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Book

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

Chapter in a book

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5. Hudson FB, Hawcroft J. Duration of treatment in phenylketonuria. In: Seakins J, Saunders R, editors. Treatment of inborn errors of metabolism. London: Churchill Livingstone, 1973, p.51-56.

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

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

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

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Original research

The effect of three different primary teeth rotary instrument systems on the amount of apically extruded debris in pulpectomy of primary teeth

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Purpose

The objective of this study is to evaluate the amount of apically extruded debris and working time during root canal treatment with three different primary teeth rotary instrument systems.

Materials and Methods

A total of 80 extracted primary second molar teeth were randomly divided into four groups (n=20) stratified by the instruments used: AF Baby Rotary, Easyinsmile Baby Rotary, Endoart Pedo Gold, and hand files. The apical extrusion of debris was collected then dried in Eppendorf tubes that were pre-weighed with 10^{-4} precision micro-balance. The incubation period was set as 14 days at 37°C. The dry weight was procured by deducting the preoperative weight from the postoperative weight. The systems' working time was calculated by chronometer. Mann Whitney U test with Bonferroni correction was used for pairwise comparison following the variance analysis with Kruskal Wallis test. Wilcoxon test was used for intragroup comparison.

Results

Although all instruments caused apically extruded debris ($p<0.001$), there was no statistically significant difference between the groups in debris extrusion. However, the longest working time was found in the manual K files, the Endoart Pedo Gold system had the shortest working time ($p<0.001$).

Conclusion

Our results demonstrate that all instrument systems caused apical extrusion of debris. Furthermore, the rotary instrument systems designed for primary teeth exhibited significantly shorter working time.

Keywords: *Apical debris, primary teeth, root canal preparation, rotary system, time*

Introduction

Maintaining the health of the primary dentition is critical for regular jawbone development, natural muscle function, clear speaking, and even the natural eruption of permanent dentition. Additionally, early loss of primary teeth has been linked to a variety of issues, including poor oral habits, alterations in arch proportions, and disruptions in the eruption sequence of permanent dentition (1, 2).

One of the most common reasons for early tooth loss is periapical infection (3). As a therapeutic option to preserve the primary tooth as a natural space maintainer, pulpectomy is being considered (4). Root canal treatment involves completely removing the pulp tissue from the primary tooth, followed by debridement, shaping, drying, and filling of the root canals with a resorbable material (5, 6).

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Although manual instrumentation has been the most common method for root canal treatment in primary teeth, it is time-consuming and often tiring for both the operator and the child (7). Numerous studies have reported the success of rotary instrument systems in conserving the original root canal anatomy of primary teeth (6, 8).

Furthermore, rotary instrument systems have minimized complications that occur during the traditional treatment process when using standard manual files (6, 8). The use of Nickel-Titanium (Ni-Ti) rotary instruments accelerates and simplifies root canal treatment while reducing file breakage. Moreover, Ni-Ti instruments provide better shaping for the cleaning procedure, irrigation, and obturation during root canal treatment (8).

In response to ongoing advancements in the field of pediatric dentistry, innovative pediatric rotary systems have been introduced (such as AF Baby Rotary, Easyinsmile Baby Rotary, Endoart Pedo Gold, etc.). These systems aim to provide more convenient and efficient pulpectomy in primary teeth by offering exclusive pediatric rotary files with modified length, taper, and tip size (9).

AF Baby Rotary files are made of Ni-Ti heat-treated material and feature advanced memory AF-H alloy with a noncutting tip. The files have a triangular cross-section design (10). Easyinsmile Baby Rotary files also utilize advanced memory AF-H alloy material and a noncutting tip. The cross-section design of these files is convex triangular (11). Endoart Pedo Gold files, on the other hand, are made of Ni-Ti heat-treated material, and they feature a guiding noncutting tip and convex triangular cross-section with advanced memory alloy material (12).

Chemo-mechanical preparation plays a crucial role in root canal treatment and involves cleaning with files and solutions. However, during canal preparation, dentine shavings, pulpal pieces, debris, and solutions are inadvertently extruded into the periapical area (13). The extrusion of these elements into the periapical tissues can lead to unfavorable outcomes such as inflammation, postoperative discomfort, delays in periapical healing, and damage to permanent teeth germs (14). Therefore, it is recommended to prevent apical debris extrusion for effective primary tooth root canal treatment (13).

Additionally, considering children's shorter attention span, working time becomes an important factor in their acceptance of root canal treatment (15). Working time is particularly critical in dental treatments conducted under general anesthesia or sedation due to the limited time available (16). The working time for root canal treatment on primary teeth varies depending on the doctor's experience, skill, and the number and type of instruments used (17).

Previous studies have compared rotary systems in primary teeth in terms of instrumentation time and apical debris extrusion. However, to the best of our knowledge, no other study utilizing the primary rotary systems used in our research has been found in the literature (18, 19). Our study aims to determine the amount of apically extruded debris and the preparation time during root canal treatment using three different rotary instrument systems for primary teeth. The null hypothesis states that there will be no difference between the rotary instrument systems in terms of working time and the amount of apically extruded debris.

Materials and methods

Ethical approval

Approval from the Scientific Research Ethics Committee of the Karadeniz Technical University Medical Faculty (2021/40) was obtained for this study.

Sample size determination:

The sample size was determined based on the study conducted by Kucukyilmaz *et al.* (20). It was concluded that 18 teeth per group would be sufficient, considering an alpha error of 0.05 and a beta error of 0.20. However, to account for possible data loss (10%), it was decided to include 20 teeth in each group for the research.

Inclusion criteria and sample preparation:

For this study, the distal canals of 80 primary second molars were selected. These canals had a single and wide canal, a smooth outer contour, and fewer intracanal branches compared to the mesial root (18). The molars were extracted from children aged 4-6 years due to periapical infection and for preventive orthodontic treatment. External debris and soft tissue remnants were removed from the teeth, which were then stored in distilled water at room temperature until the experimental process. Teeth with at least two-thirds of the root length, no pathological root resorption, no obvious root caries or fractures, and a root curvature angle of less than 20° (evaluated according to Schneider's protocol) were included in the study (21). Buccolingual and mesiodistal radiographs were taken for each tooth to confirm the root curvature angle, presence of a single canal, resorption, and canal obliteration. Before the experimental process, the mesial roots of each tooth were removed at the furcation level using a low-speed diamond borer with underwater cooling. Endodontic access cavities were prepared using a diamond bur and a high-speed handpiece. Canal patency was established using a size 10 K-file (VDW, Munich, Germany). An apical opening with a diameter of 0.12 mm was created by advancing the file 1 mm from the apical foramen, and teeth with wider apical foramens were excluded from the study. Canals larger than the International Organization for Standardization size 15 were removed, resulting in 80 teeth that met the size requirements (22). The working length was determined by advancing a size 10 K-file into the root canal until the tip of the file was visible at the apical foramen. The canal length was established by subtracting 1 mm from this point. To prevent debris extrusion from lateral canals, the distal root surfaces of the teeth were covered with nail polish up to one-third of the apical area.

Debris collection

For this study, the experimental model developed by Myers and Montgomery (23) was utilized. Eppendorf tubes without stoppers were weighed with a micro-balance (Kern ABJ, Gottingen, Germany) to an accuracy of 10^{-4} g before canal instrumentation. Each tube's weight was measured three times consecutively, and the average values were recorded. The eighty teeth were coded and divided into four groups of twenty specimens using a computer-based randomization program (Research Randomizer) (24).

Next, the stoppers of the Eppendorf tubes were perforated, and the teeth were inserted up to the cementoenamel junction. To equalize air pressure inside and outside the Eppendorf tubes and serve as a drainage cannula, a 27-Gauge (G) needle (INMED Medical Products, Istanbul, Turkey) was placed alongside the stopper. The stoppers, along with the teeth and needles, were securely attached to their respective Eppendorf tubes. The tubes were then placed in vials covered with foil to prevent the practitioner from observing apically extruded debris during the root canal treatment. The entire device was handled through the vial, and the Eppendorf tubes were never touched with fingers.

Instrumentation and working time

Hand file group: In the manual preparation of this group, a stainless-steel K-file (VDW, Munich, Germany) with a taper of 0.02 was used. The standardized technique was followed, starting with size 15 and progressing in the following order: 20.02, 25.02, and 30.02 (n=20).

AF Baby Rotary group: AF Baby Rotary files (Fanta Dental Materials, Shanghai, China) were employed for canal preparation in this group, up to a main apical size of 30 (10). The rotary files were used with an endodontic motor (COXO, Foshan, China) at 350 rpm and a torque of 2 Ncm, following the manufacturer's recommendations. The sequence of files used was 17.08, 20.04, 25.04, and 30.04. The 17.08 file was utilized to widen the canal's coronal two-thirds. The brushing action was performed gently inward and outward (n=20). Easyinsmile Baby Rotary group: In this group, Easyinsmile Baby Rotary files (Easyinsmile International Corp., Changsha, China) were employed. The files were used in the following sequence: 20.04, 25.04, and 30.04, with the same endodontic motor at a rotational speed of 350 rpm and a torque of 2.6 Ncm, as per the manufacturer's recommendations (11). The brushing action was performed gently inward and outward (n=20). Endoart Pedo Gold group: All canals in this group were prepared using Endoart Pedo Gold files (Endoart, Istanbul, Turkey). The files were used with the same endodontic motor at a rotational speed of 350 rpm and a torque of 1.5 Ncm for the 15.06 file, 1 Ncm for the 25.04 file, and 2 Ncm for the 30.04 file, according to the manufacturer's recommendations (12). The brushing action was performed gently inward and outward (n=20).

In all groups, a new set of instruments was used for each root canal, and after each file preparation, a 10 K-file was used for recapitulation. These procedures were consistently performed by the same trained operator to eliminate biases and ensure consistency. Additionally, the root canals in each group were irrigated with distilled water using a 27-G needle before preparation, between instrument changes, and at the end of the preparation. Furthermore, after the instrumentations, the surfaces of the roots were irrigated with 1 ml of distilled water to collect any adhered debris. To maintain standardization, a total of 10 ml of distilled water was used for each tooth. The working time of the file systems, including the duration of the file in the canal, file change time, and irrigation time, was measured in seconds using a chronometer.

Evaluation of apically extruded debris

The evaluation of apically extruded debris was conducted by a second examiner who was blinded to the group allocations. After the completion of canal preparation, the Eppendorf tubes were removed from the vials. Subsequently, the tubes were placed in an incubator set at 37 °C for 14 days to allow the distilled water to evaporate. Each tube was measured three times consecutively after evaporation, and the average values were recorded. The net weight of the dry debris was calculated by subtracting the original weight of the empty Eppendorf tube from the gross weight.

Statistical analysis

The Shapiro-Wilk test was utilized to evaluate the conformity of the data to a normal distribution. For pairwise comparisons, the Mann-Whitney U test and Bonferroni correction were employed following the Kruskal-Wallis test if necessary. Intragroup comparisons before and after preparation were analyzed using the Wilcoxon test. A p-value of less than 0.05 was considered statistically significant. The data were analyzed using the SPSS 17.0 program (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA).

Results

The results indicated that all instruments caused apically extruded debris ($p<0.001$). Although the difference in weight values of the apically extruded debris between the groups was not statistically significant, the highest percentages were observed in the manual K files group, while the lowest percentages were observed in the AF Baby Rotary file systems group (Table 1). Except for the Easyinsmile Baby Rotary-Endoart Pedo Gold comparison, a statistically significant

Table 1: *The weight values of the Eppendorf tubes before and after the preparation, the weight (g) of the extruded debris, and the percentage ratios*

Groups	N	Weight before preparation (Mean ± SD)	Weight after preparation (Mean ± SD)	Extruded debris weight (Mean ± SD)	Extruded debris ratio (%)	p**
AF Baby Rotary	20	0.783330±0.0082904	0.784460±0.0079219	0.001130±0.0007794	0.144048135	p<0.001
Easyinsmile Baby Rotary	20	0.783920±0.0065121	0.785155±0.0065753	0.001235±0.0009455	0.157293783	p<0.001
Endoart Pedo Gold	20	0.782735±0.0083258	0.784215±0.0084813	0.001480±0.0011719	0.188723756	p<0.001
Manual K	20	0.783785±0.0102417	0.786170±0.0109049	0.002185±0.0018508	0.27792971	p<0.001
p*		0.751	0.996	0.131		p<0.001

SD: Standard Deviation p*: Comparison between groups Kruskal Wallis test, p* <0.05, p**: Intragroup comparison Wilcoxon Signed Ranks test, p**<0.05

difference was found among the groups in terms of working time (p=0.003, p<0.001). The manual K files group had the longest working time, whereas the Endoart Pedo Gold file systems group had the shortest working time (Table 2).

time, this result is assumed to be linked to various characteristics of file systems, such as cross-sectional design, kinematic movement, cutting efficiency, flexibility, and alloy qualities, as indicated in other studies (26, 30).

Table 2: *Pairwise comparisons of working time (sec) variable*

Groups	Working Time (Mean Value± SD)		P*
AF Baby Rotary-Easyinsmile Baby Rotary	282.2975±51.83196	219.3660±48.81846	p=0.003[a]
Easyinsmile Baby Rotary –Manual K	219.3660±48.81846	375.2600±61.06917	p<0.001[b]
AF Baby Rotary-Endoart Pedo Gold	282.2975±51.83196	183.2605±13.46420	p<0.001[c]
AF Baby Rotary- Manual K	282.2975±51.83196	375.2600±61.06917	p<0.001[d]
Easyinsmile Baby Rotary - Endoart Pedo Gold	219.3660±48.81846	183.2605±13.46420	p>0.01[e]
Endoart Pedo Gold - Manual K	183.2605±13.46420	375.2600±61.06917	p<0.001[f]

*Bonferroni correction with Mann-Whitney U test. p^a=0.003; p <0.01, $p^{b,c,d,f}$<0.001, p^e >0.01.

Discussion

Apical extrusion is a potential complication that can occur during root canal treatment when an instrument is used in an apical orientation or acts as a plunger (25). The extrusion of debris in the apical region during root canal treatment of primary teeth is of critical importance due to the proximity of the permanent tooth germs (13).

The success of primary root canal treatment is influenced by various factors, including the exclusion of apical debris and working time. Therefore, the objective of our research was to compare the amount of apically extruded debris and working time among different primary rotary file systems (20). Among several methods available for measuring apical debris, we employed the method described by Myers and Montgomery, which is widely used in dental literature (23, 26). In this study, distilled water was used as a solution to prevent potential crystallization of sodium hypochlorite. This was done because sodium crystals that form after the solution evaporates cannot be distinguished from the debris, which could significantly impact the results (27). Additionally, we did not attempt to simulate the presence of periapical tissue as it may affect the results by absorbing the irrigant and debris. The use of materials such as floral foam to mimic periapical tissue can alter the outcome, and in vivo models may produce different results due to the influence of normal or pathological periapical tissue acting as a natural barrier to debris extrusion (28).

The null hypothesis of this study was accepted for apically extruded debris but rejected for working time. The results demonstrated that all groups resulted in apically extruded debris during root canal preparation (p<0.001). This finding is consistent with previous studies on apical extrusion and confirms that chemo-mechanical preparation of the root canal system always leads to the extrusion of debris (26, 29). Similar to previous research, manual K files showed the highest percentage of apically extruded debris, although the difference was not statistically significant according to the results of this study (18, 19, 29).

The lack of statistical difference between K files in our study might be linked to the fact that, unlike other studies, we used files made for primary teeth (18, 20). At the same

Rathi *et al.* (26) evaluated the cleaning efficiency and apically extruded debris during root canal treatment with Kedo-S and Pro AF Baby Gold primary file systems. Similar to this study, rotary file systems caused apically extruded debris. The apical extrusion of debris was detected less in the Pro AF Baby Gold rotary file system than in the Kedo-S rotary file system. Similarly, although it was not statistically significant in this study, AF Baby Rotary file systems had the least percentage of apically extruded debris. AF Baby Rotary file systems were files with control memory, 'AF' wire structure, inactive cutting tip, and heat-treated like Pro AF Baby Gold file systems. The reason why there was no statistically significant difference in this study, as stated by Rathi *et al.* (26), might be due to their characteristic features such as different cross-sections and working mechanisms.

Reduced instrumentation time is extremely important in influencing the behavior and cooperation of the child in the dental chair, besides lowering weariness caused by shorter working hours in the operator, resulting in speedier treatment delivery (31). This study showed that the rotary files significantly reduced working time in comparison with hand K-files, which is consistent with findings from previous clinical trials (15, 32). Kalita *et al.* (32) evaluated the cleaning efficiency and working time during canal preparation with Kedo-S, ProTaper rotary systems, and manual K files. Working time with manual files was shown to be longer than with rotary file systems, similar to the outcomes of this study. The reason for this is that the rotary file systems were used with endo motors. Also, in our study, the longer working time of the manual files was assumed to be related to that.

Shah *et al.* (15) compared Kedo-S, Pro-AF Baby Gold rotary systems, and manual files in terms of working time and root filling quality. They found that working time with manual files was longer compared to rotary file systems, which is consistent with the results of our study. The authors also reported that using rotary file systems reduced operator fatigue and increased productivity. Additionally, they observed that the reduced working time may be attributed to the decreased number of files. Similarly, in our study, the AF Baby Rotary file system, consisting of four files, resulted in a longer working time compared to the Easyinsmile Baby Rotary and Endoart Pedo Gold file systems, which only required three files.

It should be noted that the apical extrusion of debris can vary depending on whether the tooth is vital or devital. In vital teeth, the pulp tissue acts as a barrier, preventing debris and irrigation solution from being extruded into the periapical tissues (33). Therefore, the limitation of this study is that it does not fully replicate the in vivo conditions, highlighting the need for more comprehensive future studies.

Conclusion

Our results demonstrate that all instrument systems caused apical extrusion of debris. Furthermore, the rotary instrument systems designed for primary teeth exhibited significantly shorter working time.

Türkçe özet: *Üç farklı süt dişi döner eğe sistemi ile süt dişi kök kanal tedavisi sırasında taşan debris miktarının ve çalışma zamanının incelenmesi. Amaç: Çalışmamızın amacı üç farklı süt dişi döner eğe sistemi ile süt dişi kök kanal tedavisi sırasında taşan debris miktarını ve çalışma zamanını belirlemektir. Gereç ve Yöntem: Toplam 80 adet çekilmiş süt ikinci azı diş, kullanılan aletlere göre rastgele dört gruba (n=20) ayrıldı: AF Baby Rotary, Easyinsmile Baby Rotary, Endoart Pedo Gold ve el eğeleri. Apikale taşan debris toplandı ve 10^{-4} hassas mikro terazi ile önceden tartılan Eppendorf tüplerinde kurutuldu. İnkübasyon süresi 37°C'de 14 gün olarak belirlendi. İşlem sonrası ağırlıktan işlem öncesi ağırlık değerleri çıkarılarak kuru debris ağırlığı elde edildi. Eğe sistemlerinin çalışma zamanı kronometre ile hesaplandı. Gruplar arası karşılaştırmada Kruskal Wallis testi sonrası Mann Whitney U testi ve Bonferroni düzeltmesi kullanıldı. Grup içi karşılaştırmada Wilcoxon testi uygulandı. Bulgular: Bütün eğe sistemleri apikalden debris taşmasına neden olsada ($p<0.001$), eğe sistemleri arasında debris taşırmada istatistiksel olarak anlamlı bir fark bulunmamıştır ($p>0.05$). En uzun çalışma zamanı manuel K eğelerinde, en kısa çalışma zamanı ise Endoart Pedo Gold sisteminde tespit edilmiştir ($p<0.001$). Sonuç: Tüm sistemler apikal debris ekstrüzyonuna neden olmuştur. Süt dişleri döner alet sistemleri önemli ölçüde daha hızlı çalışma süreleri nedeniyle tercih edilebilir. Anahtar kelimeler: Apikal debris, süt dişi, kök kanal tedavisi,döner sistemler, zaman*

Ethics Committee Approval: Ethics committee approval was obtained from the Scientific Research Ethics Committee of the Karadeniz Technical University Medical Faculty (2021/40).

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

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

Conflict of Interest: The authors declared that they have no conflict of interest.

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Original research

Pulp tissue dissolution capacities of different irrigation agitation techniques in artificial internal resorption cavities

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Purpose
The aim of this in vitro study was to compare the organic tissue dissolution capacities of 3 different irrigation agitation techniques (IATs) in artificial internal root resorption cavities (IRCs).

Materials and Methods
Ninety freshly extracted maxillary human incisors were selected. After decoronation procedure, the roots were split longitudinally, and a standard IRC were prepared in the canals on each half of the roots. Then, the bovine pulp samples (~2,3 mg) were previously weighed and placed into the cavities. The root fragments were reassembled and cemented to create a circular IRC within the canal. Teeth samples were randomly divided into 6 groups (n=15); sodium chlorur (NaCl) and sonic irrigation (SI), sodium hypochlorite (NaOCl) and SI, NaCl and passive ultrasonic irrigation (PUI), NaOCl and PUI, NaCl and laser activated irrigation (LAI), NaOCl and LAI. After that, the teeth were decemented and the tissue samples inside the cavities were weighed again. The percentage of weight loss was calculated and statistically analyzed.

Results
SI has significantly more successful results than PUI and LAI in groups which the irrigant was NaCl. There was also a significant difference between LAI and PUI in groups which the irrigant was NaOCl (Group 6 > Group 4, p=0.003). There was no significant difference between LAI and SI with NaOCl.

Conclusion
Complete dissolution of bovine pulp tissue from IRCs was not achieved by any tested techniques. However, the LAI with NaOCl was more effective than other IATs. In addition, there is no significant difference between the LAI and SI with NaOCl.

Keywords: *Dissolution, internal resorption cavity, tooth, pulp, activation*

Introduction

Internal root resorption (IRR) in permanent teeth is a pathological progressive destruction with hard tissue loss in dental hard tissue that occurs because of clastic activation (1,2). Resorption process begins when the underlying mineralized dentine is exposed to odontoclasts as the outer protective odontoblast layer and predentin of the root canal system are damaged (3). Dental trauma and the inflammation of pulp are the most reported reason for IRR with various other etiologic factors such as tooth decays, periodontal inflammation, excessive heat generation during dental treatment, calcium hydroxide procedures, anachoresis, orthodontic movement of teeth, cracked teeth and idiopathic reasons within normal pulp tissue (4,5).

