic reviews and meta-analyses, which include only high-standard articles, bibliometric analysis aims to offer a comprehensive overview of a subject. Despite these limitations, this study still offers valuable insights into the progress and evolving trends of PEEK in dentistry over the past three decades

CONCLUSION

Based on the results of this bibliometric analysis, we can draw the following conclusions:

1. Research on PEEK has experienced a global surge, leading to extensive collaboration and citations among authors, institutions, and countries.
2. The research areas of focus included PEEK applications, material properties, and manufacturing techniques.
3. Global interest in PEEK research has rapidly grown in recent years, with China leading in paper publications and The University of Zurich in Switzerland being the top contributing institution.

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Informed Consent: Written informed consent was not required for this study.
Peer-review: Externally peer-reviewed.
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Etik Komite Onayı: Bu çalışma için etik kurul onayı gerekmemektedir.
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Evaluation of the Antimicrobial Activity of Glass Ionomer Luting Cement with Chlorhexidine Diacetate

Klorheksidin Diasetatlı Cam İyonomer Yapıştırma Çimentosunun Antimikrobiyal Aktivitesinin Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to evaluate the antimicrobial activities of glass ionomer luting cements modified with chlorhexidine diacetate.
Methods: Chlorhexidine diacetate (CXD) was incorporated into glass ionomer luting powder at 0 %, 1 %, 2%, 3% concentration and 120 round-shaped samples were prepared. Antimicrobial properties against *Streptococcus mutans (S. mutans), Enterococcus faecalis (E. faecalis), and Candida albicans (C. albicans)* were assessed with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and Cristal Violet Assay. Biofilms on samples were also observed by Scanning Electron Microscopy. One-way ANOVA and Tukey Tests were used for statistical analysis.
Results: The highest amount of antimicrobial activity was found in samples with 3% added CXD. The proportion of viable pathogens were reduced significantly with the addition of 2% and 3 % CXD (P <.05). A noticeable reduction of *S. mutans* amount was seen with incorporation of 3% CXD (P <.05). *S. mutans* and *C. albicans* were inhibited significantly by all the samples when compared to control groups.
Conclusions: Addition of 2%-3% CXD to glass ionomer luting cements increased the antimicrobial properties. As, microorganisms' growth rate was inhibited in 3% CXD group, CXD added glass ionomer luting cement can be suggested in endodontically treated teeth, in high caries-risk patients, also in immunocompromised patients.
Keywords: Antifungal, antibacterial, chlorhexidine diacetate, glass ionomer, luting cements

ÖZ

Amaç: Bu çalışma, klorheksidin diasetat ile modifiye edilmiş cam iyonomer yapıştırma çimentolarının antimikrobiyal aktivitelerini değerlendirmeyi amaçlamıştır.
Yöntemler: Klorheksidin diasetat (CXD), cam iyonomer yapıştırma tozuna %0, %1, %2, %3 konsantrasyonlarında dahil edildi ve 120 yuvarlak şekilli numune hazırlandı. Streptococcus mutans (S. mutans), Enterococcus faecalis (E. faecalis) ve Candida albicans'a (C. albicans) karşı antimikrobiyal özellikler, MTT [3-(4,5-dimetiltiazol-2-il)-2,5-difenil tetrazolyum bromür] ve Kristal Menekşe Testi ile değerlendirildi. Numunelerdeki biyofilmler ayrıca Taramalı Elektron Mikroskobu ile gözlemlendi. İstatistiksel analiz için tek yönlü ANOVA ve Tukey Testleri kullanıldı.
Bulgular: En yüksek antimikrobiyal aktivite miktarı, %3 CXD eklenmiş numunelerde bulundu. Canlı patojenlerin oranı %2 ve %3 CXD eklenmesiyle önemli ölçüde azaldı (P <,05). %3 CXD eklenmesiyle S. mutans miktarında belirgin bir azalma görüldü (P <,05). S. mutans ve C. albicans, kontrol gruplarıyla karşılaştırıldığında tüm örneklerde önemli ölçüde inhibe edildi.
Sonuçlar: Cam iyonomer yapıştırma simanlarına %2-%3 CXD eklenmesi antimikrobiyal özelliklerini artırdı. Mikroorganizmaların büyüme hızı %3 CXD grubunda inhibe edildiğinden, CXD eklenmiş cam iyonomer yapıştırma simanı endodontik tedavi görmüş dişlerde, yüksek çürük riskli hastalarda ve ayrıca immün sistemi baskılanmış hastalarda önerilebilir.
Anahtar Kelimeler: Antifungal, antibakteriyel, klorheksidin diasetat, cam iyonomer, yapıştırma simanları

Clinical Relevance

Glass ionomer luting cements modified with chlorhexidine diacetate can be preferred in crown cementations on endodontically treated teeth, as well as in high caries-risk patients and in immunocompromised patients.

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INTRODUCTION

Prosthetic dentistry aims to replace or restore missing or damaged teeth by fixed partial dentures, crowns and other prostheses. Luting cements are essential materials in fixed prosthetic restorations, as they act as a link between the prepared abutment, tooth, and dental restoration. An appropriate luting cement should be biocompatible, have low solubility, prevent plaque and caries formation, have sufficient working time, short setting time, appropriate hardness, viscosity, and film thickness.[1] The antibacterial activity of luting cements during and after curing is important because this property is effective in eliminating or reducing bacteria that remain alive on the walls of the preparation or bacteria that may reach the cavity through microleakage cracks.[2]

Glass ionomer luting cements (GIC) are acid-based cements commonly used in restorations due to their physical properties such as chemical adhesion to dental tissue, fluoride release sufficient biocompatibility, and lower thermal expansion.[3] There are studies reporting that the antimicrobial activity of GIC is related to fluoride release.[4,5] It has been reported that low pH provides more bactericidal effect during curing and almost no antibacterial activity after curing.[6,7] To overcome these problems, antimicrobial agents have been added to GIC. Chlorhexidine diacetate (CXD) is a commonly used antibacterial agent because it both inhibits and destroys both Gram-positive and Gram-negative bacteria.[8] An important reason for its success as a plaque preventer is its durability and variety of antimicrobial activity.[9] It produces a bacteriostatic environment by attaching to oral mucosa and releasing for a long time.[8,9]

According to the literature review, there are few research in which CXD is incorporated into the GIC used as a luting cement. CXD has been emphasized to be the appropriate agent in lowering *S. mutans* and *C. albicans* due to its sensitivity when compared to other pathogens.[6-8] To the best of our knowledge, there are few studies evaluating the *E. faecalis* bacteria growth rate on glass ionomer luting cements.[9] Considering the microbiology of the oral biofilm and the structure and virulence factors of *E. faecalis*, it is one of the important pathogenic bacteria associated with gingivitis, periodontitis, and root canal treatment failures.[10]

Therefore, the purpose of this study was to assess the antimicrobial activities of glass ionomer luting cement with different concentrations of chlorhexidine diacetate against *S. mutans, E. faecalis* and *C. albicans* assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Cristal Violet (CV) Assay. It was hypothesized that the addition of CXD would not impact the antimicrobial properties of GIC.

METHODS

Sample Preparation

In total, 120 samples were prepared for this study (10 mm in diameter and 3 mm thick). To modify the cements, the cement powder was incorporated with CXD at different weight proportion 0%, 1%, 2%, 3 wt % (<200 nm, Nanografi, METU Teknokent). The cement powder and CXD were mixed in a mixer (President Dental, Germany) in a 2900 rpm cycle for thirty seconds. The powder was dispensed into the liquid and mixed according to the manufacturer's recommendation. (Riva Self Cure, SDI, Victoria, Australia) The proportions of powder/ monomer/ CXD for each group were listed as follows: 0% CXD, 5 g/2.5 mL/ 0 g; 1% CXD, 5 g/2.5 mL/0.05 g; 2% CXD, 5 g/2.5 mL/0.1 g; 3% CXD, 5 g/2.5 mL/0.15 g. The cement mixture was poured into molds in the form of disks. Thirty samples were generated for every concentration. A Teflon slide was put over the mold, and it was held fixed with finger pressure. The control group was established without adding any CXD.

Surface Roughness Determination

Using a profilometer (Perthometer M2, Mahr GmbH, Gottingen, Germany), the surface roughness (Ra) of each specimen was measured. The arithmetic roughness average (Ra) was obtained by moving the stylus of the profilometer in a straight line across the surface of the specimen. The procedure was carried out twice on different regions of the resin's surface, and the results were analyzed using the mean of the two measurements. The test parameters were as follows: 5µm diamond stylus tip radius, 0.25 mm/s stylus velocity, and 0.8 mm cut off length. The same investigator performed each and every procedure. As references, surface roughness values spanning from 0.08 to 0.35 µm were acceptable.[11]

Culturing and Inoculation of Microorganisms

Strains of *S. mutans* (ATCC 25175) were grown in a chemically defined medium (CDM).[12] H_3PO_4 was used to modify the pH to 7.0. To inoculate the samples, 1000µl of CDM medium and 20µl of *S. mutans* (1.2×10^5) were added.

In order to prepare anaerobic blood agar plates (CDC, BioMérieux, Durham, North Carolina, United States), E. faecalis (ATCC 29212) was cultivated.[12] Subsequently, the bacterial cultures were cultivated in Brain Heart Infusion medium supplemented with 5 g/l yeast extract at 37°C. They were cultivated by supplementing 1000µl of BHI-YE containing 0.5% glucose.

A variety of S. mutans strains (ATCC 25175) were cultivated using a chemically defined medium (CDM). In order to adjust the pH to 7.0, 12 H3PO4 was introduced. To bring the samples to life, 20µl of S. mutans (1.2 x 105) and 1000µl of CDM medium were added to the samples.

C. albicans was grown on Sabouraud's dextrose agar for 18 hours at 37°C. Afterwards, 100 mM glucose and yeast were added to the medium containing nitrogen-based yeast. Overnight liquid cultures were extracted for yeasts in the late exponential growth phase. To prepare the yeast for adhesion and biofilm experiments, it was rinsed twice with 5 ml of phosphate buffered saline (pH 7.2; Ca2+- and Mg2+-free) solution. After being rinsed, the cells were resuspended in growth medium at a concentration of 10^7 cells/ml (optical density of at least 600 nm) for adhesion experiments or biofilm assays.

Cristal Violet Assay

In an orbital mixer at 120 rpm and 37°C, samples were incubated for 72 hours. The wells were stained for 30 minutes with 1000 µl of 0.1% CV following 72 hours of mixing at 37°C and 120 rpm. The samples were washed with 1000 l of demineralized water to remove excess dye. The optical density at 585 nm was measured. The absorbance at 585 nm was calculated using a micro titer plate reader. Each experiment was repeated three times using individually grown microorganisms.

