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

Book

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

Chapter in a book

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

Thesis

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

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

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	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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Eur Oral Res 2018; 52(3): 117-21

Original research

Comparative evaluation of apical sealing ability of different root canal sealers

Halenur Altan[1],
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Gülsüm İnci[2],
Gül Tosun[3]

Purpose
The aim of this study was to compare the short and long term apical sealing ability of different root canal sealers.

Materials and methods
Fifty-five extracted human anterior single-root teeth were used. The coronal part of each tooth was removed and the root canals were prepared with NiTi rotary instruments. Teeth were divided into 5 study groups; Group I: MTA Fillapex (Angelus, Brazil); Group II: Sealapex (Sybron-Kerr, Romulus, MI, USA) and Group III: AH Plus (Dentsply, Konstanz, Germany) (n=15) and negative and positive control groups (n=5). The quality of root canal sealing was assessed by a fluid filtration method performed at 24 h and 180-day time intervals. Kruskal Wallis and Mann Whitney U tests were used to compare the groups.

Results
At 24 h evaluation, MTA Fillapex presented significantly less microleakage than the Sealapex and AH Plus ($p<0.05$). At long term interval (180-day), Sealapex and AH Plus presented significantly less microleakage than the MTA Fillapex ($p<0.05$).

Conclusion
Sealapex and AH Plus showed significantly better sealing abilities than MTA Fillapex in the long term.

Keywords: *MTA fillapex; AH plus; Sealapex; microleakage; fluid filtration method*

Introduction

The predictable outcomes of endodontic treatment rely on mechanical instrumentation and cleaning of the root canal system, elimination of the microorganisms and organic debris, as well as filling the entire root canal (1). It is commonly accepted that microleakage between the root canal walls and root canal filling might adversely affect the outcome of the endodontic treatment (2). Consequently, sealing the entire root canal system after cleaning and shaping is of utmost importance to prevent oral pathogens from colonizing and re-infecting the root and periapical tissues (3).

In endodontic treatment, sealers are principally used to fill the irregularities of the root canal system, to provide lubricating or to attach the gutta-percha to the root canal walls (1). Endodontic sealers should meet some requirements, such as biocompatibility, dimensional stability, insolubility in oral fluids, radiopacity, ease of application, antibacterial properties, adaptability to the root canal walls, as well as the ability to produce a hermetic seal (4). However, none of the sealers currently available have all characteristics of the ideal sealer (5-7).

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Based on the superior biocompatibility and high alkaline activity of mineral trioxide aggregate (MTA), root canal sealers were manufactured (8-10). An MTA-based root canal sealer, MTA Fillapex (Angelus, Londrina, Brazil), has been introduced and is composed of synthetic Portland cement, which are dark gray nodular materials (11). According to the manufacturer's description, MTA Fillapex exhibited high radiopacity, slow setting time, sufficient working time, perfect flow to allow the filling of accessory canals, low solubility, effortless removal if re-entry is required, and easy handling with small auto mixing tips (11). MTA Fillapex was introduced in last years, but only a limited number of studies have determined the microleakage of this MTA-based sealer (12, 13). The present study was designed to compare the short-term and long-term apical sealing ability of MTA Fillapex using fluid filtration technique. The null hypothesis of this study was that there is no difference in apical sealing ability among the all tested sealers at short term and long term intervals.

Materials and methods

Ethics committee approval was obtained in conjunction with study approval by the Ethics Committee of Selçuk Universiy, Faculty of Dentistry (No: 2013/05, Date: 02.05.2013).

Canal preparation and obturation

Fifty-five single-root human anterior teeth were divided at the cervical plane to get 15-mm long sequence of root. The working length of each root was determined by a 15 K-file (Kerr Corporation, Orange, CA, USA). The canal space was enlarged with ProTaper system rotated at 250 rpm (Dentsply, Ballaigues, Siwtzerland) under a continuous 5% NaOCl irrigation. The apical length was confirmed with a size 15 K-file manually between each ProTaper instrument. Preparation of the apical third portion was finished using Ni-Ti manual instruments (Dentsply, Ballaigues, Siwtzerland) to a size 40 K-file. All samples were prepared to the same final apical size, and the same operator handled both the preparation and filling steps. After preparation was carried out, the root canals were irrigated with 17% ethylenediaminetetraacetic acid (EDTA) for 1 min. to eliminate the smear layer. After EDTA solution, 5% NaOCl used for neutralization, afterward the canal was rinsed with distilled water (5 mL) and dried with paper points.

Sealapex (Sybron-Kerr, Romulus, MI, USA), AH Plus (Dentsply, Konstanz, Germany), and MTA Fillapex were applied according to the manufacturers' instructions and located into the root canals with a Lentulo (Dentsply Maillefer, Ballaigues, Switzerland) (n=15). The composition of tested root canal sealers are listed in Table 1.

Gutta-percha points (Dia Dent, Cheongju-si, Korea) were placed at the working length (15 mm), and root canals were filled using the cold lateral compaction technique. Thereafter, excess material was removed using a heated instrument 1 mm below access opening. Five samples filled with only gutta-percha and not coated with nail varnish were used as positive controls, and five other samples filled with only gutta-percha and apical end completely covered with two coat nail varnish as negative controls. After the obturation procedures, the filled samples were stored at 37°C and 100% humidity for 24 hours to allow setting of the sealer.

Assesment of microleakage

Nail varnish was used to block the transition of fluid across the dentinal tubules and provide that any liquid flow measured was caused by flow along the interface between the root canal walls and sealer. The outer surface of roots was double coated using nail varnish except at the end of the root. Twenty-four hours after the root canal filling procedures completed, the teeth were subjected to the first fluid flow measurements. For long term evaluation, the teeth were stored in distilled water for six months at 37°C. After this artificial aging, fluid filtration measurement was carried out in a similar way, as identified above.

Fluid filtration technique

The method for measuring the fluid transition through the root canal as a demonstration of apical microleakage has been previously described (14). For microleakage measurements a fluid flowmeter was used (Figure 1). A segment of 18-gauge stainless-steel tubing was attached through the space in a Plexiglas block 2.1 x 2.1 x 0.6 cm in size and sealed to the Plexiglas with cyanoacrylate adhesive (Pattex, Henkel Gmbh, Dusseldorf, Germany). The diameter of the 18-gauge tubing enabled it to be directly connected to the root canal orifices.

With this method, the whole root canal filling and dentin interface is kept under pressure. The coronal part of each root was bonded to the other side of the Plexiglas block using cyanoacrylate adhesive. Each pattern was then connected to the fluid filtration system with polyethylene tubing (Fisher Scientific, Chicago, IL, USA). A ceiling-suspended deionized water syringe provided the hydrostatic pressure of 70 cm H_2O (6.895 kPa) through a 25-µL micropipette to the coronal part of the root canal (15). The linear movement of a 1.0 mm air bubble in the micropipette was measured in mm with an endodontic ruler graduated in 0.5 mm increments. A Gilmont microsyringe (Gilmont Instruments, Barrington, IL, USA) was used to location the air bubble in the hydraulic system (Figure 2). After procedure stabilization, the fluid transition rate of each root canal was measured for 4 min (measurement time) and repeated three times in consecution (total 12 min). The fluid flow rate was measured at 24 h and 180 days following root canal obturation. Between measurements, the samples remained fixed to the Plexiglas block and were kept in deionized water at 37°C. To avoid any contamination of the coronal part of root canal by remnants of temporary filling material, temporary filling was not used. Linear measurements were converted to microliters per minute (µL min^{-1}).