The resorption will not be progressive without bacterial contamination and cannot be diagnosed with neither clinically nor radiographically. Furthermore, the vital pulp tissue located at apical part to resorption area

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provides blood supply and nutrients for clastic cells. On the other hand, necrotic part of pulp located at coronal portion of root canal system stimulates these clastic cells (4,6). After diagnosis of IRR, whether the teeth can be restorable, root canal treatment (RCT) is indicated to remove all the vital and necrotic pulp tissue, which cause a risk in aspect of stimulation of resorptive cells, and to disinfect and filling root canal system (7).

Although there are advanced endodontic instruments and treatment techniques, there remain areas in the root canal where bacteria and debris cannot be removed due to the complexity of the root canals (4,8). Several irrigation agitation techniques (IATs) such as sonics, ultrasonics, lasers, brushes, and manual dynamic agitation were suggested to access these restricted areas and to increase dissolution capacity of organic tissue (8-10).

Sonic and ultrasonic IATs have been extensively tested and are usually employed in RCT as irrigant agitation devices (11-13). Sonic irrigation (SI) devices produce a hydrodynamic phenomenon through the oscillation of smooth and highly flexible polymer tips at frequencies of 1-10 kHz to induce bacterial elimination and pulpal debridement (11). Among these devices, the EndoActivator system (Dentsply Maillefer, Baillagues, Switzerland) is the most studied device with a frequency of 0.166 - 0.3 kHz (14). It is a cordless, battery-powered handpiece with a sonic motor. Passive ultrasonic irrigation (PUI) produces microstreaming by utilizing small noncutting files oscillating freely in shaped canals at ultrasonic frequencies (25-30 kHz) and activating irrigants through secondary acoustic microstreaming (15). An ultrasonic tip is activated in the canal up to the WL and is moved passively in and up and down motion to ensure it does not bind with the root canal walls. Due to the metal alloy of ultrasonic tips, there can be undesired deformation in root canal surfaces, if the tips touch the root canal walls.

Laser activated irrigation (LAI) is another type of IAT, based on the activation of irrigants by medium-infrared lasers (2780 and 2940 nm), and has recently become a popular option in endodontics. The radiation emitted by the laser is strongly absorbed by water-based solutions and expanded and collapsed vapor bubbles are formed at the fiber tip, which results in cavitation. These changes in the size of collapsed bubbles lead to localized shock waves and a distinct fluid movement. Therefore, secondary cavitation bubbles are triggered with subsequent laser pulses. This results in the generation of acoustic streaming of the irrigant throughout the entire root canal system (16,17). Among several laser devices, Er:YAG and Er,Cr:YSGG lasers are promising as a method for activating the irrigants. Aldeen *et al.* (17) reported that using Er:YAG laser was removed significantly more debris than PUI and conventional irrigation. One laser-induced activation is PIPS (photon-induced photoacoustic streaming), performed by a pulsed Er:YAG laser, which uses low pulse energies (10 or 20 mJ) with a short pulse length (50 μs) resulting in high peak powers and efficient cavitation (18). This technique differs from other laser IATs in that only the tip is placed into the pulp chamber, thereby preventing contact with the root canal wall. In a study PIPS was more effective in the removal of apically placed dentinal debris (19). Akçay *et al.* (20) showed that the activation of the irrigant and the creation of the streaming with the Er:YAG laser have positive effects on the irrigant penetration. But Kustarci and Er (21) have warned about more apically extruded debris.

Pulp tissue dissolution is a highly desirable property of any irrigant because it potentially enhances root canal cleansing. Therefore, the tissue-dissolving property of irrigants is the most important reason for choosing an endodontic irrigant. Sodium hypochlorite (SH) is the most widely preferred irrigant because of its spacious antimicrobial effect, the capacity of dissolving the organic part of the smear layer, and pulp tissue remnants. Thus, IATs are suggested to increase the efficacy of irrigant delivery and improve root canal cleanliness (9-11).

The aim of this *in vitro* study was to compare the organic tissue dissolution capacities of 3 different IATs (sonic, ultrasonic, and laser-activated) in the presence of saline or NaOCl irrigants in artificial simulated IRCs. The null hypothesis tested was that there are no differences in pulp tissue dissolution capacity among the used 3 IATs in the presence of 2 irrigants.

Materials and Methods

Bovine pulp tissue preparation

Thirty intact, freshly extracted, young bovine mandibular and maxillary incisors were used in this study. This study was not classified as an animal study because it did not influence the premortal fate of the animals or the slaughtering process in any way. The teeth were extracted within 24 h after purchasing the bovine jaws from a slaughterhouse and immediately placed in glass vials with distilled water and stored at -20 °C until required.

Bovine incisors were decoronated at cemento-enamel junction by using diamond fissure burs and the pulp tissues were removed carefully. All pulp tissue were irrigated with distilled water to remove excess blood and blood clot. During the experiment, pulp tissues were only manipulated by using cotton pliers to avoid errors. All testing procedures were performed at room temperature.

Root canal preparation

A total of 90 single-rooted maxillary human incisors free of resorption, restoration, immature apex, cracks or fractures were selected. The crowns were removed with a sectioning saw (Isomet; Buehler, Düsseldorf, Germany) under water cooling to standardize root length as 15±1 mm. Apical patency was achieved by inserting a size 15 K-file (Dentsply Sirona) into the root canals until the tip was visible at the apical foramen. This length was reduced by 0.5 mm to determine the WL.

ProTaper Next rotary NiTi files (X1-X5) (Dentsply Sirona) were used for the root canal instrumentation according to the recommendation provided by the manufacturer, to the full WL. The canals were irrigated with 5 mL of 2.5% NaOCl using 30-G side vented needle attached to a plastic syringe (Canal Clean; Biodent, Paju, South Korea) placed 1 mm short of the WL. Final irrigation was done with 5 mL of 17% EDTA for 1 min and 10 mL of distilled water. Then, the canals were dried with paper points (ProTaper Next X5; Dentsply Sirona).

Preparation of samples

The grooves on the buccal and lingual root surfaces were created with the diamond disc and the teeth were split longitudinally in the buccolingual direction with the help of a hammer and chisel. Under magnification, the cavities with a diameter of 1.6 mm and a depth of 0.8 mm, at 6 mm beyond from the apex in both separated root fragments, were created with diamond round burs (FD.D. 801; Gmund/Tegernsee, Germany). The cavity surfaces were etched with 37% phosphoric acid (Dia-Etch; DiaDent, Seoul, Korea) for 30 s, then flushed with distilled water and dried with blotting paper. The diameters of the created cavities were checked with a digital caliper.

Bovine pulp tissue samples were prepared by using a scalpel. They were placed in a way to completely fill the cavities and then removed. Samples were weighed 3 times on a precision balance (XB 220A; Kunz Precisa, Zofingen, Switzerland) with an accuracy of 0.0001 gr, and the average of the 3 measurements was calculated. In this way, it was tried to prepare samples with standard weights (~ 2.3 mg each). Bovine pulps were placed again into the IRCs and the fragments of teeth were reassembled with the help of glue (Pattex Super Glue, Henkel, Duesseldorf, Germany).

The teeth were divided into 6 groups (n=15) randomly according to the types of agitation and placed in alginate mold before irrigation agitation protocol. The test groups were as follows: saline with SI (NaCl + SI) group, sodium hypochlorite with SI (NaOCl + SI) group, saline with PUI (NaCl + PUI) group, sodium hypochlorite with PUI (NaOCl + PUI) group, saline with LAI (NaCl + LAI) group, and sodium hypochlorite with LAI (NaOCl + LAI) group. *Group 1 (NaCl + SI):* The root canals were irrigated with 5 mL 0.9% NaCl (I.E. Ulagay Drug Industry, Istanbul, Turkey). The EndoActivator (Dentsply Maillefer) device was used for sonic agitation. It was performed using the EndoActivator handpiece set at 10.000 cycles per min with a medium polymer tip (#25/.04). The tip of the EndoActivator was placed at the IRCs and activation was made for 1 min. This process was repeated 2 times. *Group 2 (NaOCl + SI):* 5 mL 2.5% NaOCl was used as an irrigant. The same activation procedures that were previously described for Group 1 were applied. *Group 3 (NaCl+PUI):* The root canals were irrigated with 5 mL 0.9% NaCl. A noncutting #25 file (Irrisafe; Satelec Acteon, Merignac, France) driven by an ultrasonic device (Satalec P5 Newtron XS; Satelec Acteon) was used for ultrasonic activation in root canals for 3×20 sec at 50% power for 1 min. The tip was immersed in root canal containing irrigant throughout the IRC. This process was repeated 2 times. *Group 4 (NaOCl + PUI):* 5 mL 2.5% NaOCl was used as an irrigant. The same activation procedures that were previously described for Group 3 were applied. *Group 5 (NaCl + LAI):* The Er,Cr:YSGG laser system (2780 nm wavelength) (Waterlase iPlus; Biolase Technology, Irvine, CA, USA) was used at a panel setting of 0.5 W at 20 Hz (25 mJ/pulse) without air or/water spray. The pulses were focused using a fiber tip (RFPT5) with a diameter of 580 µm and a length of 14 mm. After the root canals were irrigated with 5 mL 0.9% NaCl, Er,Cr:YSGG laser system was used for activation for 1 min. This process was repeated 2 times. *Group 6 (NaOCl + LAI):* 5 mL 2.5% NaOCl was used as an irrigant. The same activation procedures that were previously described for Group 5 were applied.

In all groups, total agitation time was 2 min, and all teeth in each group were irrigated with 5 mL of NaCl as final irrigant to prevent the prolonged effect of irrigant. After that, the teeth were separated again and the pulp residues remaining in the IRCs were removed and dried with cotton pellets. The average weight of pulp tissue was calculated by measuring 3 times on a precision balance.

Statistical analysis

The difference in weights of the tissue sample, before and after exposure to the test irrigant, was divided by the original tissue weight and multiplied by 100 to obtain the percentage of tissue weight loss. The normality was assessed by Kolmogorov-Smirnov test and all the groups showed normal distribution. The data were therefore analyzed statistically using One-way analysis of variance and Tukey's HSD post hoc tests. Statistical significance was assumed at $p<0.05$. All calculations were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

Results

Mean value of tissue weight before and after irrigation was shown in Table 1. Comparison of percentage reduction of tissue weight and p values were indicated in Table 2. SI has significantly more successful results than PUI and LAI in the groups in which the irrigant was NaCl (Group 1 > Group 3= Group 5). There was also a significant difference between LAI and PUI in the groups in which the irrigant was NaOCl (Group 6 > Group 4, p=0.003). On the other hand, the tissue weight changes of the NaOCl groups using SI and PUI techniques were not significantly different from each other.

Table 1: *Measurements of tissue weight before and after irrigation agitation protocols*

Groups	n	Tissue weight before irrigation Mean ± SD (*10-3*)	Tissue weight after irrigation Mean ± SD (*10-3*)
Group 1 (NaCl + SI)	*15*	**2.2±0.40**	0.8±0.52
Group 2 (NaOCl + SI)	*15*	2.1±0.23	0.7±0.51
Group 3 (NaCl + PUI)	*15*	2.2±0.33	2.0±1.83
Group 4 (NaOCl + PUI)	*15*	2.6±0.32	1.4±0.58
Group 5 (NaCl + LAI)	*15*	2.4±0.26	1.6±0.24
Group 6 (NaOCl + LAI)	*15*	2.7±0.84	0.7±0.66

*SD: Standard deviation

Table 2: *Pairwise comparisons, means and standard deviations of % reduction in tissue weight values*

	Groups	n	Mean ± SD (%)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Reduction in tissue weight	**Group 1 (NaCl + SI)**	*15*	61.5±23.54	-	0.998	**0.002**	0.247	0.006	0.483
	Group 2 (NaOCl + SI)	*15*	65.0±25.8	0.998	-	<0.0001	0.096	**0.001**	0.753
	Group 3 (NaCl + PUI)	*15*	30.8±16.89	**0.002**	**<0.0001**	-	0.469	1	**<0.0001**
	Group 4 (NaOCl + PUI)	*15*	44.6±21.21	0.247	0.096	0.469	-	0.66	**0.003**
	Group 5 (NaCl + LAI)	*15*	32.9±13.79	0.006	**0.001**	1	0.66	-	**<0.0001**
	Group 6 (NaOCl + LAI)	*15*	76.0±21.87	0.483	0.753	**<0.0001**	**0.003**	**<0.0001**	-

**SD: Standard deviation*

Discussion

The remaining organic tissue inside the root canal space and IRCs can cause the growth of surviving microorganisms and affect the success of RCT (22). NaOCl is the most common irrigant used in the RCTs with its tissue dissolving capacity and its high surface tension, which increase the penetration to dentin tubules and therefore its antibacterial efficacy (23). In this study, 2.5% NaOCl and 0.9% NaCl (as a control) irrigants were selected as an irrigants and maxillary central incisors with simulated IRCs were used, because IRR is most frequently seen in these teeth (24). Because of its similarity to human pulp, fresh bovine pulp was preferred to simulate the organic tissue remnants (25). De Gregorio *et al.* (26) stated that the apical preparation size and taper effect the volume and exchange of irrigant at the WL. According to these findings, the apical enlargement was completed with a suitable size and taper in this study.

Frequency of activation, amount of tissue in relation to amount of irrigant in the root canal system and surface area of tissue that was available were the dependent factors for the tissue dissolution (27). The effect of different IATs on dissolution capacity of pulp tissue in artificial IRCs were the aim of this study. The results demonstrated statistical differences among the different tested protocols. Therefore, the null hypothesis was rejected.

In the groups where NaOCl were used as irrigant, there was no significant difference between PUI and SI groups, although PUI has higher frequencies than SI. This could be explained with attenuation of activation forces because of the tip of PUI touching the root canal walls. Conde *et al.* (10) found similar results and they explained these results with plateau effect where decrease of tissue dissolution occurs when using PUI. In another study, activation with XP-Endo Finisher has higher capacity of removal of simulated organic tissue from artificial IRCs compared to ultrasonics in straight root canals. It was explained that the mechanical removal of the organic tissues by XP-Endo Finisher could have increased the tissue weight loss (28).

LAI with erbium lasers has been proven to be more effective than PUI in the removal of debris and apical smear layer but there is no study evaluating the removal of organic tissue before (29,30,31). In recent study LAI techniques have had more successful results than manual-dynamic irrigation in removing debris from simulated root canal irregularities (32). LAI has significantly more successful results than PUI in this study. These results were consistent with another study in which laser-activation was shown to be more effective for the removal of calcium hydroxide from mesial roots of mandibular molars than PUI (33). The generation of heat increase and the improved irrigant flow dynamics created by laser devices may cause enhancement of tissue dissolution capacity during LAI.

There are several limitations of this type of experiment model (34). The main limitation is that the pulp tissue was packed into the IRC area and therefore lacked any physical attachment to root canal dentin. Besides that, the presence of the same dentin in all the cases can cause a bias on buffering effect. However, this method allowed the use of the same dentin with the same anatomy of the IRC for all groups, reducing the risk of bias in anatomical differences and simulating well the results for this type of complexity. To conduct a study with multiple teeth, it would be necessary a great number of teeth with different anatomies, which would then undermine the standardized comparison of the IATs. Besides, in clinical conditions, some considerable dissolution of organic pulp tissue had already occurred during cleaning and instrumentation procedure. However, in this study, agitated irrigants were directly applied to organic tissue located in IRCs which were simulated to assess the dissolution capacity of the NaOCl agitated with the various techniques. This could be another limitation of this study.

Conclusion

Complete dissolution of bovine pulp tissue from IRCs was not achieved by any tested techniques. However, the LAI with NaOCl was more effective than other IATs. In addition, there is no significant difference between the LAI and SI with NaOCl.

Türkçe özet: *Farklı irrigasyon aktivasyon tekniklerinin simüle internal rezorpsiyon kavitelerinde pulpa dokusunu çözme kapasitelerinin karşılaştırılması. Amaç: Bu in vitro çalışmada, simüle internal rezorpsiyon kavitelerinde (İRK) 3 farklı irrigasyon aktivasyon tekniğinin (İAT'ler) organik doku çözme kapasitelerini karşılaştırmak amaçlanmıştır. Gereç ve Yöntem: Bu çalışma için 90 adet yeni çekilmiş insan maksiller kesici dişi seçildi. Dekoronasyon işleminden sonra kökler bukkolingual yönde uzunlamasına 2 parçaya bölündü. Her bir parçada kök kanalı üzerinde standart bir İRK hazırlandı. Sonra, sığır diş pulpası örnekleri (~ 2,3 mg) tartıldı ve rezorpsiyon kavitelerine yerleştirildi. Kök parçaları yeniden birleştirilerek yapıştırıldı ve dişler rastgele olarak 6 gruba ayrıldı (n=15); sodyum klorur (NaCl) ve sonik irrigasyon (SI), sodyum hipoklorit (NaOCl) ve SI, NaCl ve pasif ultrasonik irrigasyon (PUI), NaOCl ve PUI,NaCl ve lazerle aktifleştirilmiş irrigasyon (LAI), NaOCl ve LAI. Aktivasyon sonrası dişler ayrılarak kavitelerin içindeki doku örnekleri çıkarılıp tartıldı. İlk ve son ölçümler arasındaki fark hesaplandı ve analiz edildi. Bulgular: İrrigasyon solüsyonunun NaCl olduğu gruplarda SI, PUI*

ve LAI'dan anlamlı derecede daha başarılı sonuçlara sahipti. İrrigasyon solüsyonunun NaOCl olduğu gruplarda ise, LAI ve PUI arasında anlamlı fark vardı (Grup 6 > Grup 4, p=0.003). NaOCl ile LAI ve SI arasında anlamlı fark yoktu. Sonuç: Sığır pulpa dokusunun IRK'lerden tamamen çözünmesi, test edilen herhangi bir teknik ile sağlanamadı. Ancak, NaOCl ile LAI, simüle edilmiş İRK'de sığır pulpa dokusunun çözünme kapasitesinde diğer İAT'lerden daha etkiliydi. İlave olarak, NaOCl ile LAI ve SI arasında anlamlı bir fark yoktu. Anahtar kelimeler: Çözünme, internal rezorpsiyon kavitesi, diş, pulpa, aktivasyon.

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Original research

Cone beam computed tomography evaluation of c-shaped canal morphology in mandibular premolar teeth*

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Purpose
The aim of this study was to evaluate the prevalence and the morphology of c-shaped root canal(s) in mandibular premolars using cone beam computed tomography (CBCT) images.

Materials and Methods
CBCT images of 1095 mandibular premolars were examined at coronal, middle, and apical levels of the root canals. The type, the level, and the position (buccal or lingual) of the c-shaped anatomy were recorded. Absolute counts and percentages of different groups and subgroups of C-shape morphologies were calculated. The Chi-square test was used to compare the prevalence of C-shaped morphology between mandibular first and second premolars. The Z-test for proportions in independent groups was used to analyze the differences in mandibular C-shaped premolar proportions between location (left and right side) and tooth (first or second premolars) (p=0.05).

Results
C-shaped root canal morphology was present in 44 teeth. The percentage of c-shaped morphologies was 6.9% and 1.6% in mandibular first and second premolars, respectively. Comparison of the first and the second premolars showed that C1 type (p=0.008) and C4b type (p=0.013) configurations are more common in the first premolars at the coronal level. In contrast, the C2 type configuration showed significantly higher prevalence in the second premolars (p=0.009). Additionally, the C4c type configuration was significantly frequent on the right premolars at the coronal level (p=0.038).

Conclusion
C-shape canal morphology is a rare but complex anatomic feature in mandibular premolars. Therefore, clinicians should be aware of this complex root canal anatomy for the success of endodontic treatment in mandibular premolar teeth.

Keywords: *C-shaped configuration, cone beam computed tomography, mandibular premolar, prevalence study, root canal anatomy*

Introduction

Definitive knowledge of root and canal morphology, including anatomic variations, is the primary step of endodontic treatment (1). Due to their complex morphology, C-shaped canals may create great challenges during endodontic treatment with respect to shaping, debridement, and obturation (2). C-shaped canal morphology is described as the presence of fins and isthmuses connecting individual canals or merged root canals resulting in a 'C' shaped appearance at cross-sectional views (3). However, this morphology may not be continuous throughout the root length (4). As reported previously, a root canal with C-shaped morphology at the coronal third of the root may not continue as a single C-shaped canal at the middle and apical thirds (5). On the other hand, separated canals de-

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tected at the floor of the pulp chamber may merge and continue as a single C-shaped canal (6). It has been stated that C-shaped molars usually contain a fused root and a longitudinal radicular groove, which is also identified in C-shaped mandibular premolars (1). The radicular groove is defined as a developmental invagination and frequently exhibits on the proximal lingual area of the middle root of the C-shaped mandibular premolars (7). It is important that isthmuses within the root canal system may exist close to this groove, which creates a danger zone concerning a possible complication during endodontic treatment (1, 7). Therefore, the detection of canal morphology and necessary modifications in the shaping and filling of C-shaped canals are essential for successful endodontic treatment of C-shaped canals (2).

C-shaped canal morphology is a well-known anatomic variation in mandibular molar teeth and is mostly found in mandibular second molars (3, 8, 9). However, its presence has been recently reported in mandibular premolars, maxillary molars, and even in maxillary lateral incisors (2). Many studies have investigated the prevalence of C-shaped root canal morphology in mandibular molars; however, there are few studies evaluating C-shaped canal prevalence in maxillary molar and mandibular premolar teeth (3). Therefore, the aim of this study was to evaluate the prevalence and the morphology of the C-shaped root canal(s) in mandibular premolars using cone beam computed tomography (CBCT) images. The null hypothesis was that there would be no C-shaped canal morphology in mandibular premolar teeth.

Material and Methods

Ethical statement

The present study design was approved by the Ethical Committee of Ege University (2021/22-2.1T/33) and followed the principles of the Declaration of Helsinki.