MTT Assay

The MTT assay was used to evaluate the metabolic activities of living biofilm cells.[13] After incubation, the specimens had been deprived of the growth medium and rinsed three times with sterile 1xPBS. Three hours later, 1000 µl of a 5 mg/mL MTT solution was added to the specimens and they were incubated in the dark. The supernatant was discarded after the incubation period, and 1000 µL of lysate solution (10% sodium dodecyl sulfate and 50% dimethyl formamide was added. The specimens were incubated for an additional three hours in the lysis solution. After the biofilm was completely dissolved, the absorbance of the resulting solution was determined.

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) images at 1000X, 5000X and 10000X magnifications were taken with a Hitachi Regulus 8230 (Japan) scanning electron microscope.

Statistical Analysis

The statistical analysis was performed by IBM SPSS 24.0 (IBM SPSS Corp., Armonk, NY, USA). Shapiro Wilk's test was used to test the normality of data, which was displayed normal distribution. Differences between CXD groups were tested using ANOVA followed by post hoc Tukey's test. Differences between CV and MTT assays for each concentration were tested by paired t test (Table 1).

Table 1 Mean±SD cell viabilities values of the tested samples with different concentrations by MTT and CV Assays.

	CXD %	MTT (Mean±SD)	CV (Mean±SD)
E. Faecalis	0%	2.28±0.08	2.47±0.01
	1%	2.28±0.03	1.93±0.01
	2%	2.20±0.03	1.10±0.01
	3%	2.09±0.04	0.48±0.01
S. Mutans	0%	2.26±0.10	2.03±0.01
	1%	2.10±0.02	1.20±0.01
	2%	1.92±0.03	0.69±0.01
	3%	1.77±0.04	0.24±0.01
C. Albicans	0%	3.32±0.10	3.26±0.02
	1%	3.26±0.04	2.62±0.01
	2%	2.98±0.02	1.29±0.01
	3%	2.30±0.03	0.65±0.01

RESULTS

The viability of the pathogens was evaluated by using two different assays performed under the same conditions: the MTT Assay and the CV Assay in this study (Figure 1).

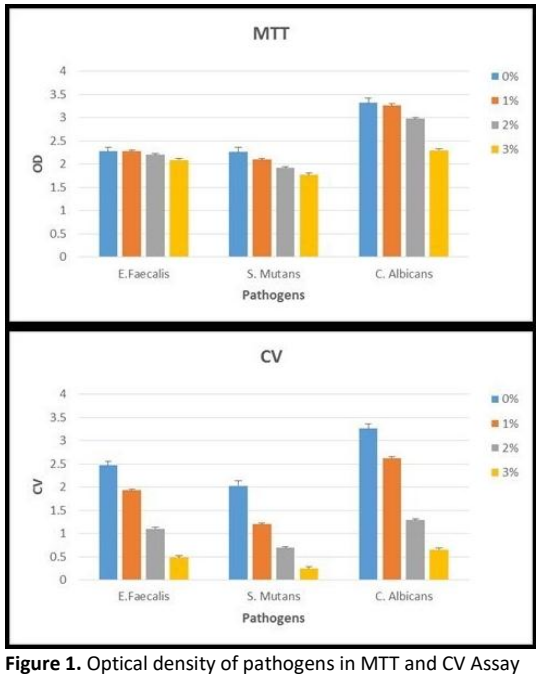

Figure 1. Optical density of pathogens in MTT and CV Assay

The standard deviation and mean values of MTT and CV are presented in Table 1. Modified groups showed significant antimicrobial properties compared to the control group. The highest quantity of antimicrobial activity was found in GIC with 3% added CXD in both MTT and CV Assays (P <.001). *S. mutans* and *C. albicans* were inhibited significantly by all the samples. The quantity of *S. mutans* and *C. albicans* growth rate was decreased with the increasing concentration. The addition of 3 % CXD reduced the proportion of *E. faecalis* (P <.001).

With the CXD concentration raised in groups, the pathogen viability and quantity decreased apparently. SEM images of groups are shown in Figure 2. In CXD groups with a high concentration, fewer pathogen colonies and smaller layers are visible.

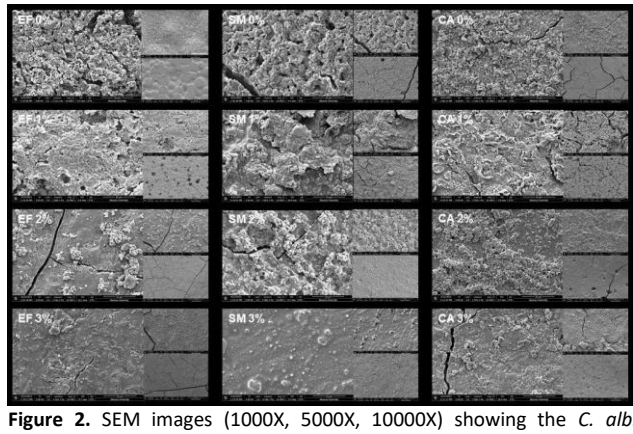

Figure 2. SEM images (1000X, 5000X, 10000X) showing the *C. albicans, S. mutans, E. facealis* biofilms at different CXD concentrations. (EF0: *E. facealis* with 0% CXD, EF1%: *E. facealis* with 1% CXD, EF2%: *E. facealis* with 2 % CXD, EF3%: *E. facealis* with 3% CXD; SM0%: *S. mutans* with 0% CXD, SM1%: *S. mutans* with 1% CXD, SM2%: *S. mutans* with 2% CXD, SM3%: *S. mutans* with 3% CXD; CA0%: *C. albicans* with 0% CXD, CA1%: *C. albicans* with 1% CXD, CA2%: *C. albicans* with 2% CXD, CA3%: *C. albicans* with 3% CXD

DISCUSSION

The present study investigated the antifungal and antibacterial effects of Glass Ionomer Luting Cement incorporated with CXD in *S. mutans, E. faecalis* and *C. albicans* in vitro. The antimicrobial activities were assessed using CV, MTT Assays, and SEM. The hypothesis was rejected, since groups containing CXD inhibited the antibacterial and antifungal activity compared to non-modified control group.

Chlorhexidine diacetate is a commonly used antimicrobial agent, and previous studies reported that luting cements modified with CXD improved their antimicrobial effectiveness.[14-16] However, there are differences in the glass ionomer cements chosen for the experiments, the chlorhexidine concentration, and the methodologies used in these studies. The current study evaluated the antimicrobial effect of CXD at 0, 1, 2, 3% concentration to glass ionomer luting cement against *S. mutans, E. faecalis* and *C. albicans.* It was reported that the addition of high concentrations of CXD increases the antimicrobial activity but decreases the mechanical properties inversely.[16,17] It has also been stated that high amounts of chlorhexidine weaken the glass ionomer network and scaffold and adversely affect the mechanical properties of GIC and high concentration of CXD can also be toxic and allergic.[17] Therefore, in this study, maximum concentration of CXD was 3%.

S. mutans is an essential factor to the development of dental caries and biofilm. In this study, *S. mutans* was significantly inhibited by CXD incorporation compared to the control group. While variations in CXD concentration and glass ionomer cement were present in this investigation, the findings are consistent with those reported by Hu et al.[18] and Marti et al.[19]. On the basis of its substantivity, it was hypothesized that CXD emitted from the material could persist in the

environment, interfering with bacterial colonization and biofilm formation while producing a bacteriostatic effect.[20,21] Davidovich et al.[21] stated that GIC inhibited the *S. mutans* growth rate. However, *E. faecalis* showed bacterial growth only after contact with 3% CXD added samples. This may be due to the resistant character structure of *E. faecalis* bacteria. Kwon et al.[22] assessed the antibacterial effects of resin containing chlorhexidine and they concluded that the growth of all microorganisms was inhibited by resin samples containing 1% CXD or higher except for *E. faecalis*. Additonally, Gro¨nroos et al.[23] reported that *E. faecalis* is less sensitive to CXD as compared with S. mutans. Therefore, low concentration of CXD does not seem to be effective in the control of *E. faecalis* infection. As *E. faecalis* plays an important role in unsuccessful root canal treatments, it is important to select the appropriate luting cement for fixed prosthetic treatments after root canal therapy. In this study, it was observed that the addition of 3% CXD reduced the growth rate of *E. faecalis*. Therefore, the use of 3% CXD concentration of GIC can be considered for crown cementation or cementation of posts after root canal treatments.

In the current study, the antimicrobial effect increased proportionally as the percentage of CXD added increased. The addition of 1% chlorhexidine diacetate was reported to be optimal in terms of antibacterial, physical, and bonding properties.[24,25] The high concentrations of chlorhexidine added into GIC increased the of antimicrobial effect on *S. mutans, C. albicans* except *E. faecalis.*. Regarding *C. albicans*, this studies results differed from those obtained by Charannya et al.[26] and Turkun et al.[17] This may be related the material used and the methodology of the bacterial test. More studies are needed to examine the antimicrobial properties of cements incorporated with different antibacterial agents.

Studies have shown that CXD interacts with fluoride and decreases its antibacterial effectiveness. CXD at a 10% concentration has been found to decrease the release of fluoride.[27] The effect of CXD addition on fluoride release may be due to the ability of CXD to soften the resin matrix pathway.[28] They explained the reason for this as that fluoride and CXD can interact and cause low solubility, and thus cause precipitation of lower concentrations of CXD. Furthermore, highly cariogenic bacteria such as *S. mutans* induce a lower pH.[29] If the antimicrobial activities improve, the pH of the biofilm in the materials used in the experiment is expected to increase. In the current study, not only the increase in the concentration of CXD, but also fluoride release from CIS may have affected the antimicrobial effect results. Therefore, more studies are needed to evaluate fluoride end CXD releases in different concentrations, and to measure the effects of pH values on microbial growth. This can be the limitation of this study.

In this study, MTT and CV assays demonstrated that the addition of CXD effectively reduced biofilm formation. CV stains biomass rather than living bacteria and thus dead bound bacteria will still be stained. MTT assay is used to evaluate metabolic activities of living bacteria in biofilms. In previous studies, activation of *S. mutans and C. albicans* was affected when CXD was at a relatively high concentration. As these assays are widely used as convenient and rapid measures of cell viability, similar results were evaluated in both assays. The number of viable bacterial colonies in the control group was more than in the CXD-modified groups, indicating that CXD shows antimicrobial activity against pathogens, according to the findings of this study. Moreover, unlike E. facialis, the results demonstrated that the growth of S. mutans is inhibited in a dose-dependent form when CXD is incorporated into the GIC at low concentrations.[30]

To evaluate the structure and morphology of the biofilm of pathogens, SEM images were also used. It revealed that in the presence of 3% concentration of CXD, the connectivity between cells was reduced, leading to a diffuse distribution. SEM images were utilized. This study assessed the antimicrobial activities of luting glass ionomer cements incorporated with CXD. The findings suggested that adding CXD would impart the antimicrobial activity against *S. mutans, E. faecalis* and *C. albicans.*

SEM images were also used to assess the structure and morphology of pathogen biofilms. It was found that in the presence of 3% CXD, the connection between cells was diminished, resulting in a diffuse distribution. According to SEM images, adding CXD would induce antibacterial activity against *S. mutans, E. faecalis, and C. albicans*.