Statistical analysis

The statistical analysis was performed using IBM Statistical Package for the Social Sciences Statistics for Windows, (Version 20.0. IBM Corp.; Armonk, NY, USA). As the data did not meet the assumptions of normal distribution, the Kruskal-Wallis test and Mann Whitney-U tests were used for multiple and pairwise comparisons, respectively. The confidence level set to 95% and p values less than 0.05 were considered significant.

Table 1. *Composition of tested root canal materials*

Materials		Composition
AH Plus® (Dentsply, Konstanz, Germany)	Epoxide paste (paste A) Amine paste (paste B)	Calcium tungstate, epoxy resins, silica, zirconium oxide, iron oxide pigments 1-adamantane amine, N,N'-dibenzyl-5-oxanonandiamine-1,9, TCD-Diamine, zirconium oxide, calcium tungstate, silica, silicone oil
Sealapex® (Sybron-Kerr, Romulus, MI, USA)		Catalyst Base Isobutyl salicylate resin N-ethyltoluenesulfonamideresin Bismuth trioxide Fumed silica (silicon dioxide) Zinc oxide Titanium dioxide Calcium oxide pigment
MTA Fillapex® (Angelus, Londrina, Brasil)		Natural resin, Nanoparticulate silica, Salicylate resin, Resin Particles in Diluting MTA, Bismuth oxide

Figure 1. *Fluid flowmeter used for microleakage measurements.*

Table 2. *Apical leakage mean (µl/min), standard deviations (SD) and means in 24h and 180 days*

Groups	24 hours (Mean±SD µL min^{-1})	180 days (Mean±SD µL min^{-1})
MTA Fillapex	0.040±0.014^{a*}	0.039±0.102^{a*}
Sealapex	0.058±0.017^{b*}	0.026±0.011^{b**}
Ah Plus	0.060±0.026^{b*}	0.032±0.011^{b**}
Negative control	0.00^{c}	0.00^{c}
Positive control	0.5^{d}	0.5^{d}

MTA: mineral trioxide aggregate
Different letters indicate stastically significant differences between sealers, significance level is $p<0.05$
Different number of * indicates stastically differences between periods, significance level is $p<0.05$

Results

Table 2 shows the apical leakage mean and standard deviations of the experimental and control groups over time. For negative control group (completely coated with varnish), measurable fluid flow was not observed within the detection limits of the model after the 24 h measurements. The positive control group, which filled with only gutta-percha, leaked significantly under pressure. All materials allowed fluid to flow throughout the sealer-root dentine interface at the short term and long term intervals.

There were significant differences in fluid leakage amongst the groups at the two time intervals ($p<0.05$). At first measurement, MTA Fillapex presented less microleakage (0.040±0.014 µL min^{-1}), statistically different than the Sealapex (0.058±0.01 µL min^{-1}) and AH Plus (0.06±0.026 µL min^{-1}) ($p<0.05$). No statistically significant difference in microleakage was observed between Sealapex and AH Plus (Table 2).

After six months of storage, Sealapex (0.026±0.011 µL min^{-1}) and AH Plus (0.032±0.011 µL min^{-1}) presented less microleakage, statistically different than the MTA Fillapex (0.039±0.102 µL min^{-1}) ($p<0.05$). No statistically significant difference in microleakage was observed between Sealapex and AH Plus. Over time, no statistically significant difference in microleakage was observed in MTA Fillapex, but significant decreases were observed in Sealapex and AH Plus ($p<0.05$) (Table 2). The values were 0 µL/ min^{-1} for negative controls and extremely high 0.5 µL/ min^{-1} for the positive control.

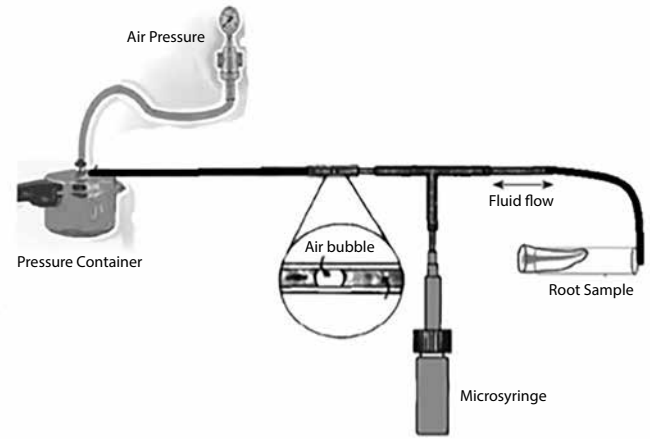

Figure 2. *Scheme of fluid filtration mechanism [adapted from Gandolfi&Prati].*

Discussion

Achieving a hermetical seal by entirely filling the root canal space decreases the risk that microorganisms left in the canal might come in contact with oral or periapical fluids (16, 17). Therefore, investigations on the sealing ability should proceed until the ideal endodontic sealer is found. In present study, the null hypothesis was rejected because MTA Fillapex showed a significant leakage from Sealapex and AH plus both at short term and long term intervals.

Sealapex is suggested as a root obturation material and includes calcium oxide which has the ability to induce hard tissue formation at the apex following root canal obturation (8).

Other sealer, the epoxy resin-based AH Plus, is well known for its sufficient flow, long-term dimensional stability, and expansive properties, and it is considered the gold standard of root canal sealers (15, 18, 19). In the present study, no significant difference was found between the apical leakage amounts of the groups filled with AH Plus and Sealapex in short term and long term intervals. Similarly, Xu *et al.* (20) reported no difference between the microleakage of Sealapex and AH Plus. Similarly, Sagsen *et al.* (21) found no difference between the apical leakages of AH Plus and Sealapex. In the other studies, different results were presented about the microleakage of AH Plus and Sealapex that were related with methodolgical differences used (15, 22, 23).

The results of the present study revealed a negative correlation between time and sealing ability of AH Plus / Sealapex. The microleakage of all sealers had high values at 24 h, but at 180 days AH Plus and Sealapex had better sealing ability than MTA Fillapex. Razavian *et al.* (24) compared apical microleakage of AH26 and MTA Fillapex, using a bacterial microleakage evaluation system. It was reported that microleakage of Fillapex increased over time and that the material had a lower sealing ability compared to AH26. Sönmez *et al.* (25) reported that MTA Fillapex had lower sealing ability than AH Plus and Pro Root MTA 7 days after obturation using dye penetration test. Different from this study; Asawaworarit *et al.* (26) reported that MTA Fillapex had more leakage than AH Plus at 7 days, but at 4 weeks, MTA Fillapex showed a better sealing ability than AH Plus. MTA Fillapex contains a high ratio salicylate resin, and which causes the long chemical reaction time (27). A possible explanation for the microleakage in short and long term may be related to extended setting time.