Sample selection

A total of 2024 CBCT images taken for various reasons between the years of January 2019 and March 2020 at the department of Oral and Maxillofacial Radiology were examined retrospectively, and images of patients with at least one mandibular premolar were included in the study. Teeth with previous endodontic treatment, internal and/or external root resorptions, immature apices, periapical lesions, full-crown restorations, and CBCT images with severe artifacts due to other restorations were excluded. Consequently, 1095 mandibular premolar images (489 first and 606 second premolars) of 586 patients (229 male and 357 female) were evaluated. The mean age of the patients was 31.8±14.6 years.

Image acquisition

CBCT scans had been previously obtained using Kodak 9000 3D (Kodak Carestream Health, Trophy, France) device at 70kV, 10mA, and 10.8s exposure time, using a 50 x 37 mm field of view (FOV) and a 76 µm isotropic voxel size.

Image assessment

All premolars were analyzed at three axial levels from the cemento-enamel junction to the anatomic apex. Two millimeters below the cemento-enamel junction was accepted as the coronal level; likewise, 2 mm above the anatomic apex as the apical level; and the halfway between the coronal and apical levels was considered as the middle level. C-canal configurations were classified according to the modified classification system described by Fan *et al.* (1). According to this classification, C1 was identified as a continuous ‘C’ with no separation or division; C2 as a discontinuation in the ‘C’ outline resembling a semicolon; C3 as two separate round, oval, or flat canals; C4 as one round, oval, or flat canal; C5 as three or more separate canals and C6 as no canal lumen or no intact canal. In addition, C4 configuration was divided into 3 subgroups (C4a, C4b, C4c) as reported by Wu *et al.* (10): C4a (round canal): The long canal diameter is almost equal to the short diameter. C4b (oval canal): The long canal diameter is at least 2 times shorter than the short diameter. C4c (flat canal): the long canal diameter is at least two times longer than the short diameter (Figure 1). As reported previously, mandibular molars and premolars containing the C-shaped canal morphology are often observed with fused roots and a longitudinal radicular groove (1, 5). Therefore, in the current study, teeth with an external radicular groove and with C1 or C2 canal configuration at any axial level were considered as having C-shaped canal morphology (Figure 2). Two oral radiologists and one endodontist made the radiographic evaluations. The type of the C-shaped canal configuration was recorded at each axial level for each mandibular premolar independently along with the number of the roots containing C-shaped anatomy (C1 or C2). In addition, buccal or lingual orientation of the C-shaped canal was recorded for each axial level.

Statistical analysis

Data analysis was performed using the IBM SPSS Statistics 20.0 (SPSS Inc., Armonk, NY, USA). Absolute counts and percentages of different groups and subgroups of C-shape morphologies were calculated for all mandibular premolar teeth. The proportion of each group was determined, as was the range for the true population proportion, to a confidence level of 95%. The chi-square test was used to compare the prevalence of C-shaped morphology between mandibular first and second premolars. The Z-test for proportions in indepen-

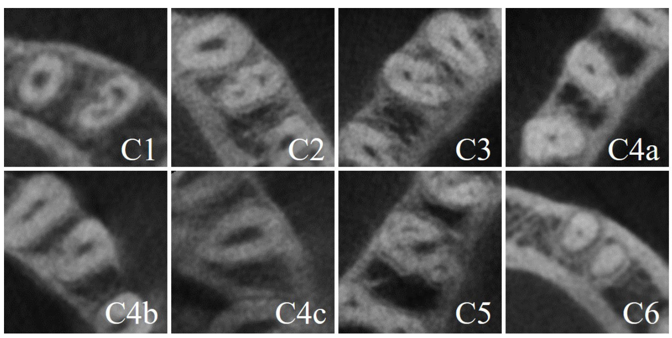

Figure 1. *Classification of the C-shaped canal configuration in mandibular premolars.*

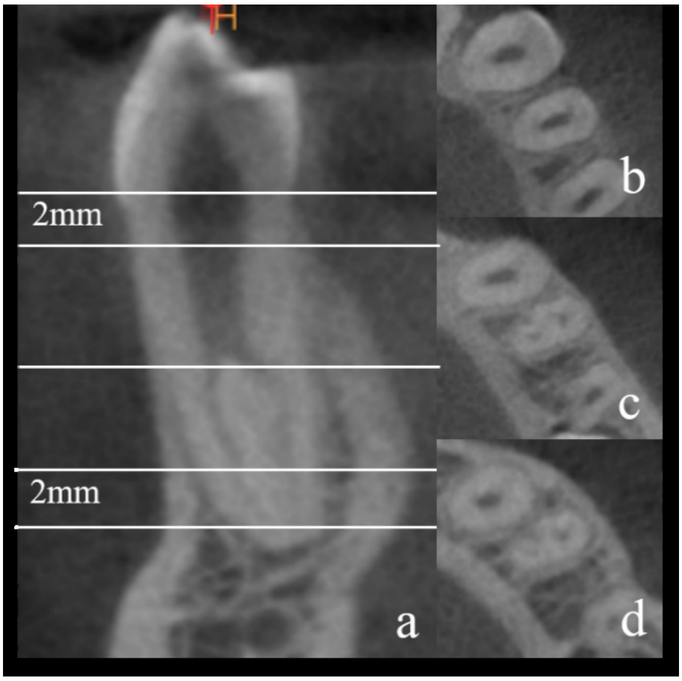

Figure 2. *CBCT image representing the diagnosis of C-shaped configuration. (a) Frontal CBCT image showing a C-shaped mandibular first premolar. (b) Axial CBCT image showing the C4c type configuration at the coronal level. (c) Axial CBCT image showing the C2 type configuration at the middle level. (d) Axial CBCT image showing the C3 type configuration at the apical level.*

dent groups was used to analyze the differences in mandibular C-shaped premolar proportions between location (left and right side) and tooth (first or second premolars). For all groups, a *p* value <0.05 was considered significant.

Results

The null hypothesis was rejected. C-shaped canal morphology was found in 32 (24 male and 8 female) of 586 patients (5.4%). The C-shaped canal percentages for male and female patients were 10.4% and 2.2%, respectively. The mean age of the patients with C-shaped morphology was 22.75±10.56 years. Among 1095 mandibular premolars, 44 teeth were identified as having C-shaped canal morphology with a prevalence of approximately 4%. All premolar teeth with C-shaped canal morphology had an external longitudinal groove, which was located in the mesio-lingual surface of the root. Thirty-four of 489 mandibular 1st premolars and 10 of 606 mandibular 2nd premolars showed C-shaped canal morphology. The prevalences for mandibular first and second premolars were 6.9% and 1.6%, respectively. The C-shaped canal configuration was observed frequently in the right first premolars and least in the left second premolars. The counts and percentages of C-shaped canal configuration for each mandibular premolar tooth were presented in Table 1.

Comparison of different axial levels for C-shaped canal configurations

The most frequent configurations at the coronal level were C4b and C4c. On the other hand, C1 and C2 configurations, which were considered as defining criteria for a C-shaped canal, were more common at the middle level. As for the apical level, the C3 configuration was more prevalent. C5 and C6 configurations were not present at the coronal level, whereas C4a, C5, C6 configurations at the middle level and C4b, C4c, C5 configurations at the apical level were not observed. Cshaped canal configuration prevalence for each axial level was presented in Table 2.

Comparison of first and second premolars

When the first and the second premolars were compared in regard to C-shaped configurations at various axial levels, it was observed that C1 (p=0.008) and C4b (p=0.013) configurations were significantly common in the 1st premolars at the coronal level, whereas C2 (p=0.009) configuration was frequent in the 2nd premolars. No significant differences were observed between the first and the second premolars in terms of C-shaped canal morphology at the middle and the apical levels.

Comparison of the left and right premolars

Comparison of the left and right sides revealed that 14 C-shaped premolars were identified on the left side while it was 30 on the right. C4c canal configuration was significantly more prevalent in the right premolars at the coronal level (p=0.038). No significant differences were observed be-

Table 1: *The counts, percentages, and location of mandibular premolars with C-shaped canals*

No & type of teeth	No. of C-shaped canals	%	Location of premolar tooth (right/left)
1095 premolars	44	4	30/14
489 1st premolars	34	6.9	24/10
606 2nd premolars	10	1.6	6/4

Table 2: *Distribution of C-shaped canal configurations* at different levels of root canal (coronal-middle-apical) of mandibular premolar teeth. *According to Fan et al. (1)*

C-shape configuration	1st premolar Axial cross-sections (n)			2nd premolar Axial cross-sections (n)		
	Coronal	Middle	Apical	Coronal	Middle	Apical
C1	5	10	9	-	3	3
C2	1	21	5	4	6	2
C3	1	1	14	-	-	3
C4a	-	-	5	2	-	2
C4b	13	1	-	1	1	-
C4c	14	1	-	3	-	-
C5	-	-	-	-	-	-
C6	-	-	1	-	-	-

tween the right and left premolars as regards to C-shape canal morphology at the middle and the apical levels ($p>0.05$).

Number of roots and position of the C-shaped canals

Among 44 C-shaped premolars, 28 first premolars and 5 second premolars were single-rooted, while 6 first premolars and 5 second premolars were bi-rooted. Except for a single 2nd premolar, the C-shaped morphology was always found in the buccal root of the bi-rooted teeth (Table 3).

Table 3: *Distribution of the number of roots and position of the C-shaped canal in mandibular premolar teeth*

Number of roots and position of the C-shaped canals	1st premolar	2nd premolar	Total
Single root-single canal	17	1	18
Single root- Buccal	11	4	15
Single root-Lingual	-	-	-
Two roots-Buccal	6	4	10
Two roots-Lingual	-	1	1

Discussion

Successful endodontic treatment requires knowledge of anatomic variations and morphologies of the root canal system (2). Canal systems with different morphologies, such as C-shaped canal morphology, may create considerable risk for treatment failures because infected debris and remnants may remain on root canal walls due to inadequate cleaning and filling (5). It has been stated that the actual number and correct configuration of root canals can be determined more accurately with 3D imaging (11). According to the joint position statement of the American Association of Endodontists (AEE) and the American Academy of Oral and Maxillofacial Radiology (AAOMR), a limited field-of-view (FOV) CBCT can be considered the imaging modality of choice for teeth with complex root canal morphology (12).

Many studies have been published evaluating the prevalence of the C-shaped canal morphology in mandibular first and second molars using 3D CBCT images (4, 13-19). However, the prevalence of C-shaped canal morphology in mandibular 1st and 2nd premolars using CBCT images had not been frequently investigated (3, 20-23).

The prevalence of C-shaped canal morphology for 1st (6.9%) and 2nd (1.6%) premolars in the present study was consistent with the previous CBCT reports showing a low prevalence of C-shaped morphology in mandibular premolars. Kaya Buyukbayram *et al.* (24) and Pedemonte *et al.* (25) reported 4.58% and 1.13% prevalence ratios ranging between 4.58-10.9% for 1st and 1-1.13% for 2nd mandibular premolars, respectively.

It was also emphasized that prevalence ratios of anatomical variations of C-shaped canal configuration for mandibular premolars might differ depending on ethnicity and geography (2, 23). Different studies based on South American and Saudi Arabian populations reported higher prevalence ratios than the present study (21, 23). On the other hand, one Iranian study failed to identify any C-shaped premolars in their study group (26). Some South Korean, Asian, and Saudi Arabian studies stated similar yet lower prevalence ratios, and, unlike the present study, a significant difference between the first and second premolars were reported (20, 22, 27). Side with the ethnic diversity, variabilities with regard to root canal morphology may also depend on the selected study groups, study design, and classification of the C-shaped morphology. Although the difference between the first and second premolars in the present study was insignificant, the higher prevalence observed in 1st premolars agreed with many previous reports suggesting that first premolars tended to demonstrate C-shaped morphology more than 2nd premolars (22, 25, 27, 28).

In the present study, males showed a significantly higher rate of C-shaped mandibular premolars than females. A previous study conducted on the Turkish population similarly found a higher prevalence in males and determined a significant relationship between gender and C-shaped canal morphology (29). However, another study conducted on the Turkish population could not find any difference between genders (24). Overall review of the literature demonstrated some studies reporting higher prevalence for males in terms of C-shaped morphology in mandibular premolars (20, 22, 28), while others showed higher prevalence for females (30). Moreover, there were many studies that could not state a significant distinction between genders in regard to C-shaped canal morphology in mandibular premolars (23, 24, 27). It is clear that there is no definite association between gender and C-shaped canal configuration in mandibular premolars. This lack of information may be related to the small sample sizes resulting from the lower prevalence of C-shaped canal configuration in mandibular premolars, as well as the use of low-resolution images with high voxel sizes for the detection of this anatomical variation. Further controlled studies are required to determine the proper relationship between gender and C-shaped canal prevalence in mandibular premolars with high sample sizes from different populations and high-quality radiographic images.

It has been reported that there is no accepted classification system for C-shaped canals for mandibular premolars. Nevertheless, it is interesting that some studies did not describe the classification system used to rank the C-shaped canal configuration (25, 31). The modified classification of Fan *et al.* (1) was preferred in the present study for mandibular premolar C-shaped canal evaluations since it was a detailed classification and predominantly preferred in recent studies that enabled objective comparisons.

It was demonstrated previously that similar to the prevalence of C-shaped morphology, the type of canal configuration might also vary depending on nationality (20). Yet, findings of the present study showing higher rates of C4b and C4c configurations at the coronal level and C1 & C2 configurations at the middle level are consistent with the result of many previous studies (21-24, 29, 30). In the mandibular molar teeth with C-shaped canal morphology, the orifice usually appears as a continuous C shape (C1) or a semicolon-like form (C2) (5). However, in mandibular premolars, C-shaped configuration appears as an oval, round or flat-shaped canals in the coronal third of the root and reveals its C-shaped canal form in the middle third (21, 23, 28). Mandibular premolars with C-shaped canals have also been distinguished by the presence of an external radicular groove frequently developing on the proximal lingual area of the middle root[7] and mesio-lingual surface placement has been reported as the most common position of the radicular groove (5, 22, 23, 27, 28). In the present study, all premolar teeth with C-shaped canal morphology demonstrated a mesio-lingual radicular groove.

Studies evaluating C-shaped canals in mandibular molars proved that C-shaped canal configuration tends to be located at the root fusion, where the radicular groove develops (5). Similarly, C1 and C2 configurations have been observed to be associated with the external radicular groove in mandibular premolars (21); which creates a danger zone during endodontic treatment (1). Owing to the close proximity of the radicular groove to the isthmuses and C-shaped canals, the dentin wall facing the groove may become relatively thin, particularly in the middle third of the root (1, 5). Gu *et al*. (32) confirmed that the dentin thickness between the root surface and the canal was the thinnest at the location of the radicular groove. Therefore, clinicians should be aware of this morphological discrepancy and should be careful to avoid stripping perforation during the instrumentation of C-shaped canals. The distinction of this morphological variation is also crucial for the planning of endodontic post placement.

Another endodontic challenge observed with C-shaped canal morphology has been stated as the unusual anatomy of the pulp chamber (2, 6). Studies evaluating the C-shaped canals in mandibular molars proved that the floor of the pulp chamber is situated deeply, and the orifice of the C-shaped canal begins below the cementoenamel junction (5). Ariciouglu *et al.* (29) reported a significant correlation between taurodontism and C-shaped configuration in mandibular molars. To our knowledge, there are no published studies evaluating the presence of taurodontism in C-shaped mandibular premolars. However, similar to the results of the present study, many studies have reported that, in the mandibular premolar teeth, the coronal third of the root of the canal begins as an oval, round or flat, and the C-shaped configuration appears in the apical half of the root (20). In addition, Vertucci type V configuration was highly associated with C-shaped canal morphology in mandibular premolars (24, 28), and the bifurcation in such teeth was usually detected at the middle third of the root (30). Therefore, clinicians should be conscious and attentive to variable canal configurations that can be seen in the middle and apical thirds of the root in the mandibular premolars, as well as possible unusual anatomy of the pulp chamber for the success of endodontic treatment.

Conclusion

According to the results obtained, C1 and C2 configurations, which were suggested as the defining criteria for C-shaped canal morphology, tend to be more frequent at the middle third of the root canal in mandibular premolar teeth. Therefore, if the presence of C-shaped morphology in mandibular premolars is suspected clinically, we recommend examining particularly the middle axial level at CBCT images. Presence of various forms of C-shaped root canals requires extra knowledge and care in terms of shaping, irrigation and obturation for successful endodontic treatment. Therefore, clinicians should be aware of diagnosing possible variations in mandibular premolar teeth.

Türkçe özet: *Mandibular premolar dişlerde c-şekilli kanal morfolojisinin konik ışınlı-bt ile değerlendirmesi. Amaç: Bu çalışmanın amacı, mandibular premolar dişlerdeki C-şekilli kök kanal(lar)ının prevalansının ve morfolojisinin konik ışınlı bilgisayarlı tomografi (KIBT) görüntüleri kullanılarak değerlendirmesidir. Gereç ve Yöntem: 1095 mandibular premolar dişe ait KIBT görüntüsü koronal, orta ve apikal kök seviyelerinde incelenerek C-şekilli anatominin tipi, seviyesi ve pozisyonu (bukkal veya lingual) kaydedildi. Farklı gruplara ve altgruplara göre C-şekilli morfolojinin dağılımı ve yüzdesi hesaplandı. Mandibular birinci ve ikinci premolar dişler arasında C-şekilli morfolojinin prevalansının karşılaştırılması amacıyla Ki-kare testi kullanıldı. Farklı konum (sol ve sağ) ve diş grupları (birinci ve ikinci premolar dişler) arasındaki C-şekilli morfoloji farklılıklarının analiz edilmesi amacıyla ise Z-testi kullanıldı (p=0,05). Bulgular: 44 mandibular premolar dişte C-şekilli kök kanal morfolojisi saptandı. Mandibular birinci ve ikinci premolar dişlerdeki C-şekilli morfolojilerin yüzdesi sırasıyla %6,9 ve %1,6 olarak hesaplandı. Birinci ve ikinci premolar dişlerin karşılaştırılması sonucu, birinci premolar dişlerin koronal seviyesinde C1 tipi (p=0,008) ve C4b tipi (p=0,013) konfigürasyonların daha yaygın olduğunu belirlendi. Buna karşılık, ikinci premolar dişlerde ise C2 tipi konfigürasyonun anlamlı olarak yaygın olduğu gözlendi (p=0,009). Ek olarak, sağ premolar dişlerin koronal seviyesinde C4c tipi konfigürasyonun anlamlı derecede sık görüldüğü saptandı (p=0,038). Sonuç: C-şekilli kanal morfolojisi, mandibular premolar dişlerde nadir olarak izlenen karmaşık bir anatomik varyasyondur. Klinisyenlerin mandibular premolar dişlerde görülen bu karmaşık kök kanal anatomisi hakkında farkındalık sahibi olması, endodontik tedavinin başarısı açısından önem taşımaktadır. Anahtar kelimeler: C-şekilli kanal morfolojisi, konik ışınlı bilgisayarlı tomografi, kök kanal anatomisi, mandibular premolar, prevalans çalışması*

Ethics Committee Approval: The present study design was approved by the Ethical Committee of Ege University (2021/22-2.1T/33).

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

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

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Original research

Effect of beverages, denture cleanser and chlorhexidine gluconate on surface roughness of flexible denture base material: an in vitro study

Sneha Saraf[1], Sunil Kumar Mishra[2], Benaiffer Agrawal[3]

Purpose

The purpose of the study was to evaluate and compare the effect of beverages, denture cleanser and chlorhexidine gluconate solution on surface roughness of flexible denture base material.

Materials and Methods

Fifty flexible denture base resin specimens measuring 50±1mm in diameter and 0.5±0.05 mm in thickness were fabricated. The specimens were divided into five groups each containing ten specimens. The specimens were immersed in distilled water (Control group A); hot coffee (Group B); cold beverage (Group C); sodium perborate containing denture cleanser (Group D) and 2% chlorhexidine gluconate solution (Group E). The specimens were immersed for 10 min daily in mentioned solutions for up to 60 days. Surface roughness (Ra) was evaluated on the 1^{st}, 20^{th} and 60^{th} day with the help of atomic force microscope. The statistical analysis was done using two-way ANOVA and Tukey's Post hoc test.

Results

The two- way ANOVA revealed that the average Ra values varied significantly depending on the type of solution used for immersion ($p<0.001$) and the duration of immersion ($p<0.001$). Variation in surface roughness with cold beverage was highest ($p=0.001$). On the 60^{th} day the surface roughness of flexible denture base resin material was higher with cold beverage (0.184 µm) and denture cleanser (0.203 µm) than that of distilled water (0.052 µm) hot coffee (0.030 µm) and 2% chlorhexidine gluconate (0.068 µm).

Conclusion

Exposure to cold beverage, which was acidic in nature and peroxide containing denture cleanser, produces much rougher surface in the thermoplastic polyamide flexible denture base resin specimens.

Keywords: *Biofilm, flexible denture, polyamide, polymethylmethacrylate, surface roughness*

Introduction

Until now, up to 95% dental prostheses were made with polymethyl meythacrylate (PMMA), because of its optical properties, biocompatibility, and aesthetics (1). To overcome the widely known limitations of PMMA like shrinkage during polymerization, less flexural strength, inferior resistance to wear and allergy to monomer, polyamide resin have been used as an alternative material. Polyamide is the polymers having thermoplastic nature, manufactured with condensation reaction among dibasic acid and diamine (2,3).

Removable partial dentures (RPDs) fabricated only with thermoplastic resin or in combination with metal is attaining greater acceptance among

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general dentists. It has good esthetics and improved comfort, so regarded as a better treatment modality compared to regular metallic clasp retained RPDs (4). Researches on denture base materials have showed a straight connection between the roughness of the surface and increase in collection of plaque and adhesion of *Candida albicans* over it. Surface roughness is a matter of concern to any denture base material and must be evaluated. Literatures have considered 0.2µm roughness of surface as a threshold level for dental restorations. (5-7).

Previously, studies were done on erosive outcome of soft drinks, coffee, red wine, freshly prepared fruit juices and denture-cleansing agents, to found the roughness occurred on the surface of regularly used dental restorations. It has been found that restorative materials showed statistically significant micro-leakage and surface roughness as the immersion regime increased (8-14). Surface topography is done to find the appropriateness of surface for a particular use. (15). For measuring surface roughness, scanning electron microscopy and profilometery were the methods being commonly used (16). Atomic force microscopy (AFM) is a primary form of scanning probe microscopy (17). Data regarding use of AFM in field of prosthodontics for studying surface topography of denture base resins in all 3 dimensions i.e. x, y and z directions with nanoscale resolution is scarce.