Within the scope of this study, 3% CXD added to glass ionomer luting cement provided the best antibacterial characteristics. More in vivo and in vitro investigations are required to examine the bonding abilities, mechanical characteristics, fluoride and CXD release measurements after incorporating CXD.

The limitation of this study is that samples were not stored in saliva as normally occurs in clinical settings, which might increase biofilm formation. Moreover, including antimicrobial additives into cement could prevent biofilm formation but could also change the chemical and mechanical characteristics of the cement. Further research is needed to study the adhesion and mechanical properties of cements.

In conclusion, CXD groups at all concentrations showed antibacterial activity against *S. mutans* and *C. albicans.* It can be concluded that antimicrobial activity against *S. mutans and C. albicans* were greater than those against *E. faecalis*. The 3 % CXD added group exhibited significant bacterial inhibition in *E. faecalis* bacteria. CIS modified with CXD can be preferred in crown cementations on endodontically treated teeth, as well as in high caries-risk patients and in immunocompromised patients.

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A Bibliometric Analysis of the Research Trends of Resin Cements in Dentistry

Diş Hekimliğinde Rezin Simanların Araştırma Eğilimlerine Yönelik Bir Bibliyometrik Analiz

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ABSTRACT

Objective: In this bibliometric analysis, it was aimed to analyze the research trends of the researchers, organizations, countries, and journals that contributed to the research focus of resin cements in dentistry.

Methods: The literature search was carried out on the basis of the Web of Science (WoS) database containing publications on resin cements between 1970 and 2022. Metadata including titles, abstracts, keywords, authors, links, countries, and references has been recorded. The obtained data were analyzed using bibliometric indicators for all interactions and collaborations via the VOSviewer software program.

Results: As a result of the bibliometric analysis, 1643 studies published between 1970 and 2022 were found in the searches made in the Web of Science database. While publications on resin cements received a total of 26871 citations, this number corresponded to an average of 20.75 per article. The H index was found to be 71. The document type of these publications was 97.60% in the article type. While the most publications on resin cements are in the Journal of Adhesive Dentistry and Journal of Prosthetic Dentistry, the most references are in the Dental Materials Journal and Journal of Prosthetic Dentistry.

Conclusion: Between 1983 and 2022, research on resin cements was on the rise. With the global increase in resin cement research, extensive collaborations and citations have been noted between authors, institutions, and countries or regions. It is predicted that resin cements will maintain their popularity in future studies.

Keywords: Article, author, bibliometric analysis, resin cement

ÖZ

Amaç : Bu bibliyometrik analizde, diş hekimliğinde kullanılan rezin simanların araştırma odağına katkıda bulunan araştırmacıların, kuruluşların, ülkelerin ve dergilerin araştırma eğilimlerini analiz etmek amaçlandı.

Yöntemler: Literatür taraması, 1970-2022 yılları arasında rezin simanlar alanında yayınların yer aldığı Web of Science (WoS) veri tabanına dayanarak gerçekleştirilmiştir. Başlıklar, özetler, anahtar kelimeler, yazarlar, bağlantılar, ülkeler ve referanslar gibi meta veriler kaydedilmiştir. Elde edilen veriler, WOSsviewer yazılım programı aracılığıyla tüm etkileşimler ve işbirlikleri için bibliyometrik göstergeler kullanılarak analiz edildi.

Bulgular: Bibliyometrik analiz sonucunda, Web of Science veri tabanında yapılan aramalarda 1970-2022 yılları arasında yayınlanmış 1643 çalışma bulunmuştur. Rezin simanlar üzerine yayınlar toplam 26871 atıf alırken, bu sayı makale başına ortalama 20,75'e karşılık gelmektedir. H indeksi 71 olarak bulundu. Bu yayınların belge türü makale türünde %97,60'tı. Rezin simanlar üzerine en çok yayın Journal of Adhesive Dentistry ve Journal of Prosthetic Dentistry'de yer alırken, en çok referans Dental Materials Journal ve Journal of Prosthetic Dentistry'de yer almaktadır.

Sonuç: 1983 ile 2022 yılları arasında rezin simanlar üzerine yapılan araştırmalar artıştaydı. Rezin siman araştırmalarındaki küresel artışla birlikte yazarlar, kurumlar ve ülkeler veya bölgeler arasında kapsamlı işbirlikleri ve atıflar kaydedildi. Rezin simanlarının gelecekteki çalışmalarda popülerliğini sürdüreceği öngörülmektedir.

Anahtar Kelimeler: Makale, yazar, bibliyometrik analiz, rezin siman

INTRODUCTION

Recent studies in dentistry have led to a gradual decrease in the need for removable partial dentures and an increase in esthetic concerns as a result of patients' increased awareness of oral health, and fixed prosthetic restorations have come to the forefront. One of the most important factors in the success of fixed prosthetic restorations is the cementation process.[1-3] Luting cements have the primary function of filling the gap between the prosthetic restoration and the tooth or implant abutment and ensuring retention of the restoration. The shortcomings of conventional cements are high solubility, lack of adhesion and inadequate esthetic properties. In order to avoid these negative aspects, resin cements have emerged.[2,4] In current clinical applications, resin cements have found more areas of application, leaving behind traditional resin cements with their chemical bonding properties, low microleakage, color options, and their contribution to aesthetics.[4] Although there are many in vitro and in vivo studies in the literature regarding resin cements,

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whose popularity and area of application are increasing day by day, there is a lack of bibliometric analysis regarding this issue. Many research fields; use bibliometric methods to investigate the impact of a particular article, by researchers, or to identify particularly influential articles in a particular research field. While bibliometric research can identify the most productive researchers in a given field, it can also reveal the dimensions of interaction between them.[5,6] Using a similar approach, bibliometric studies can also make comparisons between countries, between institutions, or between schools in different fields. The main objectives of such studies were to define the chronological trends of the analyzed subject, to show the number of citations, and to highlight the evidence-based studies.[7,8] In essence, bibliometric analysis is an analytical method used to gain insight into an academic field.[9]

The aim of this study was to perform a bibliometric analysis of the articles published on resin cements in the literature.

METHODS

The search was conducted in the Web of Science (WoS) database, originally created by the Institute for Scientific Information (ISI) and later maintained by Clarivate Analytics. The literature search was conducted in June 2022. Articles published before June 15, 2022 were included in the search. Filters were applied to improve and limit the search. First, article and compilation were selected as the document type for filtering. A total of 1643 results were obtained by entering "TS=[("resin cement") AND (dent OR prosthodont OR oral OR tooth OR teeth OR crown)]" in the advanced search section. While 1289 of these results were in the "Dentistry Oral Surgery Medicine" category, 189 of them were in the "Materials Science Biomaterials" category. The 189 results in the "Materials Science Biomaterials" category were analyzed individually and 146 publications related to dentistry were included in the study. Papers whose abstracts and keywords were not in English were excluded from the study. 87.34% of 1643 studies, or 1435 studies, were included.

All results from the final screening process were sorted from the most cited to the least cited as "highest cited" publications. The data of the publications obtained for the bibliometric analysis were saved in plain text document format ".txt" and exported. Likewise, the data of the 100 most cited publications and the first 20 of them were exported by saving them in an Excel file in ".xls" format.

For the bibliometric analysis of the obtained data, the bibliometric analysis program VOSviewer (Center for Science and Technology Studies, Leiden University) was used.[10,11] VOSviewer version 1.6.18, published on January 24, 2022, was downloaded free of charge from the official website of the program.[10] Data in ".txt" and ".xls" formats were transferred to the VOSviewer program and processed.[11-13] Collaborations and citation numbers were visualized by network type or registration with the help of VOSviewer software. In the resulting visual map, the size of the bubble reflected the number of broadcasts, the distance between the bubbles reflected the relationship between the two elements, and the color of each bubble had different meanings in different visual maps.

RESULTS

As a result of the bibliometric analysis, 1643 studies published between 1970 and 2022 were found in the Web of Science database. The number of articles published per year showed an increasing trend, with slight decreases in between. Publications on resin cements received a total of 26871 citations, with an average of 20.75 citations per article. The H-index was found to be 71. The document type of these publications was mainly articles, 97.60%. Using the information in the database, information on certain topics such as titles, authors, journals, publication dates, total citations, annual average was obtained and tables were created. Spreadsheet of the 20 most cited articles in "resin cement" research using scan targets (Microsoft Office 2020 Excel v16.35; Microsoft Corp) and shown in Table 1. The number of articles published by year between 2000 and 2022 was shown graphically in Figure 1. The distribution of the network structures formed because of the bibliometric analyses according to authors was shown in Figure 2, the distribution according to institutions in Figure 3, and the distribution according to countries in Figure 4. Information on the 10 most productive authors on resin cements was given in Table 2. According to this table, the most productive author was Valandro Luiz Felipe. There were many institutions from different parts of the world doing research on resin cement. The number of articles and citations of these countries and institutions were shown in Tables 3 and 4. Ignoring main search item, resin cement, the most used keywords were 'bond strength', 'shear bond strength', 'adhesion', and 'zirconia' (Figure 5). The distribution of the most productive countries for resin cement is consistent with the distribution of research institutions. The highest number of articles was found in the USA, followed by Brazil and Turkey. While the Journal of Prosthetic Dentistry had the most publications on resin cement, it also had the most citations (Figure 6). The three most cited authors were Ferrari M, Goracci C, and Tay FR (Table 2). Although more than one organization was at the forefront of resin cement, the universities that contributed the most were univ siena, univ sao paulo, and univ estadual paulista.