Sealing ability can be related to different factors such as micromechanic, bonding, chemical bonding (27). AH Plus and Sealapex produce rigid and strong cross-linked polymer with dentin collagens, in addition Sealapex hydration products derivate calcium hydroxide (27). Findings of the present study explain the similar ingredients in sealers have similar chemical bonding mechanism with dentinal wall.

Conclusion

Within the limitation of this study, MTA Fillapex showed the higher sealing ability in 24 hours, and Sealapex and AH Plus showed better sealing in long term. There is a correlation between sealing ability and time according to the contents of the sealer.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Selçuk University, Faculty of Dentistry (No: 2013/05, Date: 02.05.2013).

Informed Consent: Informed consent was not taken due to the in vitro study.

Peer-review: Externally peer-reviewed.

Author Contributions: ZG and Gİ designed the study. HA and Gİ generated the data. ZG and Gİ gathered the data. HA and ZG analyzed the data. HA wrote the majority of the original draft. ZG and GT participated in writing the paper. All authors approved the final version of the paper.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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Türkçe öz: *Farklı kök kanal patlarının apikal sızdırmazlık yeteneğinin karşılaştırmalı değerlendirmesi. Amaç: Bu çalışmanın amacı farklı kök kanal patlarının kısa dönem ve uzun dönem apikal mikrosızıntı özelliğinin karşılaştırılmasıdır. Gereç ve Yöntem: Çalışmada 55 çekilmiş tek köklü insan ön dişi kullanılmıştır. Dişlerin koronal kısmı kesilmiş, kök kanalları Ni-Ti döner aletler ile şekillendirilmiştir. Dişler Grup I: MTA Fillapex (Angelus, Brazil); Grup II: Sealapex (Sybron-Kerr, Romulus, MI, USA), Grup III: AH Plus (Dentsply, Konstanz, Germany) (n=15), negatif ve pozitif kontrol grubu (n=5) olmak üzere 5 gruba ayrılmıştır. Kök kanalların örtücülüğünün kalitesini değerlendirmek için 24 saat ve 180 gün aralıkla sıvı filtrasyon testi yapılmıştır. Grupların karşılaştırılmasında, Kruskall Wallis ve Mann Whitney U testi kullanılmıştır. Bulgular: Yirmi dört saatlik ölçümde, MTA Fillapex; Sealapex ve AH Plus'tan daha az mikrosızıntı sergilemiştir (p<0,05). Uzun dönemde (180 gün) Sealapex ve AH Plus, MTA Fillapex'ten daha az mikrosızıntı göstermiştir (p<0,05). Sonuç: Sealpex ve AH Plus uzun dönemde MTA Fillapex'ten daha iyi örtücü özellik sergilemiştir. Anahtar kelimeler: MTA fillapex; AH plus; Sealapex; mikrosızıntı; sıvı filtrasyon yöntemi.*

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Original research

Effects of coloring procedures on shear bond strength between resin cement and colored zirconia

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Işıl Turp

Purpose
Debonding is expected as a frequent failure type in zirconia restorations. Therefore the aim of the current study is to evaluate the shear bond strength between colored zirconia and resin cement.

Materials and methods
There were 11 groups evaluated each containing 12 zirconia discs (15 mm x 12 mm x 1.6 mm). Groups were colored with the colors A3, B1, C4, D2, and D4 of the VITA classical shade scale. Coloring procedure was carried out for either 3 second or 60 seconds for the study groups and the control group was left untreated. Specimens were then bonded to translucent resin cement having a thickness of 3 mm and width of 3 mm. The shear bond strength of the samples was measured in a universal testing machine with a crosshead speed of 1 mm per minute. Two-way analysis of variance and Tukey's HSD test were used for pairwise comparisons. Also paired t-test was used for comparing groups with the same color but having different shading times.

Results
Any significant difference was not found between the shear bond strengths of samples depending on whether color or shading times. Among the groups, B1 (60 seconds of coloring) had the highest bond strength (10.05 MPa), while A3 (60 seconds of coloring) showed the lowest bond strength (6.72 MPa). However, these differences were not statistically significant.

Conclusion
Coloring zirconia did not affect the shear bond strength between zirconia and resin cement.

Keywords: *Shear bond strength; ceramic; coloring; zirconia; resin cement*

Introduction

Zirconia has a high fracture strength of more than 1000 MPa, fracture resistance of higher than 2000 N, and fracture toughness of 9–10 MN/m$^{3/2}$ (1). In addition to these favorable mechanical properties, it is chemically durable (2), biocompatible (3, 4), and displays esthetic advantages (5) over metal-ceramic restorations. However, recent clinical trials have shown that zirconia-based ceramic restorations' decementation is a common failure. Restoration's cementation corresponds to restoration durability, and cementation is also an important factor for marginal fit and fracture strength of restorations (6, 7). There are various cementation options for zirconia framework restorations. Cementation of zirconia restorations with traditional luting cements (such as glass ionomer or zinc phosphate cements) provides adequate clinical fixation, but adhesive cementation may be preferred for better retention and marginal adaptation (7-10). In addition, resin cement shows higher compressive and tensile strength than other luting agents, and allows color selection for more esthetic restorations (11, 12).

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Silica-based ceramics and resin cement show high bond strength with the use of hydrofluoric acid and silanization (13-15). Zirconia ceramics, however, exhibit high corrosion resistance, which inhibits acid etching. This limits their potential use with resin cements (16) and resin cementation of zirconia remains as a challenging subject on which various studies have been carried out. Although zirconia frameworks are more esthetic compared to metallic frameworks, their initial opaque and whitish appearance is still a handicap. As a result of this, colored zirconia frameworks were introduced to the market to obtain a more natural color. The main advantage of colored-zirconia ceramics is that they enable the selected color to be reflected from the inner layers of the restoration as in the dentin reflecting from inside of enamel (17). Zirconia frameworks can be colored with several techniques. The addition of metallic pigments to the initial zirconia powder (before or after milling blocks are pressed), the dipping of milled frameworks into the dissolved coloring agents, or the application of liner material to sintered frameworks are some of them (18).

There are few studies evaluating the effects of these color-shading procedures on the structure of zirconia-based restorations and shear bond strengths between zirconia and veneer ceramics. These studies has shown that the shear bond strength between zirconia and veneer ceramics is affected because coloring affects the structure of the zirconia framework (19-21). The aim of the current study was to evaluate the effects of different coloring liquids and different lengths of dipping times on the shear bond strength (SBS) between the zirconia framework and the resin cement. The null hypothesis is that dipping zirconia in different coloring liquids and for various dipping times does not affect the shear bond strength between zirconia and resin cement and the failure type is not affected from coloring.