The basis for doing this research was to evaluate the surface roughness caused by using beverages, denture cleanser and 2% chlorhexidine gluconate solution on flexible denture base material using AFM. The null hypothesis assumed that there would be no variance in surface roughness of flexible denture base material with beverages, denture cleanser and 2% chlorhexidine gluconate solution.

Materials and Methods

Ethical approval

This study was performed in the Department of Prosthodontics. The ethical clearance was acquired from the institutional ethical committee number PDA/Dean/14/90A..

Specimen Fabrication

Fifty flexible denture base resin (Lucitone FRS, Dentsply, Mumbai, India) specimens of dimensions 50±1mm in diameter and 0.5±0.05 mm in thickness were fabricated according to ADA specification No.12 by the injection molding method (18). A master model of hard plastic material with precise dimensions was used for the specimen fabrication (Figure 1A). Molten wax (DPI, Mumbai, India) was poured, allowed to solidify, and then retrieved from the mold. The obtained patterns in wax were further invested in a flask with dental stone (Kalrock, Kalabhai, Mumbai, India).

The flask was put for 5min in boiling water and dewaxing was done. A layer of separating media was coated and allowed for complete drying. Single cartridge (24gm) was used for making individual specimen. The silicone was sprayed on the cartridge, and then cartridge was kept in the carrier, and put in the electric furnace for softening. The material was allowed to plasticize at 575°F for about 15 minutes. The cartridge was remove from the furnace and position over the inlet of the flask, and compressed for 1 minute at an injection pressure of 75psi with narrow piston head. Bench cooling was done for 5min before deflasking (19). The flask was opened to recover the specimens. The sprue formers were cut with the disk and finishing was done. Initially the specimens were kept at 37^0C for 24 hours in distilled water for rehydration. The specimens were divided in to 5 groups, consisting of 10 specimens each. The groups were as follows:

Group A: Flexible denture base resin specimens immersed in distilled water (control group).

Group B: Flexible denture base resin specimen immersed in hot coffee (Nescafe, Nestle, Mumbai, India) at temperature 50±1 ^{0}C.

Group C: Flexible denture base resin specimen immersed in lime juice (Nimbooz, PepsiCo, New Delhi, India) at room temperature.

Group D: Flexible denture base resin specimen immersed in denture cleansing solution (Fitty Dent, Group Pharmaceuticals, Mumbai, India)

Group E: Flexible denture base resin specimen immersed in 2% chlorhexidine gluconate solution (Safe Plus, Neelkanth enterprises, New Delhi, India)

All the finished specimens were stored in artificial saliva (MP Sai enterprises, Mumbai, India) in an incubator at 37 ^{0}C for 14 hours daily. The stored specimens were taken out from the artificial saliva and cleaned in running water for 10 seconds, and bloated dry with tissue paper. All the specimens in each group were immersed in their respective solution for 10 minutes every day. The specimens were removed and washed in running water for 10 seconds and then stored in distilled water for the rest of the day at room temperature. The same regime was followed for 60 days.

Surface roughness

Surface roughness was checked on the 1st, 20th and 60th day. Prior to testing, the specimens were cleaned in an ultrasonic cleaner for 60 seconds, blotted dry using tissue paper and air dried with an air pressure pump. As per the requirement of the testing machine, the specimens were cut in to squares of 1cm x 1cm with the help of a diamond disc (Figure 1B). The baseline readings were obtained for the surface roughness (arithmetic mean surface roughness, Ra). The surface roughness was assessed using AFM (Solver Next NT-MDT, Moscow, Russia) (Figure 2).

The AFM provides a 3D profile on a nanoscale and 3 linear scans taken across individual specimens over 30 x 30 um fields with a scan rate of 10.03 mm/s and 300 pixel resolution.

Statistical analysis

Surface roughness data obtained was subjected to two-way analysis of variance (ANOVA) for repeated measures (before and after immersion and artificial aging) and Tukey's Post Hoc test ($p<0.05$). The factors analyzed were resin, surface treatment, artificial aging, surface roughness and their interactions. The results were analyzed using software package IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp, USA.

Results

The two-way ANOVA suggested that the average Ra values differs significantly based on the type of solution used for immersion ($p<0.001$) and duration of immersion ($p<0.001$) (Table 1). The null hypothesis was rejected. A gradual increase in the surface roughness was noted when comparison was done between 1st day, 20th day and 60th day of testing for the individual test group and the control group (Figure 3).

When the mean surface roughness values were compared on 1st day, 20th day and 60th day of 4 test groups and the control group, significant variations in surface roughness of Group B (hot coffee, P=0.022), Group C (cold beverage, P=0.001) and Group D (denture cleanser, P=0.013) was observed. On the 1st day, the flexible denture base resin material had more surface roughness with 2% chlorhexidine gluconate (0.057 µm) followed by distilled water (0.034 µm), hot coffee (0.023 µm) cold beverage (0.021 µm) and denture cleanser (0.019 µm). On the 20th day the surface roughness was more with 2% chlorhexidine gluconate (0.052 µm) followed by distilled water (0.045 µm), cold beverage (0.040

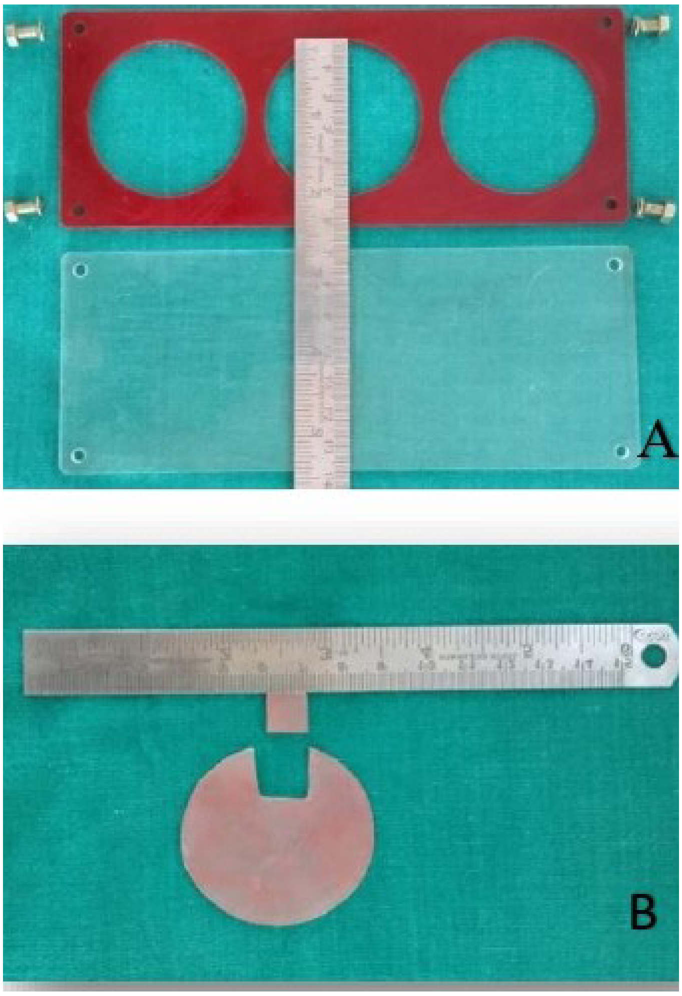

Figure 1. *A, Standardized mold for specimen fabrication. B, Specimens of size 1cm x 1cm x 0.5mm was used for testing.*

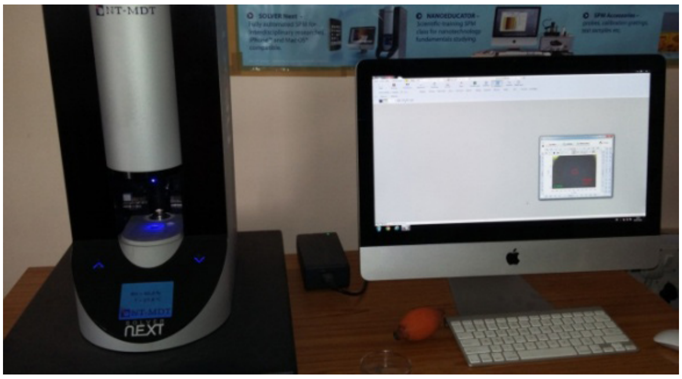

Figure 2. *Evaluation of surface roughness under atomic force microscope.*

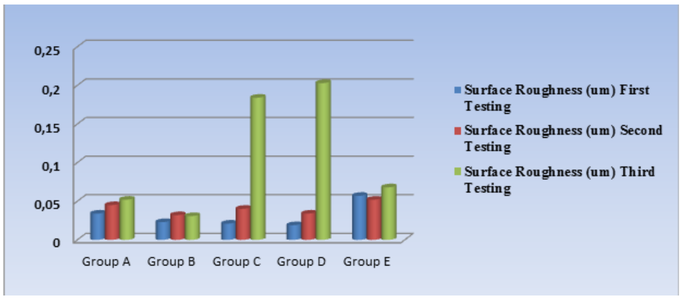

Figure 3. *Mean surface roughness on 1st day (first testing), 20th day (second testing) and 60th day (third testing)*

Table 1: *Mean surface roughness and two-way ANOVA for repeated measures on 1st day, 20th day and 60th day of immersion, *p value<0.05 was considered statistically significant. ANOVA=Analysis of variance*

Groups	Number of of specimens	Surface Roughness (µm)						Repeated measure ANOVA (F-value)	p-value
		1st day		20th day		60th day			
		Mean	S.D	Mean	S.D	Mean	S.D		
Distilled water (Group A)	10	0.034	0.005	0.045	0.021	0.052	0.036	2.444	0.152
Hot coffee (Group B)	10	0.023	0.006	0.032	0.007	0.030	0.004	7.565	0.022*
Cold beverage (Group C)	10	0.021	0.022	0.040	0.019	0.184	0.088	27.921	0.001*
Denture cleanser (Group D)	10	0.057	0.077	0.052	0.016	0.203	0.193	9.580	0.013*
2% chlorhexidine gluconate (Group E)	10	0.019	0.009	0.034	0.024	0.068	0.058	0.090	0.771
Total	50	0.031	0.037	0.041	0.019	0.108	0.120	16.958	0.001*

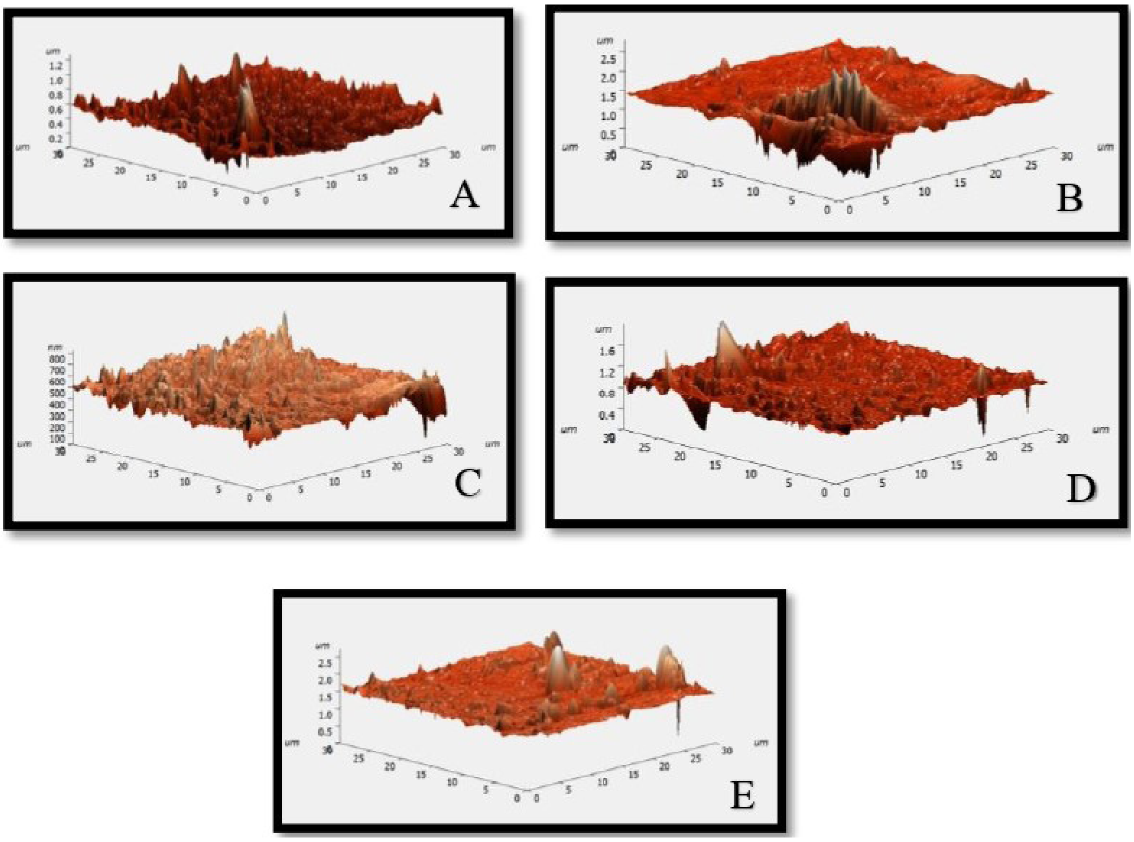

Figure 4. *Surface roughness on 60th day. A, with cold beverage. B, with denture cleanser. C, with distilled water. D, with hot coffee. E,*

Table 2: *Tukey's Post-hoc Analysis for pairwise comparisons on 1st day, 20th day and 60th day. *p value<0.05 was considered statistically significant; Ra= Surface roughness*

Groups	1st day		20th day		60th day	
	Mean Difference in Ra (µm)	P value	Mean Difference in Ra (µm)	P value	Mean Difference in Ra (µm)	P value
Distilled water (Group A) vs Hot coffee (Group B)	0.010	0.969	0.013	0.504	0.021	0.988
Distilled water (Group A) vs Cold beverage (Group C)	0.013	0.928	0.005	0.964	0.131	0.039*
Distilled water(Group A) vs Denture cleanser (Group D)	0.014	0.898	0.011	0.665	0.151	0.012*
Distilled water(Group A) vs 2% Chlorhexidine gluconate (Group E)	0.023	0.614	0.007	0.915	0.015	0.996
Hot coffee (Group B) vs Cold beverage (Group C)	0.002	1.000	0.007	0.879	0.153	0.011*
Hot coffee (Group B) vs Denture cleanser (Group D)	0.004	0.999	0.002	0.999	0.173	0.003*
Hot coffee (Group B) vs 2% Chlorhexidine gluconate (Group E)	0.033	0.255	0.020	0.122	0.037	0.917
Cold beverage (Group C) vs Denture cleanser (Group D)	0.001	1.000	0.005	0.959	0.019	0.992
Cold beverage (Group C) vs 2% Chlorhexidine gluconate (Group E)	0.036	0.187	0.012	0.565	0.116	0.089
Denture cleanser (Group D) vs 2% Chlorhexidine gluconate (Group E)	0.037	0.158	0.018	0.222	0.135	0.032*

µm), denture cleanser (0.034 µm) and hot coffee (0.032 µm). On the 60th day the surface roughness was more with cold beverage (0.184 µm; Figure 4A) and denture cleanser (0.203 µm; Figure 4B) than that of distilled water (0.052 µm; Figure 4C) hot coffee (0.030 µm; Figure 4D) and 2% chlorhexidine gluconate (0.068 µm; Figure 4E).

Pairwise comparison among groups on 1st day and 20th day did not showed any significant difference among the groups. Pairwise comparison among groups on 60th day showed significant difference ($P<0.05$) between distilled water (Group A) vs cold beverage (Group C) ($P=0.039$), between distilled water (Group A) vs denture cleanser (Group D) ($P=0.012$), hot coffee (Group B) vs cold beverage (Group C) ($P=0.011$), hot coffee (Group B) vs denture cleanser (Group D) ($P=0.003$) and denture cleanser (Group D) vs 2% chlorhexidine gluconate (Group E) ($P=0.032$) (Table 2).

Discussion

Polyamide material possesses good aesthetics, favorable gingival color, and toxicological safety in patients who are allergic to conventional resins and metals. It is flexible and has high strength, as well as resistance to chemicals and heat. Additionally, it has low porosity, low water absorption, and solubility. These properties have made polyamide increasingly popular as a denture base biomaterial (20,21).

Before applying dental prostheses orally, the surface roughness of the materials should be evaluated. A rough surface can lead to microbial colonization, biofilm formation, and discoloration of the prosthesis (22,23). In the present study, the specimens underwent artificial aging. The effect of artificial aging on the surface roughness of specimens immersed in distilled water (Control Group A) was found to be insignificant after 60 days of testing, when comparing Ra values of the 1st and 20th days. This result was similar to studies conducted by Pusz et al. (24) and Fueki *et al.* (4). Polyamide resins are injection-molded and supplied in a cartridge, which minimizes mixing errors. This provides long-term shape stability, less contraction, and improved resistance to aging (4,24).

In the present study, the surface roughness of the flexible denture base material was not significantly affected by the coffee solution (pH-5.3). This finding is consistent with the study by Navarro *et al.* (14). However, conflicting results were obtained in a study conducted by Sagsoz *et al.* (25), where an increase in surface roughness of resin specimens was observed due to extrinsic stain deposition. The discrepancy in results with coffee samples might be attributed to differences in the processing and polishing methods of the specimens. It has been proven that specimens fabricated using the injection-molded method have better physical and chemical stability compared to conventional heat and chemical processing methods (3,4).

The specimens immersed in the cold beverage Nimbooz (Group C) showed a significant alteration in surface roughness. According to a study by Constantinescu *et al.* (26), the acidity of saliva influences the surface properties of acrylic resins and increases roughness. Lemon juice, with a pH of about 2.3, is highly acidic, while the normal salivary pH ranges between 6.2 and 7.4. The type of food consumed can change the pH of saliva and cause erosion of the denture base materials' surface (27).

Considering the cleaning methods followed by patients, the resin specimens in this study were immersed in a commercially available denture cleanser and 2% chlorhexidine gluconate. The surface roughness of the specimens immersed in the denture cleansing solution increased with the duration of immersion. This result is consistent with previous studies conducted by Durkan *et al.* (28), where they also found that denture cleansers containing sodium perborate increased surface roughness. Nikawa *et al.* (29) found that denture cleansers with higher peroxide content and oxygenation levels in strongly alkaline solutions could damage denture base materials. This may be due to the chemical nature and mode of action of these cleansers. They reduce surface tension, release oxygen, and mechanically loosen debris. The oxygen bubbles aid in mechanical cleaning. Therefore, these cleansers may cause hydrolysis and decomposition of the polymerized acrylic resin itself (30).

The surface roughness of resin specimens immersed in a 2% chlorhexidine gluconate solution showed no significant variation after following a 60-day immersion regime. The result of the present study was similar to previous studies conducted by Da Silva *et al.* (31), Azevedo *et al.* (32), and Machado *et al.* (33). However, Davi *et al.* (30) obtained contradictory results in a study where they found a significant increase in surface roughness after disinfecting denture base resins with a 0.12% chlorhexidine gluconate solution. The composition of the flexible denture base resin material is chemically stable, as they are injection-molded and supplied in a cartridge, which excludes mixture errors. This provides long-term stability and resistance to aging and surface roughening (4).

One limitation of the present experiment is that it is an in-vitro study and does not completely simulate oral conditions. Further research is required with flexible dentures in patients using different beverages and denture cleansers.

Conclusion

Exposure to a cold beverage, which is more acidic in nature, and the use of peroxide-containing denture cleanser result in a much rougher surface in thermoplastic polyamide flexible denture base resin specimens. On the other hand, exposure to hot coffee does not cause a significant change in the surface roughness of the flexible denture base resin material. Therefore, 2% chlorhexidine gluconate can be considered a better option for maintaining the hygiene of flexible denture base resin material.

Türkçe özet: *İçeceklerin, protez temizleyicinin ve klorheksidin glukonatın esnek protez kaide malzemesinin yüzey pürüzlülüğüne etkisi: in vitro çalışma. Amaç: Bu çalışmanın amacı, esnek protez kaide malzemesinin yüzey pürüzlülüğüne içecek, protez temizleyici ve klorheksidin glukonat solüsyonunun etkisini değerlendirmek ve karşılaştırmaktı. Gereç ve yöntem: Çapı 50±1 mm ve kalınlığı 0.5±0.05 mm olan elli esnek protez kaide reçinesi örneği üretildi. Örnekler, her biri on örnek içeren beş gruba ayrıldı. Numuneler damıtılmış suya daldırıldı (Kontrol grubu A); sıcak kahve (Grup B); soğuk içecek (Grup C); protez temizleyici (Grup D) ve %2 klorheksidin glukonat solüsyonu (Grup E) içeren sodyum perborat. Numuneler, 60 güne kadar belirtilen çözeltilerde günde 10 dakika süreyle daldırıldı. Yüzey pürüzlülüğü (Ra) atomik kuvvet mikroskobu yardımıyla 1, 20. ve 60. günlerde değerlendirildi. İstatistiksel analiz, iki yönlü ANOVA ve Tukey's Post hoc testi kullanılarak yapıldı. Bulgular: İki yönlü ANOVA, ortalama Ra değerlerinin daldırma için kullanılan solüsyon tipine (p<0.001) ve daldırma süresine (p<0.001) bağlı olarak önemli ölçüde değiştiğini ortaya koydu. Soğuk içecek ile yüzey pürüzlülüğündeki değişim en yüksekti (p=0.001). 60. günde, esnek protez kaide reçine malzemesinin yüzey pürüzlülüğü, soğuk içecek (0.184 µm) ve protez temizleyici (0.203 µm) ile, damıtılmış su (0.052 µm), sıcak kahve (0.030 µm) ve %2 klorheksidin glukonat (0,068 mikron). Sonuç:*

Doğası gereği asidik olan soğuk içeceğe ve peroksit içeren protez temizleyiciye maruz kalmak, termoplastik poliamid esnek protez kaidesi rezin numunelerinde çok daha pürüzlü bir yüzey oluşturur. Anahtar Kelimeler: biyofilm, esnek protez, poliamid, polimetilmetakrilat, yüzey pürüzlülüğü

Ethics Committee Approval: The ethical approval was obtained from the institutional ethics committee number PDA/Dean/14/90A.