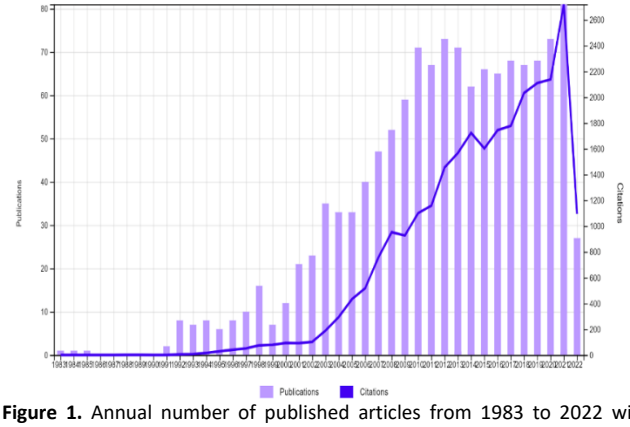

Figure 1. Annual number of published articles from 1983 to 2022 with resin cements

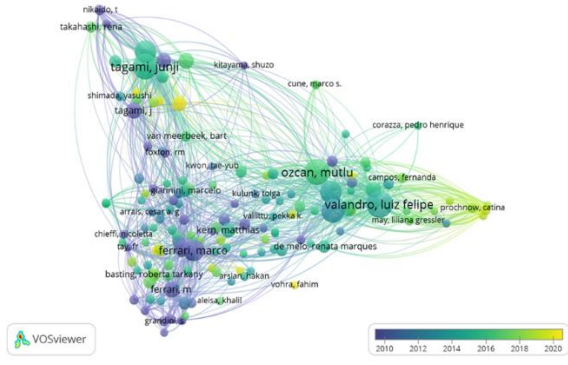

Figure 2. Collaboration networks between authors with more than 5 publications

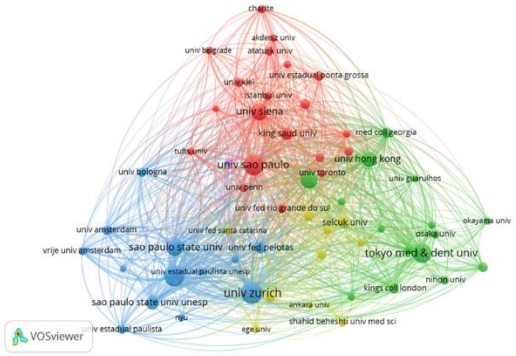

Figure 3. Collaboration networks between instituons with more than 10 publications

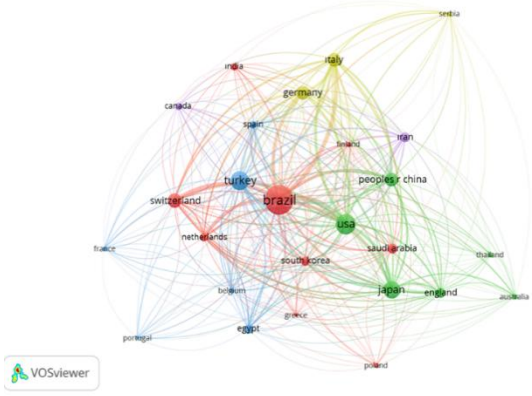

Figure 4. Collaboration networks between countries with more than 10 publications

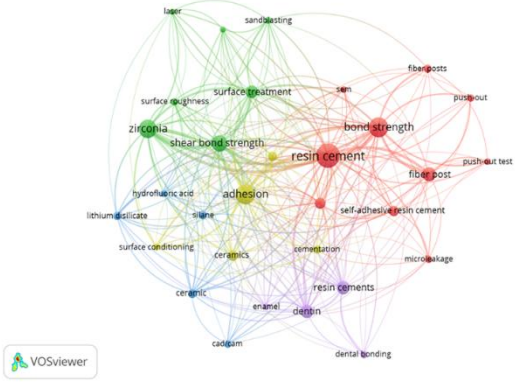

Figure 5. The term map of keywords whose total link strength was higher than 50 (*Bubble size indicates occurring frequency of term as keyword of publications. Color of bubble indicates average normalized citations*).

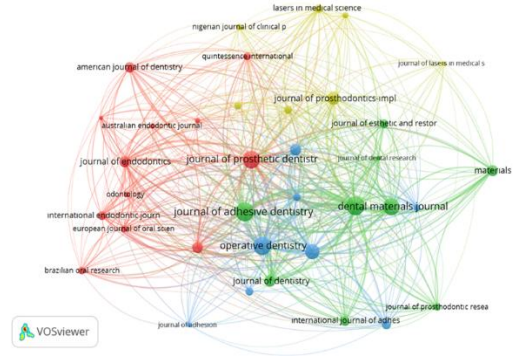

Figure 6. The citation map of journals whose total link strength was higher than 50 (*Bubble size indicates number of documents. Color of bubble indicates relative citation*).

DISCUSSION

Esthetic restorations are becoming increasingly important in prosthetic rehabilitation in dentistry. It is a fact that ceramic restorations, which are indispensable for esthetic restorations and continue their increasing trend, cannot be considered independent of cementation. The importance of resin cements is increasing according to this population. The fact that resin cements have not been studied in previous bibliometric analysis studies is the main subject and purpose of the present study, which aims to contribute to the literature.

According to the Web of Science database search, the first study on resin cements was conducted in 1985 and 1289 articles were published until June 2022. The publications showed a rapid increase in the 10-year period after 1997. In the last 10 years, 60 or more articles were published each year. The highest number of publications on resin cements was in 2021 with 81 publications. This is a numerical example of the increasing trend of resin cements in recent years. Since the data search of the study was conducted in June 2022, the number of publications in 2022 appears to be less than in previous years, but considering the linear increase in the graph, it is expected to reach the number of publications similar to previous years by the end of 2022 (Figure 1).

A review of the 20 most cited articles on resin cements included laboratory studies and clinical evaluations. The most cited studies from 2005 and earlier covered topics such as endodontic fiber post, fiber post strength, bond strength, zirconia strength, and dentin bond strength of resin cements.[14-17] The most cited articles from more recent years covered topics such as the bond strength of zirconia ceramics and the factors that affect surface treatments on resin cement bond strength.[18-20] Recent studies have investigated topics such as the translucency of zirconia and lithium disilicate and the bonding effect of surface treatments on lithium disilicate.[21,22] When the subject headings were examined by year, it was seen that resin cement is mostly related to endodontic fiber posts in the early years and has evolved to studies that contribute to zirconia bonding and esthetic materials such as lithium disilicate in the following years. It was believed that this evolution has overcome the problems such as endodontic fiber post, fiber post strength, and bond strength. One of the reasons for this situation might have been the developments in ceramics and the increase in their clinical use in recent years. In addition, problems related to resin cement, such as the bonding efficiency of CAD-CAM, zirconium oxide ceramics, the bonding strength of resin cement, are still relevant and are still a problem for us. The top 3 authors were Ferrari M, Bottina MA and Valandro LF. There was usually a parallelism between the number of publications of the authors and the highest citation (Table 1). Although the number of articles by authors such as Tay FR, Goracci C, and Kern M was low, the high number of citations they received shows that the impact rate of the articles was high (Table 1). The 3 most productive countries were USA, Brazil and Türkiye, followed by Germany and Japan (Table 2). This ranking showed that these 3 countries were the most productive countries, and the number of citations did not show any parallelism according to the number of articles. It could be seen that the most cited countries per article were Italy, Germany, and the USA. It could be said that these countries were more effective. In terms of both productivity and impact, the USA was seen to be in the lead. While Germany ranks fourth in terms of productivity, it ranked second in terms of impact; although it was relatively behind the US in terms of both impact and productivity, it was a significant data point. The fact that the United States was ahead in both productivity and efficiency might be due to the large number of research-based institutions in the country.

Table 1. 20 most cited articles in resin cement research

Title	Authors	Source Title	Publication Date	Publication Year	Total Citations	Average per Year
Resistance to fracture of endodontically treated teeth restored with different post systems	Akkayan B, Gulmez T.	Journal of Prosthetic Dentistry	Apr 2002	2002	351	16.71
Monolithic cad/cam lithium disilicate versus veneered y-tzp crowns: comparison of failure modes and reliability after fatigue	Guess Petra C, Zavanelli Ricardo A, Silva Nelson RFA, Bonfante Estevam A, Coelho Paulo G, Thompson Van P.	International Journal of Prosthodontics	Sep-Oct 2010	2010	214	16.46
Clinical behavior of zirconia-based fixed partial dentures made of dc-zircon: 3-year results	Tinschert J, Schulze Karen A, Natt G, Latzke P, Heussen N, Spiekermann H.	International Journal of Prosthodontics	May-Jun 2008	2008	202	13.47
Effects of sodium hypochlorite and rc-prep on bond strengths of resin cement to endodontic surfaces	Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH.	Journal of Endodontics	Dec 2001	2001	199	9.05
Clinical behavior of translucent-fiber posts: a 2-year prospective study	Monticelli F, Grandini S, Goracci, C.	International Journal of Prosthodontics	Nov-Dec 2003	2003	197	9.85
Fracture resistance of endodontically treated teeth restored with composite posts	Newman MP, Yaman P, Dennison J, Rafter M, Billy E.	Journal of Prosthetic Dentistry	Apr 2003	2003	187	9.35
Meta-analysis of bonding effectiveness to zirconia ceramics	Inokoshi M, De Munck J, Minakuchi S, Van Meerbeek B.	Journal of Dental Research	Apr 2014	2014	178	19.78
Bonding to oxide ceramics-laboratory testing versus clinical outcome	Kern M.	Dental Materials	Jan 2015	2015	163	20.38
In vitro retentive strength of zirconium oxide ceramic crowns using different luting agents	Ernst CP, Dose P, Cohnen U, Stender E, Willershausen B.	Journal of Prosthetic Dentistry	Jun 2005	2005	162	9
Influence of surface treatments and resin cement selection on bonding to densely-sintered zirconium-oxide ceramic	de Oyague RC, Monticelli F, Toledano M, Osorio E, Ferrari M, Osorio R.	Dental Materials	Feb 2009	2009	157	11.21
The contribution of friction to the dislocation resistance of bonded fiber posts	Goracci C, Fabianelli A, Sadek FT, Papacchini F, Tay FR.	Journal of Endodontics	Aug 2005	2005	155	8.61
Clinical evaluation of teeth restored with quartz fiber-reinforced epoxy resin posts	Malferrari S, Monaco C, Scotti R.	International Journal of Prosthodontics	Jan-Feb 2003	2003	155	7.75
Development of a clinically validated bulk failure test for ceramic crowns	Kelly JR, Rungruanganunt P, Hunter B, Vailati F.	Journal of Prosthetic Dentistry	Oct 2010	2010	152	11.69
Efficacy of different adhesive techniques on bonding to root canal walls: an sem investigation	Ferrari M, Vichi A, Grandini S.	Dental Materials	Sep 2001	2001	148	6.73
Mechanical and physical properties of contemporary dental luting agents	Attar N, Tam LE, McComb D.	Journal of Prosthetic Dentistry	Feb 2003	2003	146	7.3
Evaluation of the adhesion of fiber posts to intraradicular dentin	Goracci C, Sadek FT, Fabianelli A, Tay FR, Ferrari M.	Operative Dentistry	Sep-Oct 2005	2005	145	8.06
Microtensile bond strength and interfacial properties of self-etching and self-adhesive resin cements used to lute composite onlays under different seating forces	Goracci C, Cury AH, Cantoro A, Papacchini F, Tay FR, Ferrari M.	Journal of Adhesive Dentistry	Oct 2006	2006	139	8.18
The effect of zirconia surface treatment on flexural strength and shear bond strength to a resin cement	Qeblawi DM, Munoz CA, Brewer JD, Monaco EA.	Journal of Prosthetic Dentistry	Apr 2010	2010	133	10.23
Surface debris of canal walls after post space preparation in endodontically treated teeth: a scanning electron microscopic study	Serafino C, Gallina G, Cumbo E, Ferrari M.	Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontologist	Mar 2004	2004	126	6.63
Adhesive permeability affects coupling of resin cements that utilise self-etching primers to dentine	Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NRFA, Pashley DH.	Journal of Dentistry	Jan 2004	2004	124	6.53

When analyzed by institutions, the most productive institutions were shown in Table 3. The top 3 most productive institutions were Univ estadual paulista, Univ sao paulo, Uni siena. The fact that the first 2 of the 3 most productive institutions and the first 10 of the 5 most productive institutions were in Brazil, as well as Brazil's second place in the publication ranking of the countries, suggested an excess of interest in esthetic restorations in Brazil and an increased in this regard. According to the number of articles, the 3 most influential institutions were the University of Augusta, the University System of Georgia and the University of Siena. Based on the data, it could be said that the number of studies conducted in institutions and the rate of influence did not increase linearly (Table 3).