Materials and methods

Specimen preparation

Zirconia blocks were partially stabilized with yttrium (ICE Zirkon, Zirkonzahn, South Tyrol, Italy) were cut into discs by means of a low-speed diamond saw (Struers Ltd., Lanarkshire, United Kingdom). The sizes of 132 discs were 15 mm x 12 mm x 1.6 mm. The samples were divided into eleven groups (n=12). Ten groups were colored with coloring liquids (Colour liquid, Zirkonzahn Inc., Norcross, GA, USA), and one control group was not colored. Five groups were colored with coloring liquids based on VITA shading (A3, B1, C4, D2, D4) for 3 seconds and five groups were colored with the same coloring liquids for 60 seconds by the aid of plastic holders. After the coloring procedure of the samples, except for the control group, they were dried under a warming lamp (Zirkonlampe 250, Zirkonzahn Inc., Norcross, GA, USA) for 45 minutes. Then the samples were sintered in a sintering oven (Zirkonofen 600, Zirkonzahn Inc., Norcross, GA, USA) according to the manufacturer's instructions.

After sintering, all samples were sandblasted with 50 µm aluminum oxide (Al_2O_3) particles (10 mm distance, 20 seconds duration, 3.5 atm pressure), in order to increase surface roughness and enhance bond strength. Afterwards samples were cleaned in an ultrasonic cleaner (Quantrex 90, L&R Ultrasonics, Kearny, NJ, USA) for 10 minutes, rinsed and dried with air.

All samples were then treated with metal/zirconia primer (Ivoclar-Vivadent, Schaan, Liechtenstein) for 180 seconds prior to cement application. A PVC (polyvinyl chloride) ring (R-3603, Saint-Gobain S.A., Courbevoie, France) with an inner diameter of 3 mm and a height of 3 mm was positioned on the zirconia surface. Resin cement (Multilink Automix, Ivoclar Vivadent AG, Schaan, Liechtenstein) was mixed automatically in the syringe according to the instructions of the manufacturer and then applied to each surface of the zirconia samples by packing the inner cavity of the PVC ring (Figure 1).

Thirty minutes after irradiation, the rings around the cement cylinders were removed using a surgical blade by vertically cutting the ring into two or more fragments. Bonded samples were then stored in distilled water at 37°C for 24 hours.

Shear bond strength test

The shear bond strengths (SBS) of the samples were measured at a speed of 1 mm per minute with a universal testing machine (TSTM 02500, Elista Ltd., İstanbul, Turkey) by an experienced observer (Figure 2). The accuracy of the load cell used was ±0.5%. Following the SBS test, fracture modes were examined with an optical microscope (Olympus SZ4045 TRPT, Olympus Life Sciences, Tokyo, Japan) at magnifications of 10x and 20x, to determine types of failure. Potential fracture types were classified as cohesive, adhesive, or combined (a category which includes both cohesive and adhesive fractures).

Statistical analysis

Statistical analysis of the data was performed with Statistical Package for Social Sciences (SPSS) statistical software (SPSS PC, Vers.15.0; SPSS Inc.; Chicago, IL, USA). As all the variables were numerical and the distribution of the data met the assumptions for normality, two-way analysis of variance (ANOVA) was used to compare multiple groups having the same shading times, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for pairwise comparisons. A paired t-test was used for groups of the same color but different shading times. Confidence interval was set to 95% and p values less than 0.05 were considered significant.

Results

The SBS results of the samples, according to different color shades and coloring times, are given in Table 1. The B1 group, which was colored for 60 seconds, showed the highest SBS value of 10.05 MPa. The A3 group, colored for 60 seconds, showed the lowest SBS value of 6.72 MPa. The non-colored control group showed the second-highest SBS value, at 9.35 MPa. However there was no significant difference between groups. All fractures between the zirconia framework and the resin cement were adhesive. Cohesive or combined fractures were not observed.

Table 1. *Mean shear bond strengths of the groups with standard deviations*

Groups	Mean (MPa)	SD (±)	Min (MPa)	Max (MPa)
A3 (3 s)	7.15	3.07	4.33	15.89
A3 (60 s)	6.72	1.67	5.67	10.48
B1 (3 s)	7.68	1.38	5.09	10.45
B1 (60 s)	10.05	4.26	5.87	21.14
C4(3 s)	8.68	1.87	6.70	12.55
C4 (60 s)	8.68	2.80	6.42	14.79
D2(3 s)	8.51	3.42	5.49	16.71
D2 (60 s)	8.14	1.64	6.68	12.55
D4(3 s)	8.05	2.40	5.45	12.54
D4 (60 s)	8.49	2.73	6.42	15.21
Control	9.34	2.61	6.51	15.92

MPa: megapascal; SD: standard deviations

Figure 1. *A test sample with 3 mm thick resin layer before being subjected to shear bond strength testing.*

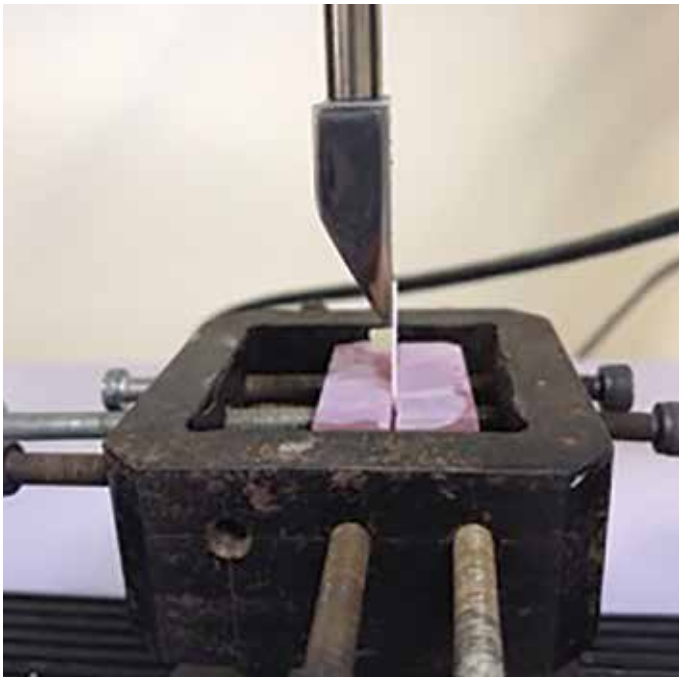

Figure 2. *The test set-up for shear bond strength testing.*

Discussion

The coloring procedures have been used for several years, however, the effect of coloring procedures on the bond strength between zirconia frameworks and resin cement has not been investigated. The results of the current study have shown that the SBS between the zirconia framework and the resin cement is not affected by either the coloring shade or the dipping time; so the null hypothesis is accepted for both SBS and fracture type.

Previous studies investigating the SBS of veneer ceramic with colored zirconia have reported that coloring procedures can affect the results (20, 22, 23). Chevalier *et al.* (24) reported that concentration of coloring pigments at grain boundaries reduces the percentage of the stabilizing element (yttrium). Reduction in the percentage of the stabilizing element would likely result in a higher frequency of tetragonal-monoclinic phase transformation, which would affect the mechanical properties of the zirconia. As a further explanation of the mechanical effects of coloring, Chen and Chen (25) have explained that the melting point of the coloring pigment (2410°C) is much lower than the melting point of yttrium and hafnium oxides (2751°C). Therefore, displacement of the stabilizing elements by the metallic pigments can occur during the sinterization of zirconia frameworks (2).