Informed Consent: Participants provided informed consent.

Peer-review: Externally peer-reviewed.

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

Conflict of Interest: The authors declared that they have no conflict of interest.

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Original research

Midface asymmetry in non-syndromic unilateral cleft lip-palate: A retrospective cbct analysis

Berkhas Tumani Üstdal[1], **Burcu Evlice**[1], **Hazal Duyan Yüksel**[1]

Purpose
The aim of this study was to determine the relationship between the zygomaticomaxillary complex (ZMC) and infraorbital foramen region (IFR) with facial symmetry in patients with unilateral cleft lip and palate (UCLP) using cone beam computed tomography (CBCT).

Materials and Methods
In this retrospective study, CBCT images of 30 non-syndromic UCLP patients were included, along with 30 age- and sex-matched control individuals. ZMC symmetry was evaluated in the axial section by comparing the right and left sides. Similarly, symmetry in the IFR was assessed in the coronal section. The significance level was set at p<0.05 for statistical analysis.

Results
The study group comprised 12 female and 18 male patients, with ages ranging from 10 to 18 years (mean age 14.1 years). Both ZMC and IFR measurements were significantly lower on the cleft sides of the study group compared to both the non-cleft sides of UCLP patients and the control group (p<0.001, p=0.022, and p=0.036, respectively). Furthermore, IFR measurements were significantly lower in the control group compared to the non-cleft sides of the study group (p=0.04).

Conclusion
This study demonstrated that individuals with UCLP exhibit asymmetry in both the ZMC and the IFR. These findings suggest a negative impact on facial aesthetics.

Keywords: *Facial asymmetry, cleft lip-palate, midface symmetry, esthetics, cone beam*

Introduction

Cleft lip and palate (CLP) is one of the most common craniofacial deformities. Although the exact etiology of CLP is not known, it is believed to be caused by a combination of genetic and environmental factors (1). Facial asymmetry, a common condition, can arise from various causes, including congenital malformations and hereditary and environmental factors (1). Among patient groups with facial asymmetry, those with CLP exhibit the most significant influence of heredity. Asymmetry can be observed in the middle and lower facial regions of these patients. Skeletal and dentoalveolar asymmetries have been documented on the cleft side of the maxilla in individuals with CLP (2). Studies have indicated that individuals with more symmetrical faces tend to have better emotional, psychological, and physiological health, and are often perceived as more attractive compared to those with asymmetrical faces (3, 4). The zygomatic complex plays a crucial role in determining the width and height of the lateral face and contributes significantly to overall facial shape (5).

The objective of this study is to investigate the relationship between the zygomaticomaxillary complex (ZMC) and the infraorbital foramen region

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(IFR) in terms of midfacial symmetry in non-syndromic patients with unilateral cleft lip and palate (UCLP) using cone beam computed tomography (CBCT). Furthermore, the study aims to compare these findings with a control group. The null hypothesis tested in this project is that there are no differences in the measurements ZMC and IFR between individuals with or without UCLP.

Materials and Methods

Ethical approval

This retrospective study was conducted in accordance with the principles of the 1964 Declaration of Helsinki and it was approved by the Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (date: 01/10/2021, meeting no: 115, decision no: 32).

Sample size determination

To determine the sample size, power analysis was conducted using pilot study data (G*Power 3.1.9.4), which indicated that 30 patients were needed for each group based on an effect size of 0.88, significance level (α) of 0.05, and power (β) of 0.90.

Study design

In this retrospective study, CBCT images of 30 non-syndromic unilateral cleft lip and palate (UCLP) patients and 30 healthy individuals were evaluated. The study group consisted of 12 female and 18 male patients with a mean age of 14.1 years (ranging from 10 to 18 years). The control group was randomly selected from retrospective images of systemically healthy patients who were matched with the study group in terms of age and gender and had undergone CBCT for various reasons such as impacted teeth and implant planning. Radiographs were excluded from the study if they had artifacts, positioning errors, or insufficient image quality for detailed examination.

Zygomaticomaxillary complex (ZMC) symmetry was evaluated by measuring the distance between the most prominent point of the zygoma (malar eminence) and the vertical line drawn from the basion point on the right and left sides in the axial section, following the methodology of Khaqani et al. (6) (Figure 1). Infraorbital foramen region (IFR) symmetry was evaluated by measuring the distance between the widest point of the infraorbital foramen and the midsagittal reference line at the crista galli on the right and left sides in the coronal section (Figure 2). All measurements were conducted by two oral and maxillofacial radiologists (BE: 15 years of CBCT interpretation experience; BTU: 3 years of CBCT interpretation experience). To assess intra-observer agreement, the observers made the measurements twice, with a one-week interval.

Imaging protocols

Radiological evaluations were performed using a 22-inch LG Flatron monitor (LG, Seoul, Korea) with a screen resolution of 1440x900 pixels and a color depth of 32-bit. CBCT images were acquired using Planmeca Promax® 3D Mid (Planmeca, Helsinki, Finland) in standard resolution mode (90 Kv, 10 mA, 27 s scan time, voxel size: 0.4 mm3). The DICOM format data were transferred to Romexis 5.2.0 software (Planmeca Oy, Helsinki, Finland), and all images were evaluated in coronal and axial sections.

Statistical analysis

Statistical analysis was performed using IBM SPSS software version 20.0 (IBM SPSS, Armonk, NY, USA). The Shapiro-Wilk test was used to assess the normal distribution of the data. Paired samples t-test was employed to compare the cleft and non-cleft sides of CLP patients, while independent samples t-test was used to compare CLP patients with the control group. The significance level was set at $p<0.05$. The Pearson correlation coefficient was used to measure the reliability of the raters' repeated measurements, and the intra-class correlation coefficient was used to test inter-rater reliability.

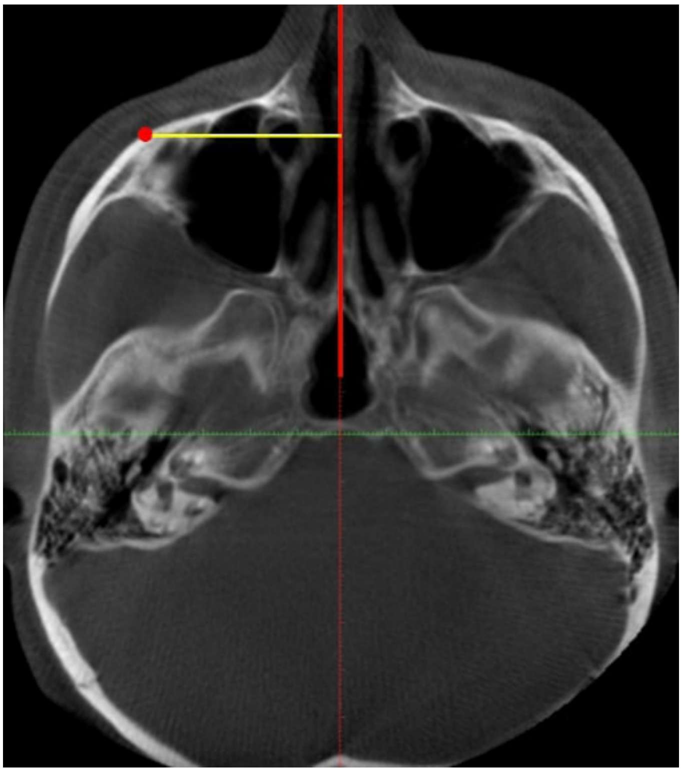

Figure 1. *Measurement of zygomaticomaxillary complex symmetry from the axial section.*

Results

In this retrospective study, ZMC and IFR measurements were performed on CBCT images from both the right and left sides of 60 patients. The measurements were calibrated by evaluating correlation coefficients. The minimum intra-rater reliability for the first and second observers was 0.83 ($p<0.001$) and 0.87 ($p<0.001$) respectively. The minimum inter-rater reliability was 0.81 ($p<0.001$) (Table 1).

Significant differences were observed in ZMC and IFR measurements between the cleft side and non-cleft side in the study group ($p<0.001$) (Table 2). Furthermore, the ZMC

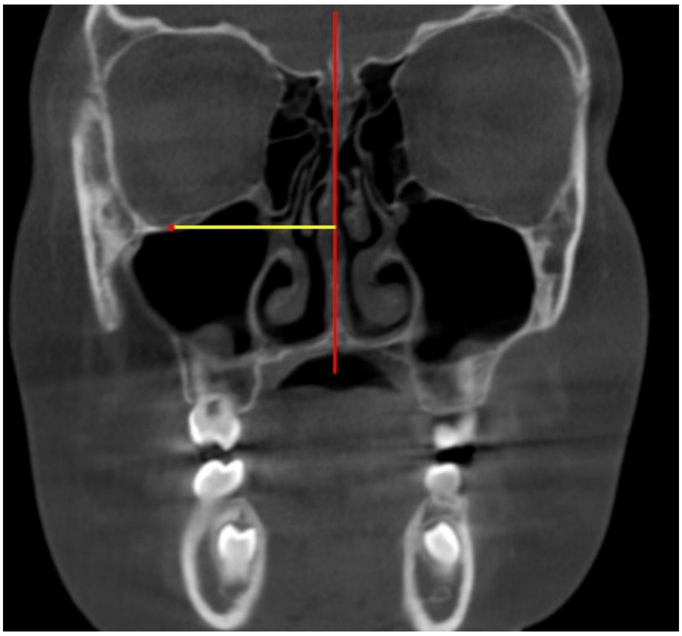

Figure 2. *Measurement of infraorbital foramen region symmetry from the coronal section.*

and IFR measurements were significantly lower on the cleft side of the study group compared to the control group, with p-values of 0.022 and 0.036 respectively (Table 3). Additionally, the IFR measurements were significantly lower in the control group compared to the non-cleft sides of the study group (p=0.04) (Table 3).

Table 1: *Correlation coefficients for intra-rater and inter-rater measures.*

Side	PCC for 1st observer	PCC for 2nd observer	ICC for inter-observer
IFR	0.832	0.871	0.825
ZMC	0.858	0.898	0.811
p<0.001 (for all measurements)			

ICC: Intra-class correlation coefficient, PCC: Pearson correlation coefficient

Table 2: *Comparison of the cleft and non-cleft sides of the study group according to ZMC and IFR measurements.*

Side	N	Mean± Std. Dev.	*p*
IFR-Non Cleft	30	33.08±2.59	**<0.001***
IFR-Cleft	30	29.79±2.11	
ZMC-Non Cleft	30	43.59±4.66	**<0.001***
ZMC-Cleft	30	40.54±5.23	

ZMC: zygomaticomaxillary complex, IFR: infraorbital foramen region

Discussion

Children born with cleft lip and palate often exhibit significant facial asymmetry resulting from the congenital deformity. Asymmetrical facial features have been extensively documented in the literature (2,7,8). The objective of this study was to investigate the relationship between the zygomaticomaxillary complex (ZMC) and infraorbital foramen region (IFR) with midface symmetry in patients with non-syndromic unilateral cleft lip and palate (UCLP).

Table 3: *Comparison of the study and control groups according to ZMC and IFR measurements.*

Side	N	Mean± Std. Dev.	*p*
IFR-Non Cleft	30	33.08±2.59	**0.004***
IFR-Control	30	31.16±2.39	
IFR-Cleft	30	29.79±2.11	**0.022***
IFR-Control	30	31.16±2.39	
ZMC-Non Cleft	30	43.59±4.66	0.600
ZMC-Control	30	43.03±3.59	
ZMC-Cleft	30	40.54±5.23	**0.036***
ZMC-Control	30	43.03±3.59	

ZMC: zygomaticomaxillary complex, IFR: infraorbital foramen region

Various methods have been employed to assess facial asymmetry, including direct measurements of anthropometric landmarks, measurements from photographs or video frames, and 3D scans (9,10,11,12-14). Similarly, various methods have been used to evaluate ZMC symmetry (5,15-18). However, the increasing availability of low-dose cone beam computed tomography (CBCT) has provided researchers with a quantitative and three-dimensional tool to assess cleft deformities and asymmetry (14). In this study, the CBCT technique was selected due to its significantly lower radiation dose and high reliability in length measurements (19-21).

Although numerous studies have examined asymmetry in UCLP patients, the evaluation of midface asymmetry remains limited (14). Our study revealed a significantly higher rate of asymmetry in the midface region of UCLP patients. Harikrishnan and Balakumaran (19) developed a 3D model of an UCLP patient's skull using CBCT and observed asymmetry not only in the maxilla but also in the orbital, zygomatic, and frontal bones, consistent with our findings. Agarwal *et al.* (22) described the maxilla and its associated bones as hypoplastic, deformed, and volumetrically reduced in UCLP patients. Since the maxillary bone is interconnected with the orbital region, the hypoplastic and defective maxillary bone may also contribute to insufficiency in the orbital region.

Patel *et al.* (23) reported significant midface asymmetry in the majority of cleft patients, including expansion to the mandible and upper midface (zygoma) in some cases. Choi *et al.* (14) utilized CBCT to compare asymmetries in the midface and dentoalveolar areas and found significant differences only in the nasolabial and dentoalveolar regions when comparing the cleft and non-cleft sides of UCLP patients. Similarly, Bugaighis *et al.* (24) discovered statistically significant differences in the symmetry of all 3D landmarks between UCLP patients and the control group, with the most significant differences observed in the nasolabial region. Another study by Yang *et al.* (25) reported significant differences between the cleft and non-cleft sides primarily around the cleft and nasal chamber, with no significant differences extending to deeper regions of the maxillary complex.

In contrast to these studies, our findings revealed asymmetry extending beyond the nasolabial region. The facial asymmetry observed in UCLP patients is believed to arise from hypoplastic and deformed bones and associated muscles, which not only impact aesthetics but also diminish the patients' quality of life. In addition to addressing functional concerns, enhancing aesthetics can improve the quality of life for individuals with UCLP, who are already disadvantaged due to the deformity.

This study had certain limitations. Firstly, the study did not encompass soft tissues, which may play a crucial role in determining aesthetic facial symmetry. Additionally, the sample size was relatively small. Future studies with larger sample sizes, including soft tissue analysis, should be conducted to provide more comprehensive insights.

Conclusion

This study demonstrates that individuals with UCLP exhibit greater asymmetry in the midface region when compared to the control group. This condition adversely affects facial aesthetics, emphasizing the importance for physicians to address both functional improvement and enhancement of facial aesthetics throughout the stages of treatment for UCLP patients.

Türkçe özet: *Non-Sendromik Unilateral Dudak-Damak Yarığında Orta Yüz Asimetrisi: Retrospektif Bir KIBT Analizi. Amaç: Bu çalışmanın amacı, unilateral dudak damak yarığı (UDDY) olan hastalarda konik ışınlı bilgisayarlı tomografi (KIBT) kullanarak zigomatikomaksiller kompleks (ZMK) ve infraorbital foramen bölgesinin (IFB) yüz simetrisi ile ilişkisini belirlemektir. Gereç ve Yöntem: Bu retrospektif çalışmaya yaş ve cinsiyet açısından uyumlu 30 non-sendromik UDDY'li ve 30 sağlıklı bireyin KIBT görüntüleri dahil edildi. ZMK simetrisi aksiyal kesitte sağ-sol taraflardan değerlendirildi. IFB'deki simetriyi belirlemek için ölçümler koronal kesitte sağ-sol taraflardan yapıldı. İstatistiksel analizde anlamlılık düzeyi p<0,05 olarak belirlendi. Bulgular: Çalışma grupları 12 kadın ve 18 erkek hastadan (yaş aralığı:10-18, ortalama yaş:14.1) oluşmaktadır. ZMK ve IFB ölçümleri çalışma grubunun yarık olan tarafında, hem UDDY'nin yarık olmayan tarafına kıyasla hem de kontrol grubuna kıyasla anlamlı derecede düşüktü [sırasıyla (p<0.001), (p=0.022) ve (p=0.036)]. Ayrıca kontrol grubunun IFB ölçümleri çalışma grubunun yarık olmayan tarafına kıyasla anlamlı derecede düşüktü (p=0,04). Sonuç: Bu çalışma, UDDY'li bireylerin hem ZMK'te hem de IFB'de asimetriye sahip olduğunu göstermektedir. Bu durum yüz estetiği açısından olumsuz bir etki yaratır. Anahtar kelimeler: Yüz asimetrisi; Dudak damak yarığı; Orta yüz simetrisi, estetik, konik ışın*

Ethics Committee Approval: The study protocol was reviewed and was approved by the Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (date: 01/10/2021, meeting no:115, decision no:32).

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

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

Conflict of Interest: The authors declared that they have no conflict of interest.

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Original research

Comparison of the Cameriere's third molar maturity index and Olze et al.'s stages of radiographic visibility of the root pulp in a Turkish population

Kübra Nur Çakan[1]

Purpose

The purpose of this study was to compare the Cameriere's third molar maturity index and Olze et al.'s stages of radiographic visibility of the root pulp in estimating the age of maturity in the Turkish population. The age of majority, which is legally significant, marks the transition from childhood to adulthood. In Turkey, the age of majority is set at 18 years. As the third molars continue to develop at this age, they can serve as an indicator of dental age.

Materials and Methods

A total of 705 panoramic radiographs obtained from individuals aged 15 to 22 years, including children and adults, were included in this study. The left mandibular third molars were evaluated on panoramic radiographs using Cameriere's third molar maturity index and Olze's method of radiographic root pulp visibility (RPV) stages. Minimum and maximum values were noted for each stage, and a median with upper and lower quartiles, as well as mean and standard deviation were calculated. Sensitivity and specificity values were calculated.

Results

In males, Cameriere's third molar maturity index demonstrated a sensitivity of 0.77% and specificity of 0.96%, while in females, it showed a sensitivity of 0.57% and specificity of 0.92%. Regarding Olze et al.'s stage 0, the sensitivity and specificity values were 0.86% and 0.79% in males, and 0.85% and 0.75% in females, respectively.

Conclusion

Although both methods can be used to distinguish individuals below or above the age of 18, the cut-off value suggested by Cameriere's method resulted in a higher rate of type 2 error (false negativity). Therefore, the method proposed by Olze et al., based on the radiographic visibility of the root pulp, can be employed to differentiate between adults and minors in the Turkish population.

Keywords: *Age estimation, radiographic root pulp visibility, legal age, third molar maturity index*

Introduction

Age estimation is a critical process conducted in both deceased and living individuals, with applications in civil and criminal law. It plays a vital role in the identification of deceased individuals during forensic investigations. In the case of living individuals, age estimation is necessary for various purposes such as job applications, school admissions, marriages involving minors, asylum and refugee claims, and cases where there is a lack of legitimate identification or proof of legal age. The significance of individual identification has been escalating, driven by the increasing number of refugees and immigrants globally, including in Turkey (1, 2).

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Determining the age of unidentified asylum seekers holds importance for both the host country and safeguarding the rights of children (3). Proper processing of asylum claims requires accurate determination of whether individuals are of legal age or not (4). The age of majority marks the transition from childhood to adulthood and holds legal significance (5). While the legal age threshold varies across countries, in Turkey, it is set at 18 years (6).

Numerous radiological dental and skeletal age estimation methods have been documented in the literature. The Study Group on Forensic Age Diagnostics (AGFAD) recommends the use of X-rays and physical examination of teeth and the left hand for age estimation in living individuals (7). Tooth development serves as a parameter for age estimation, but it becomes challenging to accurately estimate age once tooth development is complete (8). The third molars, also known as wisdom teeth, emerge last in the jaw and continue to develop until the age of 22 (9, 10). Consequently, these teeth are utilized to predict whether an individual has reached adulthood (11).

In 2008, Cameriere *et al.* developed a practical method for determining adult age using panoramic radiographs. This method evaluates the relationship between the third molar maturity index (I3M) and age by calculating the ratio of apical width to tooth length. The original study reported a cut-off value of 0.08 for determining adulthood (12). The validity of this value has been tested and confirmed in diverse populations (13-16). In 2010, Olze *et al.* introduced a 4-stage classification based on the radiographic visibility of the root pulp in the lower third molars, specifically in a German population (17). Studies conducted in other populations have validated the suitability of these stages as age markers, particularly in populations with legal age thresholds of 18 and 21 (18-20).

Despite an extensive literature review, we were unable to find a study comparing these two age estimation methods in the Turkish population. Therefore, the objective of this study is to compare Cameriere's third molar maturity index and Olze *et al.*'s stages of radiographic visibility of the root pulp in estimating the age of maturity within the Turkish population.

Materials and Methods

Ethical approval

The study protocol received approval from the Ethics Committee for Non-Interventional Clinical Research Studies at the Faculty of Medicine (approval no. 2022/45), and the study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki.

Study Sample

A total of 910 panoramic radiographs were examined, which included both children and adults ranging from 15 to 22 years of age. These individuals had presented to the Oral and Dental Health Hospital between 2019 and 2022. From this sample, 692 radiographs (Group 1: n=335; Group 2: n=357) that met the inclusion criteria were included in the study. Group 1 was utilized to test Cameriere's cut-off value of 0.08, while Group 2 was used to test stage 0 of Olze et al.'s radiographic root pulp visibility. The radiographs were obtained using the Castellini X-Radius Trio Plus 2D device, specifically the Castellini Digital Panoramic System from Bologna, Italy. Standardization was achieved as all radiographs were captured by the same dental technician. The inclusion criteria comprised the presence of the right or left mandibular third molar, good quality radiographs, a known age between 15 and 22 years at the time of radiography, and the absence of systemic diseases. Individuals without a third molar, those with malformed third molars, unknown age, systemic diseases, or bone pathologies impacting skeletal and dental development were excluded from the study. Pertinent information including the date of radiographic image acquisition, date of birth, and sex of each subject were recorded. The chronological age of each subject was determined by subtracting the date of birth from the date the radiograph was obtained.