Table 2. Top 10 most cited authors

Author	Citations	Documents	Average per Article
Ferrari M	2607	58	44.95
Goracci C	1282	27	47.48
Thai FR	1130	17	66.47
Bottina MA	860	37	23.24
Valandro LF	859	37	23.22
Tagami J	704	31	22.71
Nikaido T	574	19	30.21
Kern M	567	18	31.5
Ozcan M	518	33	15.7
Soares CJ	346	17	20.35

Table 3. 10 most contributing institutions

Organizations	Documents	Citations	Average per article
Univ estadual paulista	74	1452	19.62
Uni sao paulo	65	1528	23.51
Uni siena	63	2702	42.89
Uni estadual de campinas	50	1112	22.24
Univ federal de santa maria ufsm	45	1048	23.29
Tokyo med dent	43	1206	28.05
Univ zurich	40	686	17.15
Egyptian Knowledge Bank EKB	37	575	15.54
Augusta Uni	27	1237	45.81
Unisystem of Georgia	27	1237	45.81

Table 4. 10 most contributing countries or regions

Country	Documents	Citations	Average per Article
USA	300	8285	27.62
Brazil	298	5460	18.32
Turkey	135	2512	18.61
Germany	126	4233	33.6
Japan	112	2219	19.81
Italy	106	3687	34.78
Switzerland	64	1269	19.83
England	59	1199	20.32
Saudi Arabia	52	373	7.17
Peoples R China	45	1226	27.24

CONCLUSIONS

Based on the findings obtained from the bibliometric analysis of the studies in the literature on resin cements, the following conclusions were reached:

1. As a result of the development of esthetic prosthetic materials, there was an increase in research on resin cement on a global scale. As a result of this development, the material had become more popular, and the cooperation in research had increased, the number of citations had remained at a high level.

2. In line with the publications in recent years, the bond strength of resin cement and esthetic concerns have become popular research topics.

3. It is predicted that resin cement will maintain their popularity in future studies.

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Informed Consent: Not applicable.
Peer-review: Externally peer-reviewed.
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Financial Disclosure: The authors declared that this study has received no financial support.
Etik Komite Onayı: Etik kurul onayı gerekmemektedir.
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Exploring Genetic Susceptibility in Peri-Implantitis

Peri-implantitis Gelişiminde Genetik Yatkınlığın İncelenmesi

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ABSTRACT

The investigation of genetic factors influencing peri-implant diseases, particularly peri-implantitis (PI), offers insights into the complex interplay between host genetics and inflammatory responses within oral tissues. Numerous genes encoding cytokines, receptors, and regulatory proteins have been scrutinized for their roles in modulating immune responses and tissue homeostasis around dental implants. Notably, polymorphisms in genes encoding interleukins (*IL-1, IL-6, IL-17, IL-10*), tumor necrosis factor-alpha (*TNF-α*), and matrix metalloproteinases (*MMPs*) have emerged as potential genetic markers for susceptibility to peri-implant diseases. Additionally, genes involved in bone metabolism, such as osteoprotegerin (*OPG*), receptor activator of nuclear factor kappa-B ligand (*RANK-L*), bone morphogenetic proteins (*BMPs*), and fibroblast growth factors (*FGFs*), contribute to peri-implant diseases. Polymorphisms in these genes affect bone resorption and regeneration processes, influencing the stability of dental implants. Variations in these genes can either enhance or hinder bone regeneration, impacting the healing process and the long-term success of implants. Moreover, investigations into less-studied genes like cluster of differentiation 14 (*CD14)*, Chemokine Receptor 2 (*CXCR2)*, and Fragment crystallizable gamma receptor (*FcγRs)* have revealed additional genetic determinants implicated in the pathogenesis of peri-implant diseases. These genes can influence immune cell function and inflammatory signaling pathways, contributing to the body's response to bacterial biofilms and other factors that compromise implant health. The study of these genetic variations provides a deeper understanding of individual susceptibility to PI and may guide personalized treatment strategies, ultimately improving implant success rate.
Keywords: Peri-implantitis, polymorphism, immunity

ÖZ

Peri-implant hastalıkları, özellikle peri-implantitis (PI) etkileyen genetik faktörlerin araştırılması, konak genetiği ile oral dokulardaki inflamatuar yanıtlar arasındaki karmaşık etkileşime dair içgörüler sunar. Sitokinleri, reseptörleri ve düzenleyici proteinleri kodlayan çok sayıda gen, dental implantlar etrafındaki bağışıklık yanıtlarını ve doku homeostazını düzenlemedeki rolleri açısından incelenmiştir. Özellikle, interlökinleri (IL-1, IL-6, IL-17, IL-10), tümör nekroz faktörü-alfa'yı (TNF-α) ve matris metalloproteinazları (MMP'ler) kodlayan genlerdeki polimorfizmler, peri-implant hastalıklarına yatkınlık için potansiyel genetik belirteçler olarak ortaya çıkmıştır. Ek olarak, osteoprotegerin (OPG), nükleer faktör kappa-B ligandının reseptör aktivatörü (RANK-L), kemik morfogenetik proteinleri (BMP'ler) ve fibroblast büyüme faktörleri (FGF'ler) gibi kemik metabolizmasında yer alan genler peri-implant hastalıklarına katkıda bulunur. Bu genlerdeki polimorfizmler kemik rezorpsiyonunu ve rejenerasyon süreçlerini etkileyerek diş implantlarının stabilitesini etkiler. Bu genlerdeki varyasyonlar kemik rejenerasyonunu artırabilir veya engelleyebilir, iyileşme sürecini ve implantların uzun vadeli başarısını etkileyebilir. Ayrıca, farklılaşma kümesi 14 (CD14), Kemokin Reseptörü 2 (CXCR2) ve Fragment kristalize edilebilir gama reseptörü (FcγR'ler) gibi daha az çalışılmış genler üzerindeki araştırmalar, peri-implant hastalıklarının patogenezinde rol oynayan ek genetik belirleyicileri ortaya çıkarmıştır. Bu genler, bağışıklık hücresi fonksiyonunu ve inflamatuar sinyal yollarını etkileyerek vücudun bakteriyel biyofilmlere ve implant sağlığını tehlikeye atan diğer faktörlere verdiği tepkiye katkıda bulunabilir. Bu genetik varyasyonların incelenmesi, PI'ye karşı bireysel duyarlılığın daha derin bir şekilde anlaşılmasını sağlar ve kişiselleştirilmiş tedavi stratejilerine rehberlik ederek nihayetinde implant başarı oranlarını iyileştirebilir.
Anahtar Kelimeler: Bağışıklık, peri-implantitis, polimorfizm

INTRODUCTION

Oral and dental health issues stand among the most widespread public health challenges globally, profoundly impacting individuals' overall health and quality of life.[1] A variety of conditions, including

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cavities, periodontal diseases, tooth loss, dental fractures, dental fluorosis, and craniofacial disorders, adversely affect life quality.[2] Oral health is a crucial component of overall well-being. In developed countries, efforts to improve oral health aim to minimize social and economic inequalities and facilitate access to effective prevention programs within the community. Conversely, in developing countries, factors such as poverty, and low education levels significantly compromise oral health. [3] The primary source of all diseases in the oral cavity for both adults and children is the biofilm layer.[4] Biofilm is a layer that firmly attaches to the enamel and root surfaces of teeth as well as dental prosthetics, and oral soft tissues. It not only adheres strongly but also multiplies, creating a dense environment rich in pathogenic microorganisms that can influence oral health.[5] Recently, dental implants have become one of the most favored methods for treating or cosmetically resolving toothlessness, thanks to their high success rates.[6] Despite these success rates, dental implants designed to function like tooth roots and made of biocompatible titanium-based materials can encounter complications that lead to loss of osseointegration.[7] The primary treatment goal is to restore the patient's lost function, phonetics, aesthetics, and psychosocial well-being. However, this also introduces new artificial surfaces that can foster bacterial biofilm formation, potentially leading to inflammation of the periodontal tissues that support the teeth.[8] This problem has become more widespread as the number of implant procedures increases daily, leading to a rise in implant-related diseases.[9] The soft tissue around the implant is essential for its long-term success and develops during the healing process post-implantation.[10]

Peri-implant diseases are generally classified into two main categories: peri-implant mucositis (PiM) and peri-implantitis (PI).[11] Peri-implant mucositis is diagnosed when complications around an implant are restricted to inflammation of the mucosa. In contrast, PI is identified when this inflammation is accompanied by alveolar bone loss and the development of pathological pockets.[4] Factors influencing the development of peri-implant diseases include patient-specific factors (the host's inflammatory response, smoking, stress, and genetic diversity in related genes), the presence and condition of keratinized mucosa in the implant area, the quantity and quality of bone and soft tissues, the selected implant system and type, and the forces impacting the implant and surrounding tissues. [12] Inflammation is a critical factor affecting osseointegration and the success of implants. In PI, a significant increase in the levels of inflammatory mediators, particularly interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), interleukin-8 (IL-8), and macrophage inflammatory protein-1A peri-implant fluid has been reported.[13] It is believed that the production of inflammatory cytokines stimulates PI and bone resorption, leading to implant failure.[14] Various studies have reported PiM prevalence rates between 8% and 44%, and PI prevalence rates between 1% and 19%.[15]

Gene polymorphisms are genetic variations found in a population at a frequency greater than 1%, among which single nucleotide polymorphisms (SNPs) are the most prevalent.[16] SNPs occur in both the coding and non-coding regions of genetic sequences. SNPs located within coding sequences have the potential to modify amino acid sequences. Conversely, SNPs that are situated in non-coding regions can influence gene expression or the regulation of RNA sequences.[17] These genetic variations are risk factors for various diseases since they can influence gene expression levels and the synthesis or efficacy of proteins. [18] Studies have identified various genetic variations correlated with PI.[16,18,19] SNPs in inflammatory cytokine and various biomarker genes can create changes in expression levels or amino acid sequences, accelerating the destruction of peri-implant tissues.[19]

GENE POLYMORPHISMS AND PI

Cytokines

Cytokines are small, soluble proteins that regulate inflammation and immunity by mediating intercellular communication.[13] Secreted by various cells, they influence cell growth, mobility, and differentiation, playing a key role in disease pathogenesis.[13] Their detection is crucial for monitoring conditions like periodontal disease and peri-implant infections.[13] Cytokines like interleukins and TNF-α play crucial roles in immune responses and inflammation.