A previous study reported that light-cure resin cements and dual-cure resin cements, which are activated by a light source, cannot be sufficiently polymerized if light cannot penetrate through the material (26-28). In a recent study, Heffernan *et al.* (29) compared the translucency of different all-ceramic core materials, finding that In-ceram zirconia has the highest opacity (with a 1.00 contrast ratio) when compared to the same-value metal-ceramic specimens. Therefore, dual-cure resin cements are more reliable for low-translucency ceramics, and this study focused on a certain dual-cure resin cement only.

In the current study, the SBS values obtained were higher than those reported by Moon *et al.* (30) who had used the same resin cement and a metal/zirconia primer. However, the SBS results of the current study were lower than studies which have used resin cements based on MDP monomer (31-35). The absence of adhesive functional monomers in the resin cement may explain the lower SBS values and adhesive failure at the zirconia-resin cement interface when compared to resin cements based on MDP monomer (14, 36).

In this study, the SBS between zirconia and resin cement ranged from 6.72 MPa to 10.05 MPa. Luthy *et al.* (15) reported that a minimal bond strength of 10–13 MPa is required for an acceptable clinical bonding. The present study demonstrated a SBS lower than this clinically acceptable threshold for all groups. It should also be noted that, although coloring procedures do not affect the shear bond strength between zirconia and the resin cements used in the current study remains clinically unacceptable according to Luthy *et al.* (15).

The present study demonstrates that the coloring procedure and duration applied to the zirconia framework has no effect on the SBS of the resin cement. However, the study is limited to a single resin cement (Multilink) and a single zirconia framework system (Zirkonzahn). A future objective is to measure the effects of different types of resin cements on the SBS of other zirconia systems.

Conclusion

Different coloring liquids and dipping times do not affect the shear bond strength between the zirconia framework and resin cement. Adhesive type of fractures were observed for all samples. Still, the shear bond strength values of non-MDP containing resin cement used in this study are not enough for clinical use for both colored and non-colored zirconia frameworks.

Ethics Committee Approval: Ethics committee approval was not needed for the current *in vitro* study.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author Contributions: İT designed the study. İT and IT generated, gathered and analyzed the data. IT wrote the majority of the original draft. İT participated in writing the paper. All authors approved the final version of the paper.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Türkçe öz: *Renklendirme süreçlerinin zirkonya ile rezin siman arasındaki bağlanma dayanımı üzerindeki etkileri. Amaç: Desimantasyon zirkonya destekli restorasyonlar için sık görülen sorunlarından biridir. Bu çalışmanın amacı, renklendirilmiş zirkonyum oksit alt yapı ile rezin siman ile arasındaki makaslama bağlanma dayanımını incelemektir. Gereç ve Yöntem: Her birinde 12 zirkonya disk (15 mm x 12 mm x 1.6 mm) olacak şekilde 11 grup oluşturulmuştur. Gruplar VITA klasik renk skalasına göre: A3, B1, C4, D2 ve D4 renklerine uyumlu olarak renklendirilmiştir. Tüm gruplara önerilen 3 saniyelik renklendirme ve 60 saniyelik uzatılmış renklendirme işlemi uygulanmıştır. Kontrol grubunda renklendirme yapılmamıştır. Örneklere 3 mm kalınlığında ve 3 mm yüksekliğinde translusent rezin siman uygulanmış ve üniversal test cihazında 1 mm/dakika hızında bağlanma dayanımı testi yapılmıştır. İki yönlü varyans analizi (ANOVA) ve ikili karşılaştırmalarda Tukey HSD testi kullanılmıştır. Aynı renkte olan ancak farklı tonlama süresine maruz kalan örnekler ise t-testi ile değerlendirilmiştir. Bulgular: Makaslama bağlantı dayanımının renk ya da renklendirme sürelerine bağlı olarak anlamlı bir fark göstermediği belirlenmiştir. 11 araştırma grubunun içinde en yüksek bağlantı değeri (10,05 MPa) 60 saniye renklendirme uygulanan B1 renk grubunda; en düşük bağlantı değeri (6,72 Mpa), 60 saniye renklendirme uygulanan A3 renk grubunda elde edilmiştir. Ancak bu farklar istatistiksel olarak anlamlı değildir. Sonuç: Zirkonya alt yapı ve rezin siman arası bağlantı dayanımı zirkonyayı renklendirme işlemlerinden etkilenmemektedir. Anahtar kelimeler: Makaslama bağlanma dayanımı; seramik; renklendirme; zirkonya; rezin siman*

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Original research

Assessment of curve of spee in different malocclusions

Purpose
The aim of this study was to compare the depth of curve of Spee (COS) in Angle Class I, Angle Class II and Angle Class III malocclusions.

Materials and methods
The Samples were chosen among the diagnostic materials in İstanbul Medipol University Department of Orthodontics. Ninety plaster models were chosen, and were divided into 3 groups (n=30) according to Angle dental malocclusion classification. The depth of curve of Spee was measured on left and right sides of mandibular dental models and mean values were used as depth of curve of Spee. ANOVA test was used to evaluate normally distributed data. Comparison of the sides were performed by using paired sample t test. Significance level was set to $p<0.05$.

Results
The depth of COS was found as deepest in Class II malocclusion (2.9±0.8 mm) and was relative flat in Class III malocclusion (2.1±0.9 mm) and the difference was statistically significant ($p<0.05$). No significant difference was found between Angle Class I and Class III malocclusions.

Conclusion
Since the depth of curve of Spee is increased in Class II malocclusions, this factor should be considered in treatment planning.

Keywords: *Curve of spee; malocclusion; angle classification; mandible; orthodontics*

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Hüsamettin Oktay

Introduction

The curve of Spee (COS) was determined as an occlusal line, which is tangential part of a cylinder that begins at incisal edges of mandibular incisors and terminates at the anterior margin of mandibular condyle. The COS is an anatomic curvature and was first presented by Ferdinand Graf Von Spee in 1890 (1-3). It has been reported that overbite, mandibular incisor proclination, height of molar cusp, lower arch length, posterior contact and inclination of articular eminence are related with the arrangement of the teeth in sagittal plane (1, 4, 5). Balance of occlusal function is achieved by the consistence of these parameters and the COS (1, 5, 6). Condyle's horizontal position to occlusal plane has an effect on the COS (1). Graf Von Spee stated that maximum tooth contact could be achieved in chewing by means of this geometric arrangement (3, 7).

Chewing is the major function of maxillofacial component and effective chewing is related to crush/shear ratio of the teeth. In food processing, crush/shear ratio of buccal teeth increases, then efficient mastication can be achieved by virtue of COS. This indicates that, COS has also a mechanical function as well as its morphological function (7, 8). Although we have limited knowledge of the functional properties of the COS (1, 9), it is well known that increased depth of COS is frequently associated with deep-bite malocclusions (10).

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Andrews (11) described the six keys of the normal occlusion in 1972, and reported that the majority of the non-orthodontic normal individuals had a flat occlusion plane, therefore recommended that flattening the curve of Spee should be one of a goals of orthodontic treatment.