Measurements

The evaluation of the left mandibular third molars on panoramic radiographs involved the application of both Cameriere's third molar maturity index and Olze's method of radiographic root pulp visibility (RPV) stages. To assess these methods, two separate groups were formed from different samples. While the eruption status of the teeth was not considered in Cameriere's method, the RPV method ensured that the roots of the third molars were fully developed and that the apex was closed. To maintain consistency, maxillary third molars were excluded from the study due to the presence of anatomical structures such as the tuber maxilla and maxillary sinus, which hindered their evaluation. The measurements of the radiographic images were performed by the same investigator using Image J (version 1.50n, National Institutes of Health, Bethesda, MD, USA), an image processing software, on a 15.6-inch LED-backlit screen with a resolution of 1920 x 1080 in a semi-dark room.Cameriere's third molar maturity index.

Digital orthopantomographs (OPGs) were analyzed using the Image J software. Lower left permanent third molars (38) were evaluated. On third molars with open apex, the distance between the inner edges of the apex was recorded as A (A1+A2) and the length of the tooth as L (Figure 1). The third molar maturity index (I_{3M}) was calculated by dividing the A value by the L value (I_{3M}=A/L). In addition, if the root apex of the third molar tooth was complete, then I_{3M} was recorded as 0.0 (12). An I_{3M}<0.08 was considered as cut-off value for discriminating minors from adults, in line with Cameriere *et al.*

Radiographic visibility of the root pulp was assessed as described by Olze et *al*. (17) Digital OPGs were classified using the stages of radiographic root pulp visibility. Left lower third molar (38) were used to evaluate root pulp visibility. When the left third molar was not suitable for evaluation, the right lower third molar (48) was used instead. Root pulp visibility was evaluated in four stages (Figure 2):

- Stage 0: The pulp of each root canal is visible along the entire length of the root;
- Stage 1: The pulp of a root canal is not visible from the apex to more than half of the root;

• Stage 2: The pulp of both root canals is not visible along part of the root, or the pulp of one root canal almost the entire length of the root;

• Stage 3: the pulp of each root canal is not visible over almost the entire length of the roots.

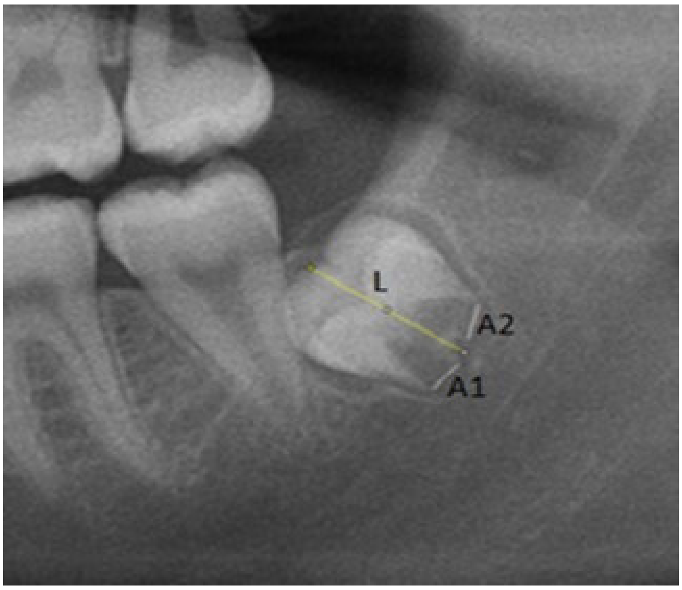

Figure 1. *Example of A1, A2 and L measurements on the third molar (Cameriere's third molar maturity index).*

Figure 2. *Schematic drawings of the radiographic visibility stages of the root pulp of the third molar.*

Statistical analysis

SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Variables including name, date of birth, sex, date of radiograph and chronological age were recorded for each subject. Minimum and maximum values were noted for each stage, and a median with upper and lower quartiles, as well as mean and standard deviation were calculated. Sensitivity and specificity values were calculated. Statistical significance level was set at 5% ($p<0.05$) for all tests.

Results

Age and sex distribution of 692 individuals are shown in Table 1. 335 OPGs were examined to verify the ability of the third molar maturity index to discriminate minors from adults, 357 OPGs to test the performance of Olze *et al.'s* stage 0. Among males, the highest number of subjects was observed among 17-year-olds for both methods. Among females, the highest numbers of subjects were observed in 16-, 18- and 20-year-olds for Cameriere *et al.*'s method, and in 22-year-olds for Olze et al.'s method. Table 2 shows descriptive statistics. The third molar maturity index values decreased with increasing age, showing increases in 17- and 21-year-old females and in the 18-year-old females. The median I_{3M} values in males 19 years of age and older were 0.00, indicating a closed apex. Similarly, the median I_{3M} values were 0.00 in women aged 20 years and older. Standard deviations differed between sexes. When the discriminatory performance of Cameriere's third molar maturity index was analyzed, the method showed a sensitivity of 0.77% and a specificity of 0.96% in males. Among females, sensitivity and specificity were 0.57% and 0.92%, respectively (Table 3). Table 4 shows descriptive statistics of chronological age by RPV stage for both males and females. In females and males, stage 0 was first seen at 16 years of age, stage 1 at 17 and 16 years of age, and stage 2 at 17 and 18 years of age, respectively. Additionally, the mean ages were 17.67 years for stage 0, 19.44 years for stage 1, and 20.41 years for stage 2 among males. The mean ages were 18.15, 19.91 and 20.23 years for stage 0, 1 and 2, respectively. In both sexes, the mean age increased as the stage increased. Stages 0 and 1 occur at a

Table 1: *Age and sex distribution of the entire sample. Numbers in brackets indicate individuals evaluated to confirm the performance of Olze et al.'s root pulp visibility (RPV) stages*

Age (years)	Male	Female	Total
15	17	23	40
16	17 (21)	27 (19)	44 (40)
17	22 (31)	20 (23)	42 (54)
18	13 (27)	27 (19)	40 (46)
19	17 (26)	23 (28)	40 (54)
20	18 (26)	27 (26)	45 (52)
21	20 (26)	23 (30)	43 (56)
22	15 (22)	26 (33)	41 (55)
Total	139 (179)	196 (178)	335 (357

later age in females, while stage 2 occurred at a later age in males. Stage 3 was not observed in both sexes. When the discriminatory performance of the RPV stage 0 to determine whether the individual was over or under 18 years of age was examined, the method showed a sensitivity of 0.86% and a specificity of 0.79% in males. Among females, sensitivity and specificity were 0.85% and 0.78%, respectively (Table 5).

They reported a cut-off value of 0.08, with a specificity of 98% and sensitivity of 70%. However, in your study, the specificity and sensitivity values were lower, with values of 94% and 65%, respectively, compared to Cameriere *et al.*'s findings.

The sensitivity results in your study were 77% in males and 57% in females, which were lower than those reported in some populations such as the Chinese (87% in males, 77% in females), Colombian (91% in males, 95% in females), French (92% in males, 74% in females), Albanian (94% in males, 75% in females), and Australian (90% in both males and females) populations (21-25). However, the sensitivity values in your study were higher than those observed in the Saudi population (52.3% in males, 51.3% in females) (26). When comparing with Sharma et al.'s study in the Indian population, your study showed lower sensitivity in females but higher sensitivity in males (66% in females, 74.7% in males) (27).

On the other hand, the specificity values in your study were higher, with values of 96% in males and 92% in females, compared to other populations such as the French (88% in both males and females), Australian (85% in males, 87% in females), and Indian (83% in males, 79% in females) populations (23, 25, 27). However, the specificity of the female population in your study was lower than that reported in the Chinese (98%), Colombian (93%), Albanian (96%), and Saudi (97%) female populations (21, 22, 24, 26).

In a previous study on the Turkish population, which examined panoramic radiographs of 293 subjects, the speci-

Table 2: *Summary statistics of third molar maturity index in females and males by ages*

	Males						Females					
Age (years)	**N**	**Mean**	**SD**	**Min**	**Med**	**Max**	**N**	**Mean**	**SD**	**Min**	**Med**	**Max**
15	17	0.55	0.31	0.19	0.43	1.96	23	0.70	0.29	0.26	0.68	1.29
16	17	0.25	0.19	0.09	0.19	0.86	27	0.41	0.31	0.00	0.27	1.38
17	22	**0.35**	0.28	0.00	0.25	0.93	20	0.18	0.16	0.00	0.17	0.72
18	13	0.11	0.2	0.00	0.05	0.75	27	0.28	0.25	0.00	0.21	0.97
19	17	0.04	0.06	0.00	0.00	0.18	23	0.16	0.21	0.00	0.10	0.97
20	18	0.02	0.05	0.00	0.00	0.2	27	0.06	0.10	0.00	0.00	0.42
21	20	**0.05**	0.1	0.00	0.00	0.42	23	0.04	0.06	0.00	0.00	0.21
22	15	0.00	0.00	0.00	0.00	0.00	26	0.01	0.02	0.00	0.00	0.09

Table 3: *Contingency table explaining the discriminatory performance of the test in females and males ([a] true positive [b] false positive [c] false negative [d] true negative)*

Sex		**Age (years)**		
		≥18	**<18**	**Total**
Male	I_{3M}<0.08	64[a]	2[b]	66
	I_{3M}≥0.08	19[c]	54[d]	73
	Total	83	56	139
Female	I_{3M}<0.08	72[a]	5[b]	77
	I_{3M}≥0.08	54[c]	65[d]	119
	Total	126	70	196

Table 5: *Contingency table explaining the discriminatory performance of the test in females and males ([a] true positive [b] false positive [c] false negative [d] true negative).*

Sex		**Age (years)**		
		<18	**≥18**	**Total**
Male	Stage 0	45[a]	26[b]	71
	> Stage 0	7[c]	101[d]	108
	Total	52	127	179
Female	Stage 0	36[a]	29[b]	65
	> Stage 0	6[c]	107[d]	113
	Total	42	136	178

Table 4: *Summary statistics of chronological age by root pulp visibility stages in females and males*

Sex	**Stage**	**N**	**Min**	**Max**	**Mean**	**SD**
Females	0	65	16	22	18.15	2.08
	1	67	17	22	19.91	1.58
	2	46	17	22	20.23	1.55
Males	0	71	16	22	17.67	1.59
	1	69	16	22	19.44	1.71
	2	39	18	22	20.41	1.40

Discussion

Many methods have been developed for age estimation based on tooth development. The study conducted by Cameriere *et al.* (12) in 2008 introduced a method for age estimation based on the relationship between the third molar maturity index (I3M) and age on panoramic radiographs.

ficity values were 100% in both males and females, and the sensitivity values were 94% in males and 85% in females, which were higher than the values observed in your study (28). It's important to consider that these differences in results may be related to genetic variations both within and across populations (29).

Using a cut-off value of 0.08 can successfully identify individuals under the age of 18 from those aged 18 or older in both males and females. However, it may lead to more false negatives in females due to the slower development of third molars in women (29). The delay in root development of third molars may result in lower sensitivity in females. Gender differences may contribute to variations in study results. Therefore, even with the same cut-off value, women over 18 years of age with incomplete root development may be misclassified as children. Using a different cut-off value could enhance the discriminatory power of the Cameriere method in females (11).

In our study, when the discriminative power of Olze *et al.*'s RPV stage 0 was tested, the specificity and sensitivity values in females were 78% and 85%, respectively. In males, the corresponding figures were 79% and 86%, respectively. High sensitivity coupled with low specificity values showed that the test itself was better in distinguishing individuals over 18 years of age. In their original study in 2010 involving 1198 individuals, Olze *et al.* (17) reported that stage 0 was first seen in females and males at the ages of 17.2 and 17.6 years, stage 1 at the ages of 21.6 and 22.4 years, and stage 2 at the ages of 24.7 and 22.3 years, respectively. In our study, stage 0 was first seen in females and males at 16 years of age, stage 1 at 17 and 16 years of age, and stage 2 at 17 and 18 years of age, respectively. In contrast to the study by Olze *et al.,* all stages were observed at younger ages in the current study. This may be an indication of closure of root apices at an earlier age in both women and men in the Turkish population. Differential results between the studies can be attributed to the differences in the age range of individuals and characteristics of the populations tested.

In a study by Akkaya *et al.* (2) in 463 individuals aged from 16 to 34 years, stage 0 first occurred at the ages of 16.43 and 16.61 years, stage 1 at 16.93 and 17.91 years, and stage 2 at 18.14 and 18.13 years in women and men, respectively. Our findings are in line with those reported in that study. In 2015, Perez-Mongiovi *et al.* (18) conducted a study on 487 individuals between the ages of 17 and 30. In their study, stage 0 was first observed at the ages of 17 and 18.2, stage 1 at the ages of 17.4 and 18.4, and stage 2 at 18.1 and 18.8 years of age, in females and males, respectively. Lucas *et al.* (19) reported similar results in their study of 100 individuals aged between 16 and 26 years. This study reported minimum values that were similar to ours. However, there are also studies which showed higher minimum values (17, 20, 30).Perez-Mongiovi *et al.* (18) also estimated the discriminatory power of the test in their study and reported sensitivity results of 79.9% and 80.7% in males and females, respectively. However, the specificity values were 27% in males and 19.6% in females. In our study, both specificity and sensitivity values were higher than those reported by Perez-Mongiovi *et al.*

In a 2019 study by Kumar *et al.* (31), the sensitivity and specificity values of the two techniques were compared in 615 individuals aged between 15 and 22 years. In their study, the cut-off value of 0.08 showed 67% sensitivity and 76% specificity in females. However, Olze *et al.*'s stage 0 showed 72% sensitivity and 91% specificity. In males, the sensitivity and specificity values were 76% and 72% respectively using the cuf-off value of 0.08 and 68% and 86% respectively using Olze et al.'s stage 0. In comparison to Kumar et al.'s study, the cut-off value of 0.08 showed higher specificity and Olze *et al.*'s RPV stage 0 demonstrated higher sensitivity in both females and males in our study.

In a study conducted on 429 third molars, Günacar *et al.* (32) compared the use of the stages of radiographic root pulp visibility in age estimation using OPG and cone beam computed tomography (CBCT). In this study, individuals in the CBCT group had stage 1 and stage 2, while stage 1, 2 and stage 3 were not found in individuals in the OPG group under the age of 18. For OPG in this study, stage 0 first occurred at the ages of 16 years in both genders, stage 1 at 20 and 24 years, stage 2 at 25 and 27 years and stage 3 at 33 and 25 years in women and men, respectively (32). In our study, only the stage 0 age value was similar to this study, the other stages were observed at younger ages. Stage 3 was not present in our study. This may be because stage 3 is seen in advanced ages. Additionally, Günaçar *et al.* (32) recommended the use of CBCT for RPV evaluation. This may be due to the 2D nature of OPG, geometric distortion, anatomical noise and superposition of oral structures. RPV evaluation may be incorrect due to the superposition of the external oblique ridge in the third molar region on the pulp tissue in 2D imaging.

When the two methods were compared in our study, Cameriere's method (I_{3M} <0.08) showed lower sensitivity in females and males compared to Olze *et al.*'s stage 0. Moreover, Olze *et al.*'s stage 0 showed higher specificity in for both sexes than the cut-off value of Cameriere. It is important to decide whether an individual is a minor or adult in order to hold them accountable for their actions and to protect the rights of children (15). Incorrectly identifying a minor as an adult or identifying an adult as a minor will result in the individual not being properly punished (33). For this reason, age estimation methods should be simple and minimize errors (34, 35).

In this study, a cut-off value of 0.08 by Cameriere *et al.* successfully discriminated individuals under 18 years of age; however, it should be noted that it causes a high rate of false negatives. Olze et al.'s stage 0, on the other hand, is successful in distinguishing individuals over the age of 18 but may cause a high rate of false positives.

Conclusion

There was no significant difference observed in the sensitivity and specificity results between Cameriere's and Olze's methods. Both methods can effectively differentiate individuals below or above the age of 18. However, Cameriere's method, with its proposed cut-off value, showed a higher rate of type 2 error (false negativity). Despite this, it is considered more acceptable. Therefore, the method proposed by Olze *et al*., based on the radiographic visibility of the root pulp, can be employed to differentiate between adults and minors in the Turkish population. It is recommended to further validate this method through studies with larger sample sizes. Additionally, considering the limitations of the two-dimensional imaging technique (OPG), the option of employing cone-beam computed tomography (CBCT) should be considered to enhance the accuracy of these methods.

Türkçe özet: *Cameriere'nin üçüncü molar olgunluk indeksi ile Olze ve ark.'nın kök pulpasının radyografik görünürlük aşamalarının Türk popülasyonunda karşılaştırılması. Amaç: Reşit olma yaşı, bireyin çocukluktan yetişkinliğe geçiş yaşı olup, hukuki öneme sahiptir. Türkiye'de reşit olma yaşı 18'dir. Yasal yetişkinlik yaşı olan 18 yaşında halen gelişmekte olan üçüncü azı dişleri diş yaşını tahmin etmek için kullanılabilir. Bu çalışmanın amacı, Türk popülasyonunda olgunluk yaşını tahmin etmek için Cameriere'nin üçüncü molar olgunluk indeksini ve Olze ve ark.'nın kök pulpunun radyografik görünürlük aşamalarını karşılaştırmaktı. Gereç ve Yöntem: Bu çalışma, 15-22 yaş arası çocuk ve yetişkin hastalardan alınan 705 panoramik radyografi üzerinde yapılmıştır. Bulgular: Cameriere üçüncü molar olgunluk indeksi erkeklerde %0,77 duyarlılık ve %0,96 özgüllük, kadınlarda %0,57 duyarlılık ve %0,92 özgüllük gösterdi. Olze ve ark.'nın evre 0'ının duyarlılık ve özgüllük sonuçları erkeklerde sırasıyla %0,86 ve %0,79, kadınlarda %0,85 ve %0,75 olarak bulundu. Sonuç: Her iki yöntem de 18 yaş altı ve üstü bireyleri ayırt etmek için uygulanabilse de, Cameriere tarafından önerilen cut-off değeri daha yüksek oranda tip 2 hata (yanlış negatiflik) üretmiştir. Bu nedenle, Olze ve ark.'nın kök pulpasının radyografik görünürlülüğü yöntemi, Türk popülasyonunda yetişkinler ve küçükler arasında ayrım yapmak için kullanılabilir. Anahtar Kelimeler: yaş tahmini, radyografik kök pulpası görünürlülüğü, yasal yaş, üçüncü molar olgunluk indeksi*

Ethics Committee Approval: The study protocol was approved by the Ethics Committee for Non-Interventional Clinical Research Studies at Faculty of Medicine (approval no. 2022/45).

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

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

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Original research

Stress distribution of four implant supported overdentures with tilted standard-sized implants and mini implants*

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Purpose

The goal of the current study is to evaluate the stress distribution when tilted implants and mini-implants are used to support a mandibular overdenture.

Materials and Methods

Three-dimensional (3D) finite element models of mandibular overdentures were established using four, axial, standard-sized implants (SA model), four standard-sized implants with the mesial ones axial and the distal ones tilted (ST model) and four mini-implants (MA model) with Locator attachments. On each model, a 100 N load was applied to the overdenture in four different directions; bilateral vertical, unilateral vertical and oblique load on the posterior region, and a vertical load on the incisors. The stresses distributed at the peri-implant bone, implants, the prosthetic components, and the overdentures were evaluated.

Results

Non-axial posterior loading caused higher stress values in the implant and the prosthetic component than axial posterior loading. Lower stress values of the implant and the prosthetic component were observed in the ST model than SA model. The stress distribution in the overdenture at posterior loads were mostly observed around the implants.

Conclusion

Less prosthetic complications may be expected when the treatment option in the ST model is used. Fatigue fractures may occur around the implants in the overdentures, precautions are advised.

Keywords: *Tilted implants, mini-implants, finite element analysis, locator, overdenture*

Introduction

The conventional rehabilitation of edentulism was used to be complete dentures. Their difficulty of use, low patient satisfaction, and reported high success of dental implants, resulted in declaration of McGill Consensus in 2002. According to the McGill consensus (1), the first treatment option to suggest to patients with edentulous mandible is complete dentures supported with two implants. Number of implants can be increased to three or four in order to create an angular relationship and prevent the movement of the denture towards the soft tissues (2). Overdentures supported by three or four implants are recommended when increased retention is needed in situations such as high muscle attachment or prominent mylohyoid ridges (3).

Rehabilitation with an implant-supported fixed restoration is primarily preferred to a rehabilitation with an overdenture if possible. It is stated that edentulous mandible can be rehabilitated with a fixed restoration by four implants in the interforaminal region, which tends to have substantive residual alveolar bone when the rest has been resorbed, with imme-

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diate loading when tilted but long implants are used distally (4,5). But unfortunately, fixed rehabilitation does not always meet the needs of the patient. Sometimes an implant-supported overdenture can be preferred because of its lower cost or to replace other lost tissues as well as teeth to obtain labial and buccal support to achieve esthetics. Using distally tilted implants instead of axial implants to support mandibular overdentures with non-splinted, single attachments has commercially came up with as they are used to support fixed prosthesis with the advantage of reduced cantilever length (6). But any study about this treatment concept has not been published yet therefore it lacks evidence.

Mini implants have been used for several years in partially edentulous situations when the interdental space is inadequate or adjacent teeth roots are convergent limiting the implant area. They are also preferred in edentulous situations when the crest is narrow and atrophic without any bone augmentation procedures (7). But there is limited number of studies about the stress of bone or prosthetic components generated by mini-implant supported overdentures and the application is not thoroughly justified yet (8).

The goal of the current study is to evaluate the stress distribution when locator attachments are used on tilted, interforaminal, standard-sized implants and axial mini-implants by using a finite element analysis (FEA). The influences of bilateral vertical loading, unilateral vertical loading, unilateral oblique loading and loading from the incisal site are investigated.

Materials and Methods

An edentulous mandible was modeled according to the tomography examination file of a patient who already had computed tomography examination from the database of the clinic of Bezmialem Vakıf University (Planmeca ProMax 3D Mid, Planmeca OY, Helsinki, Finland). 1 mm cross-sections were recorded in Digital Imaging and Communications in Medicine (DICOM) format. This file was opened with 3D Slicer software (9,10). The mandible was segmented by manual and automatic methods and the mesh-cleaning of the Standard Triangle Language (STL) data was carried out with MeshLab software (11).