Interleukin-1

Inflammatory cytokines known as interleukins (ILs) play a critical role in regulating the immune response. They initiate the breakdown of the extracellular matrix and bone tissue, and excessive production of these substances plays a significant role in the development and clinical symptoms of inflammatory diseases.[20] There is substantial literature indicating a strong association between various IL-1 gene polymorphisms and PI.[21,22]

The IL-1 family comprises at least 11 cytokines that are crucial for immune function.[22] These cytokines are mapped to the long arm of chromosome 2q and the most extensively researched gene regions within this family include *IL-1α* −889 C/T (rs1800587) and *IL-1β* +3954 C/T (rs1143634). [23] Besides IL-1α and IL-1β, the antagonist IL-1RN also plays a role by binding to the receptor and blocking IL-1 mediated signals, exerting an anti-inflammatory effect. The gene encoding IL-1RA, IL-1RN allele 2, is associated with PI. [24] The effect of IL-1 is derived from the balance between IL-1α, IL-1β, and IL-1RA. IL-1RA binds to the IL-1 receptor, blocking the effects of IL-1α or IL-1β. [25] Genetic variations at -889 in *IL-1α* and +3954 in *IL-1β* lead to higher IL-1 levels, resulting in an increased inflammatory response. Conversely, the presence of *IL-1RN* polymorphisms results in lower levels of this molecule, allowing more molecules to join the IL-1 receptor and thereby enhancing inflammation. Consequently, depending on the alleles an individual possesses, the production of IL-1α, IL-1β, and IL-1RN can increase or decrease. [26] Various studies have found evidence of a combined effect between *IL-1* gene variations, peri-implant bone loss and smoking.[27,28]

Periodontitis, a process similar to peri-implantitis, shares common inflammatory pathways with peri-implantitis, which specifically affects dental implants.[29] A study conducted in the Turkish population investigated *IL-1α* (rs1800587) and *IL-1β* (rs1143634) polymorphisms in periodontitis. [30] While no association was observed between IL-1 *α* and periodontitis, the C allele of *IL-1β* (rs1143634) was found to be more prevalent in healthy individuals, indicating a potential protective role.[30] Another study on PI found that the *IL-1 α* (−889) and *IL-1 β* (+3954) gene polymorphisms had a higher frequency of mutated alleles (T allele) in the PI group compared to the control group. This suggests that these genetic polymorphisms may increase susceptibility to developing PI.[31]

Interleukins-10

As an important anti-inflammatory cytokine, Interleukins-10 (IL-10) plays a vital role in protecting supportive tissues from damage and reducing the production of pro-inflammatory molecules by activating nuclear factor-kappa-B ligand (RANKL) and matrix metalloproteinase (MMP) receptors. [32] Polymorphisms in the 5′ region of the *IL-10* gene are crucial for regulating its production, with the most common variations being −1082 adenine (A) > guanine (G), −819 thymine (T) > cytosine (C), and −592 A > C. Among these, the −1082 A>G variation is most significantly associated with the development of PI, although evidence also suggests a link for the −819 and −592 polymorphisms. [33] However, due to the limited number of studies, their status as definitive risk factors remains unconfirmed.[33] Research by Saremi et

al.[34] emphasized that specific gene polymorphisms like *IL-10* −819 T>C and *IL-10* −592 A>C could play roles in the pathogenesis of PI. Additionally, a study by Taha[35], involving 15 PI patients and 35 individuals with successful implants, identified a correlation between the *IL-10* −1082 A>G polymorphism and PI.

Interleukins-17

A pro-inflammatory cytokine, Interleukin-17 (IL-17), is produced by CD4+ Th17 cells. It includes six distinct isoforms and interacts with five specific receptors.[36] It plays a crucial role in promoting autoimmune and inflammatory conditions by activating immune responses that increase the production of pro-inflammatory cytokines, neutrophils, and monocytes.[37] The *IL-17* gene, located on chromosome 6p12.1, is particularly important for its ability to stimulate the synthesis of key inflammatory mediators such as RANKL, GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor), ICAM1 (Intercellular Adhesion Molecule-1), and prostaglandin E2, which are essential in driving inflammatory processes within the body.[37] A study involving 15 participants in Iraq found that individuals with PI were more likely to possess the *IL-17* (rs2275913) AA or GA genotypes, with the AA genotype significantly more common than the GG genotype and G allele.[37]

Interleukin -6

Interleukin -6 (IL-6) is a key cytokine in the early immune response, produced by various blood cell types to support the body's defense mechanisms.[38] Its levels rise significantly in response to trauma, infections, and chronic inflammation.[38] Alongside IL-1β, IL-6 plays a crucial role in promoting bone resorption by increasing the local secretion and activity of metalloproteinases, leading to the degradation of peri-implant tissues and triggering bone resorption mechanisms.[38] Variations in the promoter region of the *IL-6* gene, especially a substitution from guanine to cytosine, have been associated with elevated plasma levels of this pro-inflammatory cytokine.[38] Although there are only a few studies on the link between this polymorphism and PI risk, some research has shown its impact. [38,39]

A study by Petkovic-Curcin et al.[38] revealed that the *IL-6* (rs1800795) polymorphism showed significant differences in genotype and allele frequency. In patients with PI, only the wild-type GG genotype was present, while the GC and CC genotypes were absent, suggesting that the C allele may have a protective role, though further research is needed to confirm this.[38] These findings were supported by Agrawal et al. [39], who also found that patients with the GG genotype exhibited increased peri-implant bone loss, likely due to an overactive inflammatory response driven by IL-6 after implant placement.

Tumor Necrosis Factor-Alpha

As a key inflammatory cytokine, Tumor Necrosis Factor-Alpha (TNF-α) is important for managing immune response regulation. [40] It not only promotes bone resorption and induces fibroblast apoptosis but also inhibits the limited repair of periodontal tissue by enhancing the local release and activity of metalloproteinases that degrade connective tissue. [41,42] The link between TNF-α and PI is believed to arise from its ability to increase local metalloproteinase release and activity, thereby damaging peri-implant tissues and triggering mechanisms of bone resorption.[43] Specifically, the rs1800629 polymorphism at position +308 in the *TNF-α* gene, which results in a guanine to adenine change, leads to direct alterations in TNF-α secretion.[38] Research indicates that the AA and AG genotypes are more frequently observed in patients with PI compared to the GG genotype and are linked to a higher risk of implant failure.[38]

Matrix Metalloproteinases

Matrix Metalloproteinases (MMPs) constitute an important subgroup of zinc- and calcium-dependent endopeptidases.[44] The human MMP family includes more than 23 types, mainly responsible for the degradation of various substances, including the extracellular matrix (ECM), which is crucial for cell development and morphogenesis, as well as growth factors, cytokines, chemokines and adhesion proteins.[45] Type I collagen serves as the primary component of the ECM in periodontal diseases, making three collagenases (MMP-1, MMP-8, and MMP-13) and gelatinases (MMP-2 and MMP-9) especially significant. Other MMPs, such as MMP-7, MMP-12, and MMP-14, along with other proteases, are regarded as moderately effective.[46] MMP-8 is capable of breaking down collagen types 1, 2, and 3, but it degrades type 1 collagen more efficiently than other MMPs.[46] It is believed that excessive activation of MMP-8, in conjunction with other MMPs, may induce inflammation linked to tissue degradation in periodontal and other inflammatory diseases.[47] Costa-Junior and his team, [48] examined the connection between the rs11225395 polymorphism in the *MMP-8* gene and early implant failure. Their results indicate that having the T allele and the TT genotype might be risk factors for early-stage failure of implants. [48] Additionally, de Araujo et al. [49] studied the effects of four different SNPs; *MMP-8* (rs11225395), *MMP-1* (rs1144393), *MMP-1* (rs1799750), and *MMP-3* (rs3025058), and found that the SNPs *MMP-1* g.-1607 G > GG (rs1799750) and *MMP-8* g.-799 C > T (rs11225395) each influenced implant loss in their own way. The 2G allele of *MMP-1* g.-1607 G > GG (rs1799750) was a risk factor, while the C allele of *MMP-8* acted as a protective factor. [49]

BONE METABOLISM GENE POLYMORPHISMS AND PI

Bone metabolism-related genes, such as *Bone morphogenetic proteins* (*BMPs)*, *osteoprotegerin* (*OPG*), and *receptor activator of nuclear factor kappa-B ligand* (*RANK-L*), are crucial for bone regeneration and the stability of dental implants.

Osteoprotegerin

OPG, a cytokine receptor, belongs to the TNF receptor superfamily.[50] This secreted glycoprotein, which is crucial in regulating bone resorption, has been linked to PI.[51] The *OPG* gene hosts several polymorphisms, and research often focuses on two functional single nucleotide polymorphisms (SNPs): 950 C/T (rs2073617) and 1181 G/C (rs2073618). [51] A study by Kadkhodazadeh et al. [52] including 77 patients with chronic periodontitis, 40 patients with PI, and 89 periodontally healthy individuals, found that the CC genotype of the 1181 G/C polymorphism was significantly more common in patients with PI than in the other groups. However, when analyzing the 950 C/T genotype, no statistically significant differences were found between the groups.[53] Research conducted by Zhou and Zhao, [51] in the Chinese population indicated that the rs2073618 polymorphism in the *OPG* gene might increase susceptibility to PI, but no link was found with the rs2073617 polymorphism. Additionally, another study in the Chinese population demonstrated a significant association between the allelic distribution of the 1181 G/C polymorphism and PI. [54]

Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor beta (TGF-β) superfamily and consist of over 30 osteogenic proteins. They are mainly found in osteoblasts and are encoded on chromosome 14q22-23. [55] These proteins are present in both mineralized and nonmineralized tissues, indicating their versatile roles in the body.