Considering the importance of flattening the COS for orthodontics, knowing the relationship between dental malocclusions and COS will allow us to treat these cases more successfully.

This study aims to compare depth of COS in Angle Class I, Angle Class II and Angle Class III malocclusions. The null hypothesis of this study was that there was no significant difference in the depth of curve of Spee between Angle Class I, II, and III cases. The null hypothesis was repeated 2 times, please delete the second sentence.

Materials and methods

Specimen characteristics and sample size calculation

Ninety plaster models were selected among the diagnostic materials of Istanbul Medipol University Department of Orthodontics. Ethics Committee of the same university approved the study with the number 10840098-604.01.01-E.15421. G*Power Ver. 3.0.10. was used for power analysis and it was found that 20 samples in each group would give the 80% power. Three study groups in equal numbers (n=30) were formed based on Angle classification (Class I, II, and III). The casts having fully erupted dentition except the third molars were enrolled in the study. Exclusion criteria for this study were as follows; previous orthodontic treatment, severe craniofacial disorders, posterior cross-bite, tooth anomaly, tooth wear, presence of occlusal fillings, presence of cusp fillings, presence of prosthetic restorations, and temporomandibular disorders. Mean age of the subjects was 13.60±1.03 years.

Model measurement

Manual measurement of the depth of COS was done with a digital caliper (Mitutoyo Corp, Kanogawa, Japan) and a plate (a flat plane). The plate was set on the mandibular plaster model as it was so as to touched the distal cusps of the second molars and incisal edges of the central incisors as described previously (4). The deepest cusp tip to the plate was recorded, using the digital caliper, and the depth of COS were recorded for each side. The right and the left sides were measured and mean value of them was used as the depth of COS. The measurement technique on models and cephalometric radiographs of the sample cases were presented in Figure 1 and 2. One hundred eighty measurements were performed for this study. Two weeks after the first measurement, 30 plaster models were randomly remeasured again by the same researcher to assess the method error (12).

Statistical analysis

A Statistical Software (IBM Corp. Released 2013. IBM Statistical Package for the Social Sciences Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.; USA) was used to evaluate the data. Shapiro-Wilks test was performed to check for normality. As the distribution met the assumptions of normal distribution, one way analysis of variance (ANOVA) test for multiple comparisons and Tamhane T2 or Tukey HSD post-hoc tests for detecting pairwise differences in the COS between malocclusions were used. Comparison of sides was performed by using paired samples t test. The results were presented as mean values (millimeter=mm) with standard deviations. The reliability of the measurements were checked with intra-class correlation (ICC) coefficient. Confidence level was set to 95% and $p<0.05$ was considered statistically significant.

Results

Errors of the measurements were 0.3 to 0.5 mm and ICCs were 0.941-0.973. Descriptive and analytic statistics for measurement of the COS are given in Table 1. In Class I malocclusion, mean depth of COS was 2.3±0.6 mm, in Angle Class II malocclusion mean depth was 2.9±0.8 mm and in Angle Class III malocclusion mean depth was 2.1±0.9 mm. Mean value both left and right sides in Angle Class II was found higher than that of the other classes ($p<0.001$). In Angle Class II malocclusion the COS was deepest and was flat in Class I and III malocclusion. The differences between Angle Class II malocclusion and the other Classes were statistically significant ($p<0.001$). No significant difference was found between Angle Class I and Class III malocclusions.

Discussion

Most orthodontic problems in orthodontic patients are accompanied by an increased curve of Spee. When planning an orthodontic treatment, the depth of curve of Spee should be added to the amount of space needed to correct the crowding. During fixed orthodontic treatment, crowding will be corrected and the curve of Spee will flatten. As a result of this, extra space for leveling and aligning of the teeth will be needed. Andrews (11) and other authors (13, 14) mentioned that intercuspation would be optimal in the presence of a flat plane of occlusion and therefore flattening of the COS should be one of the goals of orthodontic treatment, especially in deep overbite cases. The evaluation of COS in orthodontics is important because the depth measurement of COS is a part of space analysis, which is directly related to the planning of orthodontic treatment (14, 15). Although there are different opinions about the development of the COS, it is important to know in which type of malocclusion this curve is more severe. Therefore, this study was performed to assess the relationship between different sagittal dental malocclusions and the depth of the COS.

Curve of Spee measurements can be performed by using different orthodontic diagnostic materials. Plaster models, photographs, 3D study models can be used for measurements (16-18). The plaster models were used in our study due to easy measurement of that materials. Various methods have been reported to measure the depth of COS in the literature but there is little consensus about the measurement methods. Techniques such as taking the perpendicular distance on

Table 1. *Mean values of the depth measurement of curve of Spee stratified by malocclusions and sides*

	Combined Mean±SD (mm)	p	Right Mean±SD (mm)	Left Mean±SD (mm)	p
Class I	2.3±0.6[a]	NS	2.3±0.8[a]	2.3±0.7[a]	0.968
Class II	2.9±0.8[b]	<0.001	2.9±0.9[b]	2.9±0.7[b]	0.543
Class III	2.1±0.9[a]	NS	2.1±0.9[a]	2.0±0.9[a]	0.801
p			0.002	<0.001	

a, b: There is no difference in the measurements which were marked with the same letter; NS: Non-significant

Figure 1. *Positioning of the flat surface over the model (upper left) and measurement of the depth of the Spee curve with the digital caliper (lower left) in a patient with Angle Class II malocclusion (right).*

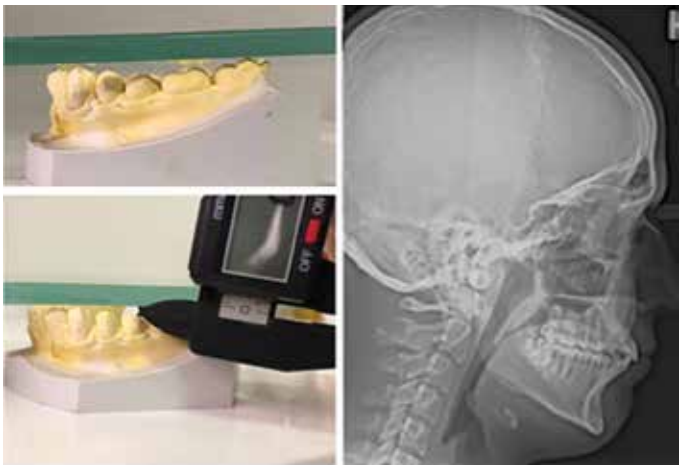

Figure 2. *Positioning of the flat surface over the model (upper left) and measurement of the depth of the Spee curve with the digital caliper (lower left) in a patient with Angle Class I malocclusion (right).*

left and right sides, perpendicular distance's average to each cusp tip (19), the sum of the perpendiculars (17) or the average of maximum depth on left and right sides (4, 13) were used previously to measure depth of COS. In the present study, the technique of Braun (4) was used.

Previous studies have stated that there was no significant change in the COS between the adolescence and adulthood periods. Therefore, models of the patients in permanent dentition period were included in the study (19-21).