The implants (Bluesky implants and miniSKY implants, Bredent medical GmbH & Co.KG, Senden, Germany) and the locator attachments (SKY locator, SKY locator angled, miniSKY locator; Bredent medical GmbH & Co.KG, Senden, Germany) used in the models were modeled according to the STL files received from the manufacturer. A mandibular overdenture was then designed with a cusp angle of 30°.

Three different models were constructed using the structures mentioned above only with the differences in implant type and implant inclination. All models included four implants with locator attachments to support the overdenture. The implants of all models were positioned in the interforaminal region with their apical end 13.5 mm away from each other. In the first model (SA model: Model with standard, axial implants) the implants having a length of 12 mm and diameter of 4.1 mm were positioned vertically to the occlusal plane. In the second model (ST model: Model with standard, tilted implants), the mesial implants having the same dimensions as model 1 were positioned vertically to the occlusal plane and the distal implants with a length of 16 mm and a diameter of 4.1 mm were tilted 35° distally elongating inter-implant distance. The distance between the mesial implants was 13.5 mm as in other models and the distance between the mesial and distal implants 17.5 mm in the crestal region. In third model (MA model: Model with mini, axial implants), four mini-implants having a length of 12 mm and diameter of 2.8 mm were positioned vertically to the occlusal plane to support the overdenture 13.5 mm away from each other (Figure 1).

The three-dimensional geometries of the mandible, implants and locator attachments were modeled and meshed in ANSYS Revision 14.5 software (ANSYS Revision 14.5, Canonsburg, Pennsylvania, USA). The meshing was performed with 3D four-node tetrahedron elements. The total number of elements and nodes are given in Table 1.

The edentulous mandible consisted of a constant 2 mm thick cortical bone covering the trabecular bone, covered by the mucosa with a thickness of 2 mm. The locator attachment consisted of three parts; the abutment on the implant, nylon replacement and the titanium cap in the denture. The implant, abutment and the cap were made of Ti-6Al-4V titanium alloy. All material properties were obtained from the literature (Table 2) and all materials were considered to be isotropic, homogeneous and linearly elastic. A total implant-bone osseointegration was assumed so a mechanical-

Figure 1. *The 3D FE models of the mandible and the prosthetic components: The SA model is four axial, standard-sized implants model; the ST model is four standard-sized implants model with the mesial ones axial and the distal ones tilted and the MA model is four mini- implants model.*

Table 1: *The total number of elements and nodes.*

	SA model	ST model	MA model
Node number	844831	630526	1082712
Element number	552145	408912	751121

Table 2: *The material properties used in the study.*

	Young's modulus (MPa)	Poisson's ratio	Reference
Ti-6Al-4V	103400	0.35	Sertgöz and Güvener (12)
Cortical bone	13700	0.3	Barbier *et al.* (13)
Cancellous bone	1370	0.3	Barbier *et al.* (13)
Overdenture	4500	0.35	Brunski *et al.* (14)
Mucosa	1	0.37	Menicucci *et al.* (15)
Nylon	28.3	0.4	Liu *et al.* (16)

ly perfect interface between the two structures existed in the models letting no movement to occur during analysis. To reproduce the clinical situation, contact was applied at the overdenture–mucosa interface and between parts of the attachments. The analysis was carried out with the FEA software ANSYS Revision 14.5. Von Misses equivalent stresses was calculated for all components.

Temporomandibular joints and masticatory muscles of temporal, masseter, medial pterygoid and lateral pterygoid were integrated in the models. The temporal muscle attached to the coronoid process of the mandible, the masseter muscle attached to the angle and lower half of the lateral surface of the mandible ramus, the medial pterygoid attached to the lower and back portion of the medial surface of the ramus and mandible angle, and the lateral pterygoid attached to the neck of the mandible condyle (Figure 2). Each muscular area was defined with 10 nodes of the elements. The temporomandibular joints were fixed in the upper parts of the condyles resulting in a rigid contact of the condyles and the glenoid fossae. Movements of rotation and translation were not allowed as in a previous study (17).

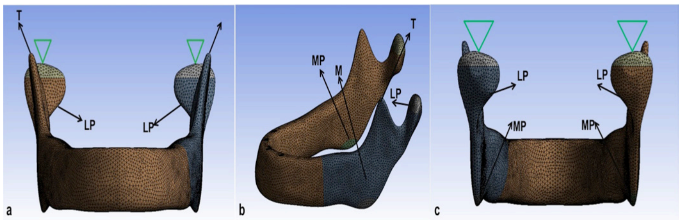

Figure 2. *The boundary conditions of the models. Arrows indicate the direction of the forces applied by the muscles (T: temporal; LP: lateral pterygoid; MP: medial pterygoid; M: masseter). The green triangles show the temporomandibular joint fixations. a- frontal view, b- lateral view, c-lingual view.*

The stress distribution of the related structures was evaluated at four different loading conditions that happen during different phases of chewing and biting. The loading conditions were; 1- The denture was loaded with vertical loads bilaterally on the first molar teeth region (BV load: bilateral vertical load). 2- The denture was loaded with a vertical load unilaterally on the first molar tooth region (UV load: unilateral vertical load). 3- The denture was loaded unilaterally at the posterior region. The direction of the load had an angle of 30° long axis of the teeth to simulate the early phase of mastication when the bolus is placed on the working side and there is no contact on the nonworking side (17) (UO load: unilateral oblique load). 4-The denture was loaded on the incisal surfaces of the incisal teeth as in the act of biting (Figure 3) (AV load: anterior vertical load). A recent systematic review has declared that mean maximum bite forces of implant supported overdentures from various studies ranged between 78.5 and 132.01 N (18). The total static load of 100 N was used at all conditions because it was in the declared range and 100 N was used in the literature of similar studies (5,17,19-21). The load was applied on the center of the occlusal surface in BV, UV and UO loading conditions.

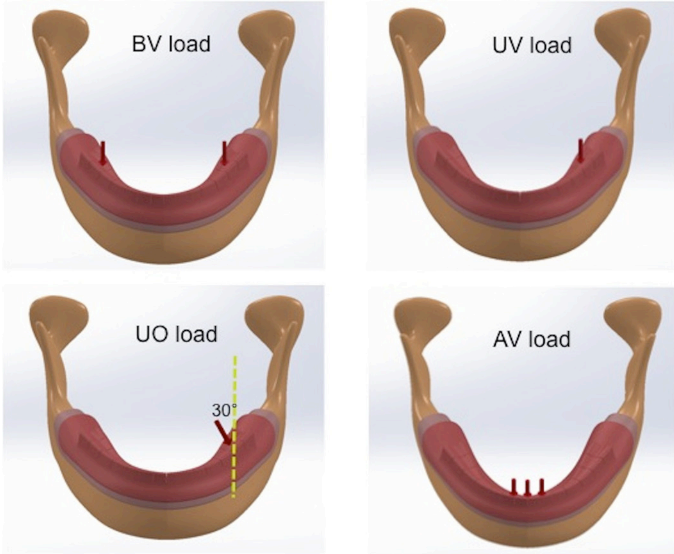

Figure 3. *The application of the masticatory force of 100 N at various places and with different inclinations. All four loading conditions are shown with their abbreviations.*

Results

Stress distribution in the peri-implant bone

The highest stress values of all structures are shown on Figure 4. The stress distribution of periimplant bone can be seen in Figure 5. Higher stress values were observed in the cortical bone than the trabecular bone. UO load and AV load caused higher stress levels (Fig 4).

The highest stress value of the cortical bone was observed when the ST model was loaded with UO load (31.29 MPa) followed by ST model loaded with AV load (28.08 MPa) (Figure 4 and 5). The lowest stress value of the cortical bone was observed when SA model was loaded with BV load followed by MA model and ST model loaded with BV load (5.06 MPa and 5.11 MPa respectively). When ST model was loaded with BV load and UV load, its highest stress values were around the mesial implants, differing from the other models having their highest stress values around distal implants.

Stress distributions on the implants and the prosthetic components

The stress levels of the implants and the prosthetic components and their distributions can be seen in Figure 4 and

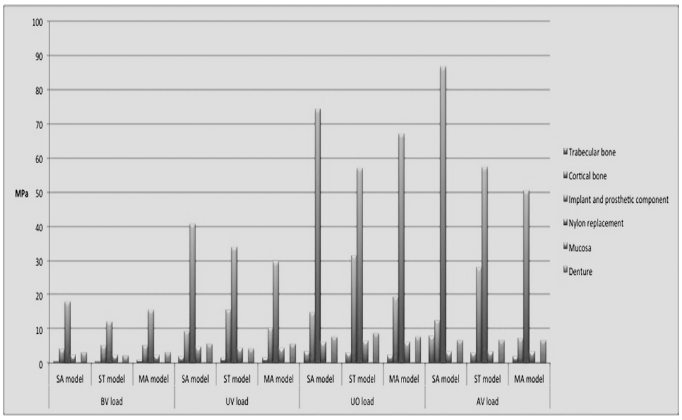

Figure 4. *The maximum stress levels of the structure of the models at different loading conditions.*

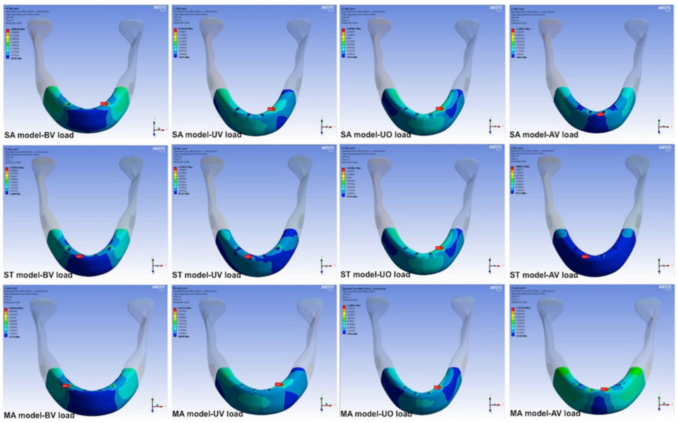

Figure 5. *The stress distribution of cortical bone of SA model, ST model and the MA model when loaded with BV load, UV load, UO load and AV load.*

6. The implant and the locator component displayed the highest stress values among other structures for all models at all loading conditions (Figure 4). The highest stress level was in SA model loaded with AV load (86,61 MPa). The SA model had higher stress levels for all loading conditions. According to the stress distribution, the stress areas were wider on the distal implants when the models were loaded posteriorly although the highest stress of the models was sometimes observed on mesial implants of the loaded side. Only ST model loaded with UV and UO load was an exception, having highest stress levels on the mesial implant of the unloaded side (Figure 6).

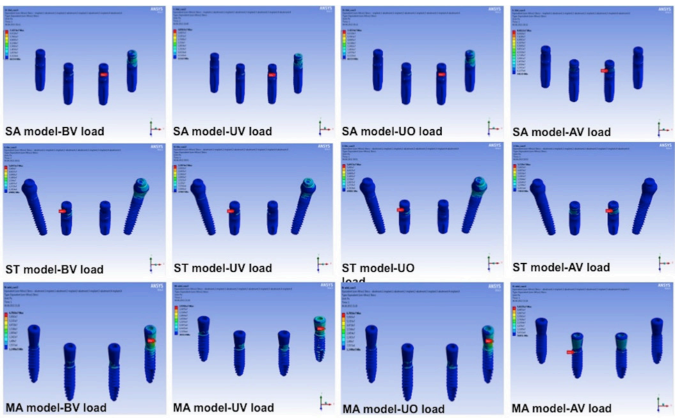

Figure 6. *The stress distributions of the implants and the prosthetic components of SA model, ST model and MA model when loaded with BV load, UV load, UO load and AV load.*

Stress distribution in the mucosa, nylon replacements and overdenture

Stress levels in the mucosa was quite low in all models for all loading conditions; it did not exceed 0.25 MPa (Figure 4). Any remarkable difference between the high stress values of nylon replacements was not observed between the three models for all loading conditions. The stress values differed in the range of 2.31 MPa (under BV load) and 6.58 MPa (under UO load) (Figure 4). There was not any remarkable difference between stress distribution of overdentures of the models at all loading conditions. Higher stresses developed when ST model was loaded with UO load (8.6 MPa) (Figure 4). The stress of the overdenture under posterior loads were mostly observed around the implants, not at the loading positions (Figure 7).

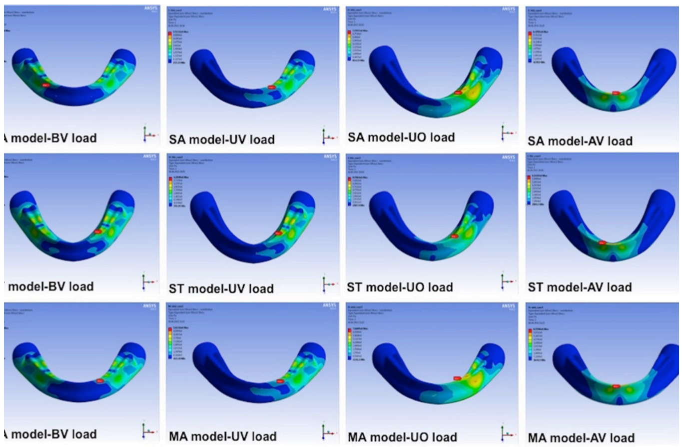

Figure 7. *The stress distributions of the overdenture of the SA model, ST model and MA model when loaded with BV load, UV load, UO load and MI load.*

Discussion

Overload is explained as forces exceeding the mechanical or biological capacity of load bearing of the structures as the bone, oral implants or the prosthesis causing mechanical failure or loss of osseointegration (22). The stress generated from overloading can be related to several consequences in the peri-implant bone, implant and the prosthetic component, mucosa, nylon replacement of a locator attachment and the overdenture. In the peri-implant bone, stress is related to implant's longevity and support (5). When implant is loaded, stress is transferred to its first material contact; peri-implant cortical bone; explaining the marginal bone loss (22). Naert *et al.* (23) has shown the correlation between marginal bone loss and high occlusal stress on the implants. This study has evaluated von Misses equivalent stresses which is applicable to ductile materials such as implants, prosthetic components and overdentures. Because of its non-ductile feature, bone should be evaluated with principal stress. However, the stress distribution in the bone was not totally excluded, but its limitations should be borne in mind. The results of the stress distribution of the bone are approximate.

The SA model in the study is overdenture supported by four interforaminal implants; a treatment modality that has been investigated and pleasing results were obtained (24). This accepted treatment modality is compared with overdentures supported by four standard-sized implants with distally tilted ones (ST model) and by four mini-implants (MA model). The ST model also displayed higher stress concentrations than SA model and MA model in other loading conditions (Figure 4). This finding probably depends on that in the ST model the overdenture is more implant-supported than the other models. Liu *et al.* (16) has suggested that as the number of implants increase, strain in the peri-implant bone also increases because more of the chewing force is shared by the implants. In the current study it can be claimed that the locator attachments on the distal implants were more distal than the models with axial implants (SA and MA); so, the mucosa-supported part of the overdenture was shorter for the ST model (Figure 1). That caused the mucosa support of the overdenture decrease and the implant support of the overdenture increase and more of the load to be borne by the implants than SA and MA models. Takahashi *et al.* (25) has stated that with All-on-4 concept the stress in the cortical bone around the implants decreased. The differences in these findings can be attributed to different prosthodontic applications The prosthesis evaluated in the aforementioned study was fixed and only implant-supported, while the prosthesis evaluated in the current study is an overdenture utilizing mucosa support in addition to implant support. Therefore, the part of the overdenture on the distal side of the implants cannot be considered as an actual cantilever. According to this result, it can be presumed that patient satisfaction for this treatment option can be higher than the other options due to more implant support than SA and MA models. The authors suggest further investigation of this assumption for future studies.

When the unilateral posterior loading conditions are compared, non-axial oblique UO load caused higher stress values in the denture and the implant and the prosthetic component than UV and BV load (Figure 4). This finding is in accordance with other studies stating that non-axial loading is more detrimental than axial loading for oral implants (26,27). For implant-supported overdentures, compressive forces are best to maintain the implant integrity, but shear and tensile forces tend to distract or disrupt the implant-bone interface (28). These harmful non-axial forces can also affect the denture, implants and prosthetic components. However, evaluating oblique forces on dental implants is stated to be more realistic than axial loading in stress analyzing studies of dental implants because function generates non-axial forces. It is also emphasized that excessive horizontal loading should be avoided for implant-supported restorations (29). In order to decrease the non-axial forces, the authors would like to advise using lingualized occlusion as the occlusal scheme of the implant-supported overdentures as it is also advised in the literature (28,30). In lingualized occlusion, the lingual cusp of the maxillary posterior teeth glides in the shallow central fossae of the mandibular posterior teeth providing buccolingual stability and thus diminishing lateral forces[24]. Besides its biomechanical advantages in implant-supported overdentures, it is indicated that masticatory performance and patient satisfaction increases with lingualized occlusion where the occlusal wear over time decreases (31,32).

The lower stress value of the implant and the prosthetic component assembly is remarkable in the ST model under all loading conditions when compared with the SA model (Figure 4). The more implant-borne denture in the ST model may have led to higher stress in the cortical bone but the smaller distal extension length has probably led to a smaller lever arm thus less stress in the implant and the prosthetic component. This finding can be interpreted as less complication related to the implant and the prosthetic component, such as screw loosening, can be expected when the overdenture is supported with four standard-sized implants including distally tilted ones. But this assumption should also be supported by further clinical studies.

The stress concentration on the mesial implants and prosthetic components of the unloaded side observed in the ST model under UV and UO loads are also probably due to varying lever formations (Figure 6). Higher stress levels in the implant and the prosthetic component may be related to complications of the unloaded side within this assembly. Using both sides during chewing can be advised to the patient to eliminate this complication risk. Or repeated prosthetic complications can lead the clinician think that the patient is using the non-damaged side of the overdenture while chewing when this treatment option is used. However, these assumptions should be verified with clinical studies.

According to the findings of the study, the nylon replacements have similar stress levels for all three models at all loading conditions (Figure 4). It can be presumed that deterioration of the nylon replacement will not differ between SA, ST and MA models; and there will not be a more frequent need to change the nylon replacements for any of the treatment modalities. There is a need for clinical studies to verify this finding.

No remarkable stress values were observed in the mucosa in any models at any loading conditions (Figure 4). This finding is probably due to less mucosa support of the four-implant supported overdentures when compared with overdentures supported with a fewer number of implants such as one or two (16).

The stresses distributed in the overdentures at loads exerted at the posterior regions were mostly observed around the implants; so, it can be predicted that denture fractures may occur around the implants (Figure 7). Although locator attachments have a low profile among other single attachments, the acrylic resin is still thin because of the bulk of the attachment and prone to fracture (33). Reinforcing the acrylic resin can be considered at the regions around the implants when these treatment modalities are applied.

There are several limitations for this study. The material properties about the biological tissues are assumed to be constant where it is quite variable from one individual to another or even within the same individual. The transversely isotropic and inhomogeneous cortical bone is taken as homogeneous, isotropic and linearly elastic (16). Peri-implant tissues are complex and their simulation with this method is an approximation (34). Also, a complete osseointegration was assumed in the models and this assumption may not be reflecting the clinical situation with varying osseointegration percentages from 30% to 70% (35). So, comparing the stress concentration areas and relative values of different treatment modalities are aimed in FEA studies instead of reporting the absolute values of stress (36). Von Misses stresses were evaluated in the current study although it is not applicable for non-ductile bone tissue. It is appropriate only for ductile materials such as the implants, prosthetic components, nylon replacement and the overdenture yet the bone was not excluded completely. The stress values observed in the study do not exceed the strength of the materials but fatigue analysis could not be carried out. So conclusions about fatigue were only assumptions. Only one mandible was evaluated but different mandibles could have variations in the stress distribution and a statistical analysis could not be carried out. To reproduce the clinical situation, contact was applied at the overdenture–mucosa interface and between parts of the attachments but any friction coefficient was not applied which is another limitation for the study.

Conclusion

Non-axial posterior loading cause higher stress values in the implant and the prosthetic component than axial posterior loading in the overdenture treatments supported by four standard-sized axial implants (SA model), by four standard-sized implants with distal ones tilted (ST model) and by four mini axial implants (MA model). Lower stress values of the implant and the prosthetic component were observed in the overdenture treatments supported by four standard-sized implants with distal ones tilted (ST model) than overdenture treatments supported by four standard-sized axial implants (SA model) under bilateral vertical (BV), unilateral vertical (UV), unilateral oblique (UO) and anterior vertical (AV) loading conditions. Stress distribution of the overdentures was observed around the implants for all three treatment options (overdenture treatments supported by four standard-sized axial implants (SA model], by four standard-sized implants with distal ones tilted [ST model] and by four mini axial implants [MA model]) under posterior loading conditions (bilateral vertical [BV], unilateral vertical [UV], unilateral oblique [UO] loading).

Türkçe özet: *Açılı standard implant ve mini implant destekli tam protezlerde gerilimin değerlendirilmesi Amaç: Bu çalışmanın amacı mandibular implant üstü tam protezleri desteklemek için açılı implantlar ve mini implantlar kullanıldığındandaki gerilim dağılımını değerlendirmektir. Gereç ve Yöntem: Dört adet, düz, standart büyüklükte implanta sahip (SA model); dört adet, mezialdekiler düz ve distaldekiler açılı, standart büyüklükte implanta sahip (ST model) ve dört adet, düz mini implanta sahip (MA model) Locator tutuculu implant üstü mandibular tam protezlerin üç boyutlu (3D) sonlu eleman modelleri oluşturuldu. Her modelde implant üstü tam proteze bilateral dik, unilateral dik, unilateral oblik ve kesicilerden dik olacak şekilde dört farklı yönde 100 N kuvvet uygulandı. Peri-implant kemikte, implantta, protetik komponentlerde ve protezde dağılan gerilimler değerlendirilmiştir. Bulgular: Dik olmayan posterior yükleme, dik yüklemeye göre implantta ve protetik komponenette daha yüksek stress değerlerine sebep olmuştur. İmplant ve protetik komponentte ST modelde SA modele göre daha düşük gerilim değerleri gözlenmiştir. Posterior yüklemelerde implant üstü tam protezde gerilim dağılımı implantların etrafında daha yoğun gözlenmiştir. Sonuç: ST modeldeki tedavi seçeneğinin kullanıldığı durumlarda daha az protetik komplikasyon beklenebilir. İmplant üstü tam protezlerde implantların etrafında yorgunluğa bağlı kırılmalar meydana gelebilir. Anahtar kelimeler: Açılı implantlar, mini implantlar, sonlu elemanlar analizi, locator, implant üstü tam protez.*

Ethics Committee Approval: Not required.