The roles of BMPs extend beyond tissue formation, repair, wound healing, and cellular differentiation; they are also essential for periodontal regeneration and maintaining tooth integrity. [55] In bone regeneration, certain BMPs such as BMP-2, BMP-4, BMP-6, BMP-7, and BMP-14 are particularly crucial as they stimulate new bone formation.[55] They also contribute to periodontal health by promoting cell growth needed for tissue repair and maintenance. [55]

Among them, BMP-2 stands out as a potent inducer of osteogenesis and is the most well-researched. [56] In dental practice, vectors carrying *BMP* genes, *recombinant human BMP-2 (rhBMP-2)* has been successfully used in procedures like maxillary sinus grafting and bone grafting in extraction sockets to enhance bone regeneration.[57] This is typically achieved by delivering *rhBMP-2* to the defect site using synthetic bone graft materials like β-tricalcium phosphate or collagen sponges. [55-58] This tissue engineering approach has also been applied in various PI pre-clinical and clinical treatment studies, where *rhBMP-2* aids in regenerating bone around dental implants, improving the success rate of these treatments.[59]

At the molecular level, the *BMP-4* gene has been linked to PI, with certain polymorphisms influencing the risk of developing this condition. Specifically, the TT genotype of the *BMP-4* rs2761884 polymorphism was found to be associated with healthier peri-implant conditions, while some *BMP-4* haplotypes have been connected to an increased risk of bone loss around dental implants.[60] This indicates that variations in the *BMP-4* gene can impact bone remodeling and contribute to the body's response to implants, potentially affecting the development of PI. [60]

Receptor Activator of Nuclear Factor Kappa Beta

RANKL, also referred to as TNFSF11, is a part of the TNF ligand superfamily.[61] Its signaling pathway plays a crucial role in the differentiation of monocytes into osteoclasts. This process is initiated when RANKL binds to its receptor, RANK, which is present on the surface of pre-osteoclasts and osteoblasts. Osteoblasts are vital in forming bone stromal cells, while RANKL expression by activated T helper lymphocytes also supports the pathway's function. [62] RANKL has been recognized as a significant genetic factor in the regulation of inflammation within peri-implant tissues.[63] During inflammatory events, the production of RANKL increases, leading to a rise in osteoclast activity and, as a result, an increased rate of bone loss. [64]

Studies have investigated the *RANK* gene polymorphisms rs35211496 (C/T) and rs3018362 (A/G) in individuals with PI, chronic periodontitis, and those with healthy periodontal status within an Iranian population.[64] Results showed that the CC genotype of the rs35211496 polymorphism is significantly associated with PI, indicating it as a potential genetic risk factor for the disease.[64] On the other hand, the distributions of the GG, GA, and AA genotypes of rs3018362, as well as their allele frequencies, did not show any statistically significant differences between the groups.[64] In a separate study involving a Brazilian cohort, researchers examined the *RANK* rs3826620 (G>T) and *RANKL* rs9594738 (C>T) polymorphisms among individuals with healthy and diseased peri-implant tissues.[65] They found no significant association between these polymorphisms and the presence of PI.[65] Furthermore, analysis of *RANKL* gene polymorphisms rs9533156 (T/C) and rs2277438 (A/G) revealed that the CT genotype of rs9533156 has a significant correlation with PI in the Iranian population, indicating its potential as a genetic marker. [62] Conversely, another study concluded that the rs2277438 (A/G) polymorphism did not have a significant association with the failure of dental implants. [66]

Fibroblast Growth Factors

The fibroblast growth factors (FGFs) comprise a family of 22 cell signaling proteins, with 18 functioning as ligands that bind to FGF receptors (FGFRs), a type of tyrosine kinase receptor. This binding triggers the dimerization of FGFRs, leading to the activation of downstream signaling pathways through their intracellular domains.[67] FGFs play important roles in tissue repair and regeneration, with certain members of the FGF family, such as FGF1, FGF3, FGF7 and FGF10, particularly known for and supporting tooth development. [68]

A study of Coelho and his colleagues suggests that the roles of *FGF3* and *FGF10* in supporting tooth structures could potentially contribute to peri-implant failures, as these growth factors are crucial in maintaining the health and integrity of dental tissues.[60] The study showed the relationship between the genes *BMP4*, *FGF3*, *FGF10*, and *FGFR1* and peri-implant bone loss.[60] It identified an association between the *FGF3* rs4631909 (TT+CT genotype) and the control group, while the C allele frequency of *FGF3* rs4631909 showed a potential link to PI. Additionally, *FGF10*-CCTG haplotypes were found to be associated with PI. [60] Notably, this is the only study that has examined the impact of *FGF* gene polymorphisms on PI, emphasizing the need for further research with larger sample sizes from diverse populations and a deeper understanding of the genetic factors contributing to peri-implant bone loss. [60]

OTHER GENES

Cluster of Differentiation 14

PI is a disease influenced by a multitude of genetic factors, encompassing both well-studied genes and others that have been less frequently examined. Among these, cluster of differentiation 14 (CD14) is a glyco-phosphatidylinositol-linked protein found on neutrophils, monocytes/macrophages, and fibroblasts, which can influence the production of pro-inflammatory cytokines.[69] CD14 functions as a versatile high-affinity receptor for binding endotoxins like lipopolysaccharides (LPS). It aids in the transfer of LPS to Toll-like receptors, which in turn triggers LPS-induced bone resorption. [70]

Two studies examining the CD14 gene polymorphisms, 1619A/G (rs2915863) and -159C/T (rs2569190) were conducted in the Serbian population, both indicating that the CC genotype of the -159C/T polymorphism (rs2569190) is linked to a higher risk of developing PI. [71] Later, Li et al. [70] find out these same genetic variations in a Chinese population, finding that the -159C/T polymorphism (rs2569190) of the CD14 gene was significantly associated with an increased susceptibility to PI. [70] In this study, the GG genotype and the G allele were identified as risk factors for PI development, consistent with previous findings. [70]

Chemokine Receptor 2

Chemokine Receptor 2 (CXCR2), which is also referred to as cluster of differentiation 192 (CD192), is situated on chromosome 2q35 and features gene polymorphisms linked to the susceptibility of different conditions. [72] It is found in oral epithelial cells and is associated with the development of chronic periodontitis. Given the similarities between chronic periodontitis and PI, it is hypothesized that *CXCR2* might also be involved in the progression of PI. [72] Additionally, Qi et al. [72] examined two variations in the *CXCR2* gene, responsible for binding IL-8, among the Chinese population. Their results showed a greater frequency of the CT genotype associated with the rs2230054 polymorphism, as well as the AG genotype and G allele for the rs1126580 polymorphism in PI patients. [72]

Fc Gamma Receptor

Fragment crystallizable gamma receptor (*FcγRs*) are receptors found on leukocytes that bind specifically to the Fc region of IgG antibodies.[73] This interaction initiates various immune responses, including phagocytosis, antibody-dependent cytotoxicity, and mast cell degranulation, which are crucial for host defense.[73] In humans, these receptors are encoded by eight genes located on the long arm of chromosome 1 (1q21-24).[74] A polymorphism of two alleles has been

identified in four subclasses of Fcγ receptors: FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb. This variation is considered a potential hereditary risk factor for both autoimmune and infectious diseases.[75] These polymorphisms significantly influence the response of periodontal and peri-implant tissues, thereby playing a vital role in the progression of these conditions. [76]

A study conducted in Iran successfully demonstrated that gene polymorphisms can influence susceptibility to PI.[73] Specifically, the presence of allele R in *FcγRIIa* (rs1801274), allele V in *FcγRIIIa* (rs396991), and allele NA2 in *FcγRIIIb* (rs1050501) were shown to reduce the ability of phagocytes to engulf microbial products, impairing their capacity to contain and control infections.[73] The same polymorphisms and their relationship with PI were studied in 2024 by Peng Li and his colleagues, [77] in the Chinese population. Their results showed that the *FcγRIIIa* rs396991 polymorphism was not associated with PI. Consistent with previous research, the *FcγRIIa* rs1801274 polymorphism has been significantly linked to PI in the Han Chinese population, indicating that the RR genotype might serve as a genetic risk factor for developing PI.[77] It is crucial to recognize that the distribution of *FcγR* genotypes and their allele frequencies can vary among individuals from different ethnic backgrounds.

CONCLUSION

In summary, ongoing genetic research is crucial for understanding the molecular pathogenesis of PI. Currently, the most extensively studied proteins linked to PI in the literature include *IL-10, IL-1, OPG, TNFα*, and the *MMP* family. These proteins play pivotal roles in the development of PI, but the impact of other cytokines could also reveal additional influential factors due to their interactions. Although existing data seem to support the presence of genetic predispositions, there is still a need for further studies with larger sample sizes across various ethnic groups to clarify this issue. Additionally, while genetic factors can serve as diagnostic markers, when combined with general risk factors for PI such as smoking, prior periodontal issues, diabetes, inadequate restorations, and poor maintenance, they can quickly lead to implant loss. Looking ahead, genetic polymorphisms and variations in cytokine expressions may offer promising avenues for new drug interventions, but more research is required in this field.

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The Effect of Baking Soda and Vinegar on Tooth Wear

Karbonat ve Sirkenin Diş Aşınması Üzerindeki Etkisi

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ABSTRACT

Alternative products used for tooth whitening teeth cause serious wear on tooth surfaces. Knowing the factors that cause tooth wear and their localizations plays an important role in reducing risk factors and treatment**.** In this case report, 2 patients who presented to the clinic with the complaint of tooth wear are presented. Case 1 used a mixture of vinegar and baking soda to whiten her teeth, while Case 2 used baking soda and whitening toothpaste. The wear on the tooth surface deepened in the cervical area. Both patients had horizontal tooth brushing and bruxism. Reduction of risk factors and direct composite restoration were described in the treatment. Alternative products that can be used to whiten teeth cause serious wear on teeth. Elimination of risk factors plays an important role in the treatment of these wear. The location and depth of the lesion play an important role in the treatment.

Keywords: Vinegar, baking soda, tooth wear, cervical

ÖZ

Dişleri beyazlatmak amacı ile kullanılan alternatif ürünler diş yüzeylerinde ciddi aşınmalara sebep olmaktadır. Diş aşınmalarının meydana gelmesine sebep olan faktörlerin ve lokalizasyonlarının bilinmesi risk faktörlerinin azaltılmasında ve tedavide önemli rol oynamaktadır. Bu olgu raporunda, diş aşınması şikayetiyle kliniğe başvuran 2 hasta sunulmuştur. Olgu 1 dişlerini beyazlatmak için sirke ve karbonat karışımı kullanırken, Olgu 2 ise karbonat ve beyazlatıcı diş macunu kullanmıştır. Diş yüzeyinde meydana gelen aşınmalar servikal bölgede derinleşmekteydi. Her iki hastada da horizontal diş fırçalama ve bruksizm vardı. Tedavisinde risk faktölerinin azaltılması ve direkt kompozit ile restorasyonu anlatıldı. Dişleri beyazlatmak amacı ile kullanılabilen alternatif ürünler dişlerde ciddi aşınmalara sebep olmaktadır. Bu aşınmaların tedavisinde risk faktörlerinin elimine edilmesi önemli rol oynamaktadır. Tedavisinde lezyonun konumu ve derinliği önemli rol oynamaktadır.