Marshall *et al.* (7), evaluated the difference in the depth of COS between left and right sides of the arches and noted that there were no significant differences in the mean values of COS between the sides. In this study, we measured on the left and right sides as in the study of Marshall *et al.* (7) and our findings were consistent with their study. Previous researches presented no significant differences in depth of COS between genders (20, 22). Hence, the gender difference was not considered in our study.

Shannon and Nanda (23), evaluated the relationships between the Frankfort plane angle, deep bite, Class II molar relationship, mandibular plane angle, overjet, and depth of the COS in their study. They have observed a relationship between Class II molar relationship and depth of the COS and stated that significantly deeper curves were found in Class II molar relationship than that of Class I ones. In the present study the mean value of COS in Class II patients was found to be deeper than that of the other ones and this finding supports the results of Shannon and Nanda's study (22).

Veli *et al.* (22) assessed the relationship between COS and vertical eruption of teeth in different malocclusions and they indicated that the depth of COS was found as deepest in Angle Class II division 1 group, followed by Class II division 2, Class I and Class III groups. The authors have found difference between Angle Class I and Angle Class III groups, which is not consistent with our study (22). Veli *et al.* (22) also assessed the depth of COS for both of the left and the right sides, and reported no significant difference in depth of COS between the sides. This result was similar with the findings of the present study.

The increased depth of COS has previously been shown to have a major role in development of dental deep-bites (7). Baydaş *et al.* (24) assessed the relationships between incisor positions, overbite, overjet, lower anterior crowding and COS in their longitudinal study and they have found significant correlations between overjet, overbite and the COS. In a previous study, it was reported that increased overbite was present in Class II division 2 patients, therefore these patients may have deeper COS. (22). The relationship between increased overbite and the COS was revealed in previous reports and this finding emphasizes the need of correcting the COS in orthodontic treatment. Orthodontists should diagnose the amount of COS as a contributing factor of deep bite and try to flatten this curve especially in Angle Class II patients.

This study was designed as a cross–sectional retrospective research in which pre-treatment models were evaluated. Both the design of the study and measurement materials were the limitations of this study. Evaluation of longitudinal changes of the depth of COS could give more detailed knowledge about the issue. Furthermore, radiographic measurements could be added to model measurements to increase the effect of the study.

Conclusion

Curve of Spee in patients with Class I and Class III malocclusion are more likely to be flat but those with Class II malocclusion demonstrated deeper curve of spee with no side-related difference. Therefore, it can be concluded that the malocclusion in sagittal direction has an impact on the depth of curve of Spee.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Medipol University (Approval number: 10840098-604.01.01-E.15421).

Informed Consent: Written informed consent was obtained from patients/parents of the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: GS designed, generated and gathered the data. GS and HO analyzed the data. GS wrote the majority of the original draft. HO participated in writing the paper. All authors approved the final version of the paper.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Türkçe öz: *Farklı maloklüzyonlarda Spee eğrisinin değerlendirilmesi. Amaç: Bu çalışmanın amacı, Angle Sınıf I, Angle Sınıf II ve Angle Sınıf III malokluzyonlarda Spee eğrisinin derinliğini karşılaştırmaktır. Gereç ve Yöntem: Çalışmada kullanılan örnekler, İstanbul Medipol Üniversitesi Ortodonti Anabilim Dalı'nda bulunan tanı materyalleri arasından seçilmiştir. Angle dişsel maloklüzyon sınıflamasına göre seçilen doksan tane alçı model, 3 gruba (n=30) ayrılmıştır. Spee eğrisinin derinliği mandibular dental modeller üzerinde sağ ve sol tarafta ölçülmüş ve ortalama değerler Spee eğrisinin derinliği olarak kullanılmıştır. Normal dağılım gösteren verileri değerlendirmek için ANOVA testi kullanılmıştır. Taraflara göre değerlendirmede eşleştirilmiş örneklem t testi kullanılarak yapılmıştır. Anlamlılık düzeyi p<0,05 olarak ayarlanmıştır. Bulgular: Spee eğrisi derinliği Sınıf II maloklüzyonda (2,9±0,8 mm) en derin, Sınıf III maloklüzyonda (2,1±0,9 mm) göreceli olarak düz bulunmuştur ve aralarındaki fark istatistiksel olarak anlamlı bulunmuştur (p<0,05). Angle Sınıf I maloklüzyon ile Angle Sınıf III maloklüzyon arasında anlamlı farklılık bulunmamıştır. Sonuç: Spee eğrisi derinliği, Sınıf II maloklüzyon vakalarında artmış olduğu için bu vakaların tedavi planlamasında bu faktörün göz önünde bulundurulması gerekmektedir. Anahtar kelimeler: Spee eğrisi; maloklüzyon; Angle sınıflaması; mandibula; ortodonti*

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Original research

Evaluation of temperature rise following the application of diode and ErCr:Ysgg lasers: an *ex vivo* study

Purpose
Erbium, chromium: yttrium, scandium, gallium, garnet (ErCr:Ysgg) lasers have been frequently used in oral surgical procedures and are almost seen as alternatives to diode lasers. The aim of this comparative study was to analyze in an animal model the thermal elevation induced by ErCr:Ysgg and diode lasers in soft tissue and bone.

Materials and methods
Thirty freshly dissected sheep mandibles containing bone and soft tissue were divided into 120 equal parts. Gallium-aluminum-arsenide (Ga-Al-As) diode laser (λ=940 nm) with 1, 2 and 5 W output powers and ErCr:Ysgg laser (λ=2780 nm) with 2.75, 4.5 and 6 W output powers were used on soft and bone tissues separately for 3 seconds with point application. Mean temperature values before and after application of the lasers were compared in soft tissue and bone.

Results
The minimum mean temperature value was observed with 2.75 W ErCr:Ysgg laser while irradiation with 5 W diode laser created the maximum values ($p<0.05$).

Conclusion
ErCr:Ysgg laser (λ=2780 nm) with 2.75 W power generates low levels of heat compared to diode lasers and may provide safer surgery in soft and bone tissues without destructive effects of temperature increase.

Keywords: *Bone; diode laser; ErCr:Ysgg lasers; soft tissue; temperature rise*

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Introduction

Laser devices are alternative surgical instruments which are frequently used in oral surgical procedures such as frenectomy, periodontal and peri-implant surgery, gingival surgery and excision of soft tissue tumors (1-5). They emit coherent and homogeneous light which shows reflection, absorption, transmission and scattering when applied on biological tissues. Absorbed energy is tolerated by the tissue or transforms into other forms of energy such as heat and photochemical reactions (6).

Laser light may induce thermal damage in the surrounding tissues both on the horizontal and vertical plain in the oral cavity (1, 7). Increased heat causes structural changes and retraction in biological tissues up to 600°C. Protein denaturation and coagulation occurs when the temperature arises above 600°C, while tissue carbonization and charring are observed between 900-1000°C. Tissue ablation occurs when the temperature rises above 1000°C. Thermal destruction of the surrounding tissues by the laser light may lead to delayed wound healing compared to scalpel incision (7). An ideal laser should maintain the thermal threshold in acceptable levels and should not provoke thermal damage to the surrounding tissues.