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Original research

Presence of candida in the dental plaque and saliva of patients with severe early childhood caries and early childhood caries: a pilot study

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Purpose
The aim of this study is to evaluate the presence of candida, which is one of the etiological factors contributing to early childhood caries (ECC) and severe early childhood caries (S-ECC), in the dental plaque and saliva of children aged 6 years and younger.

Materials and Methods
Our study involved 60 participants who met the inclusion criteria. Based on clinical examinations, we divided them into three groups, each consisting of 20 children: S-ECC, ECC, and caries-free groups. We collected dental plaque and saliva samples from the children during clinic visits. In the laboratory, we assessed these samples for the presence of candida using the Liofilchem® – ChromaticTM Candida (Roseto degli Abruzzi, Italy) medium and identified Candida species.

Results
The presence of Candida in the saliva of children with S-ECC (40%) and ECC (30%) was statistically significant compared to children without caries ($p<0.05$). Observationally, we found a higher presence of candida only in the dental plaque of children with S-ECC (25%) and ECC (15%) compared to children without caries ($p>0.05$). In the S-ECC group, we detected *Candida albicans, Candida glabrata, Candida krusei,* and *Candida tropicalis in saliva,* while *Candida albicans* was found in dental plaque. In the ECC group, *Candida albicans, Candida glabrata,* and *Candida krusei* were detected, whereas *Candida* was not detected in children without caries.

Conclusion
It is important to consider the presence of *Candida* in both saliva and dental plaque, as it potentially plays a role in the pathogenesis of ECC. These findings suggest that identifying and preventing *Candida* colonization may be valuable for individual risk assessment and could contribute to reducing ECC.

Keywords: *Candida, dental plaque, caries, saliva*

Introduction

ECC is a global public health concern (1). Despite preventive strategies aimed at reducing tooth decay, over 600 million children are affected by ECC (1, 2). Early childhood caries (ECC) is defined as the presence of one or more decayed (non-cavitated or cavitated lesions), missing, or filled (due to caries) surfaces in any primary tooth of a child younger than six years old (1). Any sign of smooth surface caries in children younger than three years old indicates severe early childhood caries (S-ECC). Between ages three and five, one or more cavitated, missing teeth (due to caries) or filled surfaces in primary maxillary anterior teeth, or a score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces, constitutes S-ECC (3).

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The primary risk factors for ECC include substrate, host, microorganism, and time (4, 5). Streptococcus mutans is the most significant cariogenic microorganism associated with ECC, which is a biofilm-dependent disease (1, 4). The ECC flora also comprises Streptococcus mutans, Lactobacillus spp., Bifidobacteria spp., Actinomyces spp., Veillonella spp., Scardovia wiggle, and Candida spp. species (4). Candida spp. is an opportunistic pathogen that is commonly found in oral flora (6). Oral and systemic predisposing factors contribute to the pathogenicity of Candida, including poor oral hygiene habits, tooth decay, immunosuppression, malnutrition, a high-carbohydrate diet, long-term antibiotic therapy, cytotoxic therapy, and dental prosthesis (7, 8). Virulence factors such as adhesion, biofilm formation, germ tube formation, dimorphism, toxin production, enzyme synthesis, phenotypic switch, and the presence of genes encoding virulence are significant contributors to the pathogenicity of Candida (9, 10). Candida spp. affects the caries process due to its properties of carbohydrate fermentation, aciduricity, acidogenicity, adhesion to tooth surfaces, biofilm formation on hydroxyapatite surfaces, penetration of dentin tubules, and extracellular enzyme synthesis (9, 11, 12). Therefore, the increased levels of Candida in the oral flora of children with S-ECC and ECC suggest its potential role in ECC etiology (7). Moreover, considering the high incidence of Candida in dental plaque among individuals with S-ECC, it has been identified as a risk factor for S-ECC and has been shown to be a more reliable indicator of S-ECC than many behavioral factors (13).

Studies have reported a higher presence of Candida in the dental plaque and saliva of children with ECC compared to caries-free children (7, 14, 15). However, information regarding its presence in S-ECC is limited. Our study aimed to investigate the presence of Candida, which is among the etiological factors of S-ECC and ECC, in the saliva and dental plaque of children aged six years and younger. The null hypothesis is that early childhood caries does not exhibit any significant differences in relation to the presence of Candida.

Materials and Methods

Ethical statement

This study was conducted with the approval of the Uşak University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Approval No. 148-01-16; 17.06.2020). The guardians of the children who participated in the study were informed about its purpose, and written consent was obtained.

Participants

The study involved 60 randomly selected children (32 boys, 28 girls) who visited the Uşak University Faculty of Dentistry Department of Pediatrics between June 2020 and August 2020. Participants were children aged 6 years and younger who met the following criteria: they were systemically healthy, had not taken any medication (steroids, antibiotics, antifungals, or immunosuppressive drugs) in the past 3 months, had no intraoral pathology, had not undergone previous dental treatment, did not use intraoral appliances, were cooperative, and agreed to participate in the study. We categorized them into three groups, each consisting of 20 children: The S-ECC group, the ECC group, and the caries-free group. The S-ECC and ECC groups were categorized based on recommended criteria (1, 3), while the caries-free group comprised children without caries.

Clinical examination

A single dentist conducted a clinical examination under artificial light using a dental mirror and a periodontal probe to record the number of caries in all groups. Additionally, we recorded the gingival index (16) and plaque index (17) based on predetermined criteria to assess the relationship between the presence of Candida and both plaque and gingival health.

Dental plaque and saliva collection

Dental plaque from the S-ECC and ECC groups was collected from carious surfaces using a sterile cotton swab, while that from the caries-free group was obtained from the labial surfaces of all teeth near the gingiva. Children were instructed not to eat for one hour before saliva collection. We collected one milliliter of unstimulated saliva from the children in the morning, between 10:00 and 11:00 a.m., by having them directly spit into Eppendorf tubes.

Microbiological assessments

Following the collection of samples from dental plaques, the cotton swab tips were placed in microcentrifuge tubes containing 100 µL of saline solution. These microcentrifuge tubes, containing dental plaque and saliva samples, were labeled, stored at -80°C, and transported to the microbiology laboratory following the protocol described by Thomas *et al.* (6).

Once all the samples had reached room temperature, dental plaque samples with a total volume of 500 µL were diluted using sterile saline. The diluted dental plaque and saliva samples were homogenized using a vortex. From each sample, 100 µL was inoculated onto ChromaticTM Candida chromogenic medium (Liofilchem®, Italy) and incubated at 35 °C for 48 hours. Candida colonies were identified according to the manufacturer's instructions. Green colonies were identified as Candida albicans, beige colonies as Candida glabrata, pink colonies with pale margins as Candida krusei, and blue colonies as Candida tropicalis (see Figure 1).

Statistical analysis

In this study, descriptive statistics, including numbers, frequency, standard deviations, means, maximum, and minimum values, were provided. The Shapiro-Wilk test was used to assess the normality of the data distribution. The independent samples t-test was employed to examine differences in means between two independent groups with a normal distribution. The Mann-Whitney U test was used to compare means between two independent groups without a normal distribution. The Kruskal–Wallis test was applied to examine the relationship between the averages of variables in cases with more than two independent groups and non-normal distribution. The Pearson chi-squared test was used for the analysis of categorical variables. In cases where

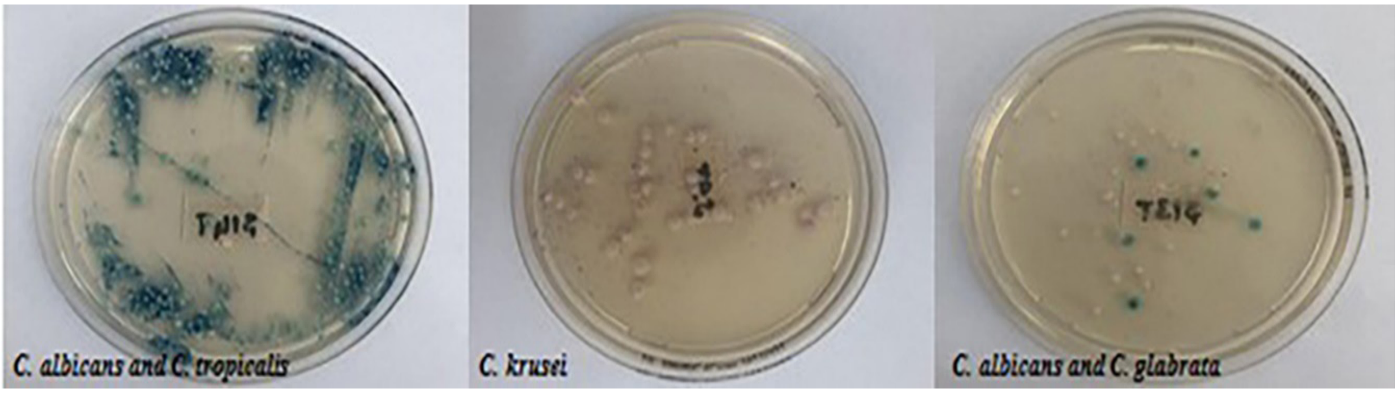

Figure 1. *Candida species seen on the chromogenic medium.*

the sample size assumption was not met, Fisher's Exact Test was performed. Data were analyzed using IBM SPSS Statistics 25.0 (IBM SPSS, Inc., NY, Armonk, USA). A p-value below 0.05 was considered statistically significant for the analysis.

Results

A total of sixty participants, comprising 32 boys and 28 girls, underwent examination. Subsequently, three distinct groups were established: ECC group, S-ECC group, and caries-free group. The characteristics of these groups are presented in Table 1. Based on our findings, the mean age of the ECC group was significantly higher than that of both the caries-free group ($p<0.05$) and the S-ECC group ($p<0.05$).

The presence and species of Candida in the children are detailed in Table 2. We observed significantly higher Candida levels in the saliva of the S-ECC and ECC groups compared to the caries-free group ($p<0.05$). However, there was no statistically significant relationship between the groups in terms of the presence of Candida in dental plaque. The presence of Candida in the dental plaque of the S-ECC group was only marginally higher than that in the ECC group, similar to its presence in saliva. Candida albicans, Candida tropicalis, Candida glabrata, and Candida krusei were detected in saliva, with Candida tropicalis being absent in dental plaque. Candida albicans was the most commonly identified Candida species in both the S-ECC and ECC groups. In the S-ECC group, it was found concurrently with Candida albicans in saliva, Candida krusei in two cases, Candida tropicalis in one case, and Candida glabrata in one case.

Table 1: *Gender, age and caries experience of the groups.*

Characteristic	S-ECC group	ECC group	Caries free group
Total number of children	20	20	20
Boys	10	12	10
Girls	10	8	10
Age in months			
Mean ± SD	56.35 ± 5.30	63.40 ± 8.91	50.80 ± 15.13
Median (min-max)	27.20 (42-63)	40.90 (42-71)	23.40 (17-70)
Number of caries			
Mean ± SD	8.55 ± 2.60	3.30 ± 1.52	0.00 ±0.00

Table 2: *Comparison of species distribution of Candida among S-ECC, ECC and caries-free groups*

	S-ECC group n (%)	ECC group n (%)	Caries free group n (%)	p value
Saliva				
Presence of Candida	8 (40)[a]	6 (30)[a]	0[b]	0.005*
Candida albicans	6 (30)[a]	4 (20)[a]	0[b]	0.026*
Candida krusei	2 (10)	1 (5)	0	0.766
Candida glabrata	1 (5)	1 (5)	0	1.000
Candida tropicalis	2 (10)	0	0	0.322
Dental plaque				
Presence of Candida	5 (25)	3 (15)	0	0.81
Candida albicans	5 (25)	2 (10)	0	0.058
Candida krusei	0	1 (5)	0	1.00
Candida glabrata	0	1 (5)	0	1.00

Fisher's Exact test, *$p<0.05$

When comparing the caries-free group with the ECC and S-ECC groups, a significant correlation was observed solely for the presence of Candida albicans in saliva ($p=0.026$). However, the distribution of all Candida species identified in dental plaque among the three groups was determined to be statistically nonsignificant ($p>0.05$).

Table 3 shows data on the presence of Candida in relation to the plaque index and gingival index. The plaque scores of the S-ECC and ECC groups were significantly higher than those of the caries-free group ($p<0.05$). A similar result was observed for the gingival index ($p<0.05$). However, just like the plaque index, there is also no significant relationship between the gingival index and the presence of Candida. It's worth noting that we excluded one child from the S-ECC group with a "3" gingival index code and one child from the caries-free group with a "2" gingival index code from the dataset due to an insufficient sample size for analysis.

Discussion

In recent years, evidence has emerged suggesting that Candida may play a role in the development of ECC (Early Childhood Caries) (7, 14, 18). Studies support a potential positive

Table 3: *Comparison of plaque index and gingival index among the groups*

		Candida Presence	0 (n%)	Gingival Index 1 (n\%)	2 (n\%)	p value
Saliva	S-ECC Group	-	0	2 (18.20)	9 (81.80)	0.603
		+	0	3 (37.50)	5 (62.50)	
	ECC Group	-	0	4 (28.60)	10 (71.40)	1.00
		+	0	2 (33.30)	4 (66.70)	
	Caries Free Group	-	7 (36.80)	12 (63.20)	0	-
		+	0	0	0	
Dental Plaque	S-ECC Group	-	0	5 (33.30)	10 (66.70)	0.53
		+	0	0	4 (100.00)	
	ECC Group	-	0	5 (29.40)	12 (70.60)	1.00
		+	0	1 (33.30)	2 (66.70)	
	Caries Free Group	-	7 (36.80)	12 (63.20)	0	-
		+	0	0	0	
Total	S-ECC Group		0	5 (26.30)	14 (73.70)	0.000*
	ECC Group		0	6 (30.00)	14 (70.00)	
	Caries Free Group		7 (36.80)	12 (63.20)	0	
		Candida Presence	**1 (n\%)**	**Plaque Index 2 (n\%)**	**3 (n\%)**	**p value**
Saliva	S-ECC Group	-	2 (16.70)	7 (58.30)	3 (25.00)	0.476
		+	0	7 (87.50)	1 (12.50)	
	ECC Group	-	3 (21.40)	11 (78.60)	0	1.00
		+	1 (16.70)	5 (83.30)	0	
	Caries Free Group	-	17 (85.00)	3 (15.00)	0	-
		+	0	0	0	
Dental Plaque	S-ECC Group	-	2 (13.30)	11 (73.30)	2 (13.30)	0.554
		+	0	3 (60.00)	2 (40.00)	
	ECC Group	-	3 (17.60)	14 (82.40)	0	1.00
		+	1 (33.30)	2 (66.70)	0	
	Caries Free Group	-	17 (85.00)	3 (15.00)	0	-
		+	0	0	0	
Total	S-ECC Group		2 (10.00)	14 (70.00)	4 (20.00)	0.00*
	ECC Group		4 (20.00)	16 (80.00)	0 (0.00)	
	Caries Free Group		17 (85.00)	3 (15.00)	0 (0.00)	

Fisher's Exact test, *p<0.05

correlation between oral Candida carriage and caries experience in children (19, 20). When Streptococcus mutans and Candida coexist in the oral biofilms of children with ECC, several factors come into play, including an increase in the extracellular polysaccharide matrix, bacterial accumulation, hypha formation of Candida, and increased biofilm virulence. These factors contribute to the development of rapid-onset caries, such as ECC (21). However, the number of studies comparing the presence of Candida in children with S-ECC (Severe Early Childhood Caries) and ECC is limited. Therefore, this study aimed to investigate the presence of Candida in children with S-ECC, ECC, and those without caries experience.

Lozano Moraga *et al*. (14) examined the unstimulated saliva of children and found that Candida was present in 35.5% of children without caries, 50% of children with moderate caries, and 72.2% of children with severe caries. Their findings indicated that Candida carriage increased with the severity of caries (14). Beena *et al.* (15) reported that Candida was detected in 84% of the dental plaque of children with ECC and 24% of children without caries, with this difference being statistically significant. In our study, significantly higher levels of Candida were observed in the saliva of the S-ECC and ECC groups compared to the caries-free group ($p<0.05$). The presence of Candida in dental plaque in the S-ECC and ECC groups was only marginally higher than that in the caries-free group ($p>0.05$). These findings align with the existing literature, suggesting that children with S-ECC and ECC tend to have more Candida in their oral flora compared to children without caries. It has been proposed that factors such as adhesion, biofilm formation, penetration into dentinal tubules, and enzyme production may contribute to the cariogenicity of Candida (9, 11, 12). It should be noted that our study found Candida presence rates to be lower than those reported in other studies (14, 15). This difference might be attributed to various factors influencing Candida colonization. It is also worth mentioning that, while more Candida was observed in the saliva and dental plaque of the S-ECC group compared to the ECC group, this difference was not statistically significant. In studies with a larger number of participants, such distinctions between the groups may become statistically significant.

Under appropriate conditions, dental plaque creates a diffusion-limiting environment, encourages microbial accumulation, and serves as a physical scaffold that facilitates the adhesion of microorganisms to teeth (22). Saliva contains proteins that act as receptors for Candida species and play a role in Candida attachment to enamel and oral bacteria (23). Several factors have been reported to influence Candida colonization, including the diversity of molecules in saliva, saliva pH, saliva flow rate, saliva buffering capacity, high sugar concentration in carious lesions, carbohydrate-rich diets, oral hygiene practices, the severity of carious lesions, sample size, and the collection of plaque on the surface (11, 14, 22, 24). Our study has revealed that Candida is more prominently present in saliva than in dental plaque. Considering potential variations in Candida colonization based on the locations from which dental plaque is collected, saliva may serve as a superior marker for Candida detection in future studies.

Xiao *et al.* (25) reported that Candida albicans were detected in saliva and dental plaque of children with S-ECC at rates ranging from 77% to 83%, while in children without caries, the detection rate was only 12% to 6%. Furthermore, they identified 6% Candida tropicalis, 6% Candida glabrata, and 17% Candida krusei in the saliva and dental plaque of the S-ECC group. Similarly, Thomas *et al.* (6) found significantly higher levels of Candida albicans in the saliva and dental plaque of children with S-ECC compared to those without caries. In our study, Candida albicans were also found to be statistically significantly higher in the saliva samples of the S-ECC and ECC groups when compared to the caries-free group ($p<0.05$). It is worth noting that statistically significant results for other Candida species might be achievable in studies with larger sample sizes.

Several limitations in our study include the inability to perform advanced typing of Candida species, a small sample size, the sensitivity of sampling and laboratory practices. Further research is required to strengthen the evidence supporting the relationship between oral Candida and S-ECC and ECC. This research should aim to determine whether the presence of Candida can serve as a risk factor or risk indicator for the development of S-ECC and ECC and to understand whether Candida growth is influenced by factors involved in the caries process. Additionally, exploring differences in contagiousness among Candida species and understanding potential inter-species relationships is essential. The low presence of Candida in all groups compared to other studies and its absence in the non-carious group might be associated with various factors, including storage and transport conditions, and further investigation is needed.

Conclusion

The rates of Candida carriage in the S-ECC and ECC groups were higher than those in the caries-free group. This suggests that Candida may play a role in the initiation and progression of S-ECC and ECC. Further studies are required to investigate the potential vertical and horizontal transmission of Candida infections.

Türkçe özet: *Şiddetli Erken Çocukluk Çürüğü ve Erken Çocukluk Çürüğü Bulunan Hastaların Dental Plak ve Tükürüklerinde Candida Varlığı: Pilot Çalışma. Amaç: Bu çalışmanın amacı, erken çocukluk çağı çürüklerinin (ECC) ve şiddetli erken çocukluk çağı çürüklerinin (S-ECC) etyolojik faktörleri arasında yer alan Candida varlığının, çocukların dental plaklarında ve tükürüklerinde değerlendirmektir. Gereç ve Yöntem: Çalışmamız dahil edilme kriterlerini uygun 60 katılımcı ile gerçekleştirildi. Klinik muayeneye göre her biri 20 çocuktan oluşan, S-ECC bulunan, ECC bulunan ve diş çürüğü bulunmayan üç grup oluşturuldu. Çocuklardan diş plağı ve tükürük örnekleri alındı. Alınan örnekler, laboratuvarda, Candida varlığı ve Candida türleri açısından Liofilchem® – ChromaticTM Candida (Roseto degli Abruzzi, İtalya) besiyeri kullanılarak değerlendirildi. Bulgular: Candida varlığı, S-ECC (%40) ve ECC (%30) bulunan çocukların tükürüğünde, çürük olmayan çocuklara göre istatistiksel olarak yüksek görüldü (p<0.05). S-ECC (%25) ve ECC (%15) bulunan çocukların diş plaklarında, çürük olmayan çocuklara göre gözlemsel olarak daha fazla Candida belirlendi (p>0,05). S-ECC grubunda tükürükte Candida albicans, Candida glabrata, Candida krusei ve Candida tropikalis, diş plağında ise Candida albicans tespit edildi. ECC grubunda Candida albicans, Candida glabrata ve Candida krusei gözlenirken, çürük olmayan çocuklarda Candida görülmedi. Sonuç: ECC patogenezinde potansiyel rol oynayan, tükürük ve dental plakta Candida varlığının bireysel risk değerlendirmesi açısından önemli olabileceği düşünülmektedir. Çalışmanın sonuçlarına göre, Candida geçiş yollarının tanımlanmasının ve önlenmesinin ECC'yi azaltmada faydalı olabileceği görülmüştür. Anahtar kelimeler: Candida, dental plak, diş çürüğü, tükürük*

Ethics Committee Approval: This study was conducted with the approval of Uşak University, Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (Approval No. 148-01-16; 17.06.2020).

Informed Consent: The parents of the participants provided informed constent.

Peer-review: Externally peer-reviewed.

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

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