Anahtar Kelimeler: Sirke, karbonat, diş aşınması, servikal

INTRODUCTION

The basis of tooth discoloration is the dissolution of the enamel surface and the accumulation of coloured molecules on the surface. Intake of beverages such as wine, coffee, tea, alcohol, and similar substances can play a role in tooth discoloration. The basis of these discolorations is the dissolution of the enamel surface and the accumulation of coloured molecules on the surface. The whitening of teeth has been well described by researchers.[1] This process can basically be achieved by wearing the stains on the enamel surface with abrasive-based materials such as carbonate or oxidation of chromogen double bonds with chemical-based agents such as hydrogen peroxide.[2] Due to the high cost of in-office whitening and the necessity of its application by professionals, patients may turn to alternative products easily found at home to lighten their tooth colour.[2] Examples of these products include whitening toothpaste, over-the-counter products (OTC), and mixtures obtained by adding lemon concentrate.[3] These products, which can be preferred to whiten teeth, may cause wear and sensitivity on tooth surfaces. Studies analysing dental wear occurring without caries have reported that the number of cases has increased significantly in recent years.[4,5]

Dental erosion is as the loss of hard tissues eroded from the tooth surface by acid or chelating agents.[6] While the chemical deterioration of the tooth surface structure is a corrosion event, it is also frequently used as erosion in the literature.[7] In recent years, instead of explaining dental wear with a single mechanism, researchers have described them as complicated lesions that may occur under the influence of multiple factors.[8] The incidence of dental wear, which can occur due to different factors without caries, increases with increasing age and as the teeth remain in the mouth for a longer period. However, when wear cause aesthetic problems, sensitivity, advanced mobility, migration, decrease in facial height, loss of occlusal stability in the posterior, and frequent fracture of teeth or restorations, they are considered pathological and treatment is required. In cases with severe wear, it is important to know the etiology, localization and severity of wear to achieve treatment success.

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The aim of this case report is to evaluate the effects of the long-term use of alternative whitening products on teeth. The etiology and treatment methods of non-caries cervical lesions caused by long-term use of products that can be used to whiten the tooth surface are discussed.

CASE PRESENTATION

This study was conducted in the Inonu University, Faculty of Dentistry, Department of Restorative Dentistry. In Case 1, a 53-year-old woman presented to our clinic with aesthetic concerns and tooth sensitivity. She reported that her teeth had recently become darker in colour and were also sensitive to hot and cold contact. The patient was a housewife and was not engaged in any occupation that would cause external wear of her teeth. She was very careful about her oral hygiene and mentioned that she had been using a special mixture to keep her teeth white. It was a mixture of grape vinegar and baking soda, which whitened his teeth considerably. For the last 3 years, she has been brushing her teeth horizontally for 10 minutes every day in the evening. The formula of the mixture consisted of half a teaspoon of baking soda and 1 teaspoon of vinegar (Figure 1). In general, wear defects were observed on the outer surfaces of all teeth (Figure 2). While vinegar is a medium-strength acidic substance, baking soda is a high-strength basic substance. When the pH value of the mixture made according to the formula applied by the patient was measured, the result was approximately 6.9 (Figure 3). The enamel surfaces of all teeth were worn and smooth. In the cervical areas, where the enamel is structurally thinner and less resistant to wear, deep defects were formed and became wedge-shaped compared to the other parts. The patient also suffered from bruxism and heavy occlusal forces were applied on the teeth. This is one of the important factors in increasing the depth of the lesions in the cervical area. Multiple factors were involved in the formation of lesions and multifactorial pathodynamic mechanisms are involved in the etiology as described by Grippo et al.[9]

Figure 1. Half a teaspoon of baking soda, one teaspoon of vinegar and its mixture

Figure 2. Right, front and left views of the teeth of a 53-year-old female patient

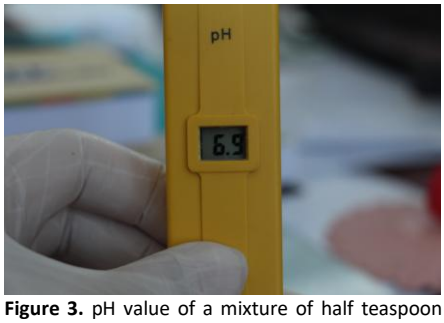

Figure 3. pH value of a mixture of half teaspoon baking soda and 1 teaspoon vinegar

In Case 2, the patient was 61 years old and presented to our clinic with the complaint of dental wear. After the clinical examination, all teeth had visible non-caries cervical area wear and shiny enamel surfaces (Figure 4). In addition, severe atrision was present. The patient had no occupational history that could cause external wear on the teeth. He had a long-standing history of using hard toothbrushes and whitening toothpaste. He also said that he occasionally brushed with baking soda before his appointments. During the treatment planning, the patient was asked to return to the clinic the next day and to bring the toothbrush and toothpaste with him. The toothbrush was hard and had bent bristles indicating that a lot of pressure was applied during brushing (Figure 5). The toothpaste were 2 toothpastes, both of which were whitening toothpaste. When the contents of both toothpaste were analyzed, they contained hydrated silica and calcium carbonate which can erode the tooth surface.[10] The patient also had bruxism, which increased the depth of the defects in the cervical area. The teeth had abrasion due to hard toothbrush and horizontal brushing, wear due to prolonged use of baking soda and whitening toothpaste, and abfraction and attrition defects due to bruxism.

Figure 4. Right, front and left views of the teeth of a 61-year-old male patient

Figure 5. Toothbrush with hard bristles

In both cases, the factors that caused wear on the surfaces of the teeth before the restoration stage can be listed as horizontal brushing, use of abrasive chemical agents, and bruxism. Oral hygiene habits, brushing frequency and duration, whitening agents, and brushes used were analyzed in detail. Instead of whitening agents, toothpaste containing desensitizing agents and not intended for whitening were recommended. Instead of horizontal brushing movement, brushing with Modified Bass technique and toothbrush with medium hardness were recommended.[11] Reduction of risk factors constitutes the first stage of treatment for the prevention of non-caries cervical lesions.[12]

After the periodontal treatment was completed, the restoration phase was started. Studies show that restorations tend to fall off as a result of vertical and horizontal forces in this area and the failure rate is high.[8,13] Regular check-ups have been recommended for teeth with a depth of less than 1 mm.[12] Treatment of lesions in the cervical area

ensures the continuity of oral hygiene. It also reduces the thermal sensitivity of the tooth, strengthens its structure, and gives an aesthetically good appearance. In the restoration of both cases, it was decided to use a restorative material (A3 Estelite Asteria, Tokuyama, Japan) with a high filler ratio and low elastic modulus. The restorative material with nanohybrid particles was effective because the cervical area of the tooth is a dynamic movement area and it has an elastic modulus that can compensate for tooth movements caused by this dynamism.[14] The area where the composite restoration would be applied was isolated with the help of a rubber dam. Thus, fluid flow from the mouth and gingival groove was prevented. The enamel surfaces close to the cavity were beveled to increase the retention of the restoration. Diamond-pointed end mills were used for bevelling. The enamel surface was then roughened with an acid (Scotchbond Universal Etchant, 3M ESPE, USA) for 20 seconds and washed. The enamel and dentin surfaces were prepared for composite application with 6th-generation self-etch adhesive (Clearfil SE Bond, Kuraray, Japan) according to the user instructions. Afterward, the nanohybrid composite was placed in the cavity and polymerized with the help of an LED light emitting 1200 mW/cm^2 light (Woodpecker, Guilin, China). After the restoration was completed, the rubber dam was removed. Firstly, a fine-grained diamond bur was used for correction and the excess was removed. Care was taken to protect the periodontal tissue during the finishing phase of the cervical restorations. Then, the surface of the composite restoration was polished with polishing discs and rubbers (Figures 6 and 7). After the completion of the restorative treatment phase in Case 1, splint treatment was decided to alleviate the occlusal forces caused by bruxism and to protect the teeth. In case 2, the prosthetic restoration phase was started for the treatment of missing teeth.

For the oral and written data used in this study, both patients were informed verbally and in writing. Consent forms were signed.

Figure 6. Direct composite restoration of case 1

Figure 7. Direct composite restoration of case 2

DISCUSSION

In the cases presented, severe tooth wear was observed as a result of prolonged and incorrect use of teeth whitening agents. Case 1 was using a mixture of vinegar and baking soda for bleaching. As a result of the general chemical reaction between vinegar and baking soda, carbon dioxide gas, water, and sodium acetate are formed.[15] In general appearance, there is a foaming mixture and this may have encouraged the patient to bleach (Figure 1). The patient used this mixture for 10 minutes a day for 3 years, resulting in severe wear on the outer surface of the teeth. The researchers considered the baking soda and vinegar mixture in the group of peroxide-free tooth whiteners and tested their effectiveness. In their in-vitro studies, they reported that these agents did not whiten as effectively as those containing peroxide.[16,17] Contrary to these studies, significant tooth wear occurred in Case 1. The reasons for this may be that the formula of the mixture applied by the patient was different from the formula in the studies, horizontal brushing, and heavy occlusal forces caused by bruxism. Case 2 had been brushing with whitening toothpaste and occasionally with baking soda for many years. The outer surfaces of the teeth were significantly worn and the crown lengths were shortened due to atrision defects. The wear on the outer surface were visible throughout the teeth and increased in severity in the cervical area. When the whitening toothpaste used by the patient were analyzed, they contained calcium carbonate and hydrated silica as abrasives. These active substances increase roughness on the surface of the teeth.[10] The patient was using a toothbrush with hard bristles. Horizontal tooth brushing movement caused the formation of abrasion defects. In recent years, it has been suggested that multiple factors play a role in the etiology of tooth surface wear occurring without caries. These effects are multifactorial.[9] The depth of the defects formed on the outer surface of the teeth occurred mostly in the cervical area. Studies have shown that the stress caused by compression and tension forces on the teeth accumulates in the cervical area.[18] The thickness of the tooth enamel decreases towards the cervical area. As a result of the incoming forces, it breaks and the underlying dentin is exposed. The fact that dentin wear more easily than enamel explains the reason for deep defects and tooth sensitivity in this area.

In the study, both patients were using highly abrasive chemical agents to clean the tooth surfaces and brushing their teeth incorrectly. These products had been used for a long time and non-caries lesions had formed on the teeth. Direct composite restorations were preferred for the treatment of these lesions. The follow-up visits after the completion of the restorations were short and satisfactory. The lack of long-term control sessions in both cases is one of the limitations of this study. Control sessions are necessary for patients to abandon their habits. In addition, the surface integrity of the direct composite restoration treatment should be examined. Fractures and discoloration should be re-evaluated.

In conclusion, the following recommendations should be made to raise awareness of patients about the use of tooth whitening products and brushing: limiting the use of alternative tooth whitening products, providing training on tooth brushing, and ensuring the correct selection of toothpaste.

Informed Consent: For the oral and written data used in this study, both patients were informed verbally and in writing. Consent forms were signed.
Peer-review: Externally peer-reviewed.
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Hasta Onamı: Bu çalışmada kullanılan sözlü ve yazılı veriler için her iki hastaya da sözlü ve yazılı bilgilendirme yapıldı. Onam formları imzalatıldı.
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