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Diode and erbium, chromium: yttrium, scandium, gallium, garnet (ErCr:Ysgg) lasers are two different types of lasers which produce light in different wavelengths. It is reported that diode laser light induces thermal changes in the surrounding area of the related application site (8, 9). ErCr:Ysgg lasers have been frequently used in oral surgical procedures and are almost seen as alternative to diode lasers (9, 10). ErCr:Ysgg lasers are used with water irrigation and considered to create heat generation in decreased levels by the help of the irrigation system (10, 11). Although histologic effects of diode and ErCr:Ysgg lasers are well-known, the heat generation after the application of diode and ErCr:Ysgg lasers has not been studied yet. The aim of this study was to compare the thermal changes after the application of ErCr:Ysgg and diode lasers in soft tissue and bone. The null hypothesis tested in this research is that there is no difference in the thermal changes occurring in the soft tissue and bone after the application of ErCr:Ysgg and diode lasers.

Materials and methods

Study design

Ethical approval was obtained from the local Animal Research Ethics Committee of the Akdeniz University (Antalya, Turkey) with approval number 556. Thirty freshly dissected sheep mandibles with residual soft tissues of muscles were divided into 120 equal parts and were placed into a water tank of which the temperature was adjusted to 35-37°C using a glass heater (Kenis K-366; Kenis, Osaka, Japan) and hygro-thermometer (Nimomed; Estar Electronic Co., Ltd., Changshan, China) (Figure 1) to simulate oral tissues at body temperature. Each specimen was obtained from the body of the mandibles and comprised at least 5 mm soft tissue. Laser applications were performed in 6 hours after the specimens were obtained in order to maximize the usability of specimens. Diode and ErCr:Ysgg lasers with 3 different energy outputs were performed during 3 seconds on soft and bone tissue of the specimens separately. Gallium-aluminum-arsenide (Ga-Al-As) diode laser (Epic; Biolase, Irvine, CA, USA) (λ=940 nm) with 300 µm fibre tip was used with 1,2 and 5 W output powers. ErCr:Ysgg laser (λ=2780 nm) (Waterlase iPlus; Biolase, Irvine, CA, USA) was used with G4 fire tip (600 µm diameter, 6 mm length) at 2.75, 4.5 and 6 W with a 40% water and 20% air spray during irradiation. The temperature of irrigation water was adjusted to 25°C, according to the manufacturers' specifications. The dispersion of the specimens to the groups were shown in Table 1.

Probe of the thermocouple device (Keitley 2000 Digital Multimeter; Keithley Instruments, Inc., Cleveland, OH, USA) was inserted into the bone and soft tissue separetely at a distance of 3 mm to the laser application point (Figure 2). Each measurement was repeated 3 times before and immediately after the application of lasers and a mean value was calculated for all groups.

Statistical analysis

Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 22 (SPSS IBM Corp.; Armonk, NY, USA). The normality of distribution and the homogeneity of variances of the sample were established using visual inspection of histograms, QQ-plots, box plots and Shapiro-Wilk's test ($p<0.05$), respectively. The comparison of the mean temperature values of specimens before and after the application of diode and ErCr:Ysgg lasers was carried out by using t-test. The analysis of the significance between the mean temperature values of bone and soft tissues before and after the application of lasers was also performed with t-test. ANOVA was performed for comparison of the mean temperature values of specimens before and after the application of different power settings (1, 2, 5, 2.75, 4.5 and 6 W) of diode and ErCr:Ysgg lasers. Sidak's correction test was utilized for the post-hoc analysis. One-way multivariate analysis of variance (MANOVA) was also performed for determining whether the mean temperature was different between bone and soft tissue, laser types and several power settings. Post-hoc analysis was carried out with Bonferroni's correction test. The confidence interval was set to 95% and $p<0.05$ was considered significant.

Results

No statistically significant difference was observed between initial temperature measurements before the application of each laser. The temperature of specimens irradiated with diode laser was significantly increased compared to specimens irradiated with ErCr:Ysgg laser ($p<0.05$) (Table 2). There was no statistically significant difference between the mean temperature values of bone and soft tissues before and after laser application (Table 3).

Figure 1. *A thermal controller was used to adjust the temperature of the water close to the body temperature.*

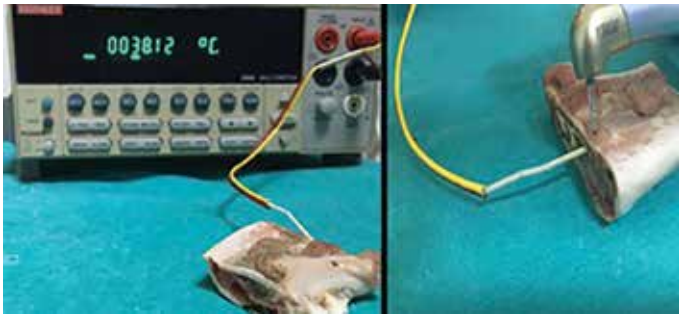

Figure 2. *3 mm distance was provided between thermocouple probe and laser application point.*

Table 1. *Duration and power settings of the laser devices applied on specimens*

Application Period	Laser Device	Tissue	Power
3 seconds	Diode (N=60)	Soft Tissue (N=30)	1W (N=10)
			2W (N=10)
			5W (N=10)
		Bone (N=30)	1W (N=10)
			2W (N=10)
			5W (N=10)
	ErCr:Ysgg (N=60)	Soft Tissue (N=30)	2.75W (N=10)
			4.5W (N=10)
			6W (N=10)
		Bone (N=30)	2.75W (N=10)
			4.5W (N=10)
			6W (N=10)

N: sample size; W: watt

Table 2. *Comparison of the mean temperature changes of diode and ErCr:Ysgg lasers before and after application*

Temperature	Tissue	N	Mean	SD	t	p
Initial	Bone	60	37.26	0.89	-0.42	0.68
	Soft Tissue	60	37.33	0.80		
Post-application	Bone	60	40.51	2.74	0.94	0.35
	Soft Tissue	60	40.04	2.76		
Difference	Bone	60	3.25	2.68	1.12	0.26
	Soft Tissue	60	2.72	2.54		

p<0.05 was determined as statistically significant: p value in bold emphasis shows that there was statistically significant difference between diode and Er, Cr:YSGG laser after the application; SD: standard deviation

Table 3. *Comparison of the mean temperature values of bone and soft tissues before and after the laser application*

Temperature	Laser	N	Mean	SD	t	p
Initial	Diode	60	37.43	0.74	1.69	0.09
	ErCr:Ysgg	60	37.17	0.93		
Post-application	Diode	60	41.53	3.06	5.57	0.00
	ErCr:Ysgg	60	39.03	1.64		
Difference	Diode	60	4.10	2.96	5.17	0.00
	ErCr:Ysgg	60	1.86	1.57		

SD: standard deviation; p<0.05 was determined as statistically significant

There was no statistically significant change in the mean temperature values between 6 different power settings of diode and ErCr:Ysgg lasers before the application (p>0.05). However, statistically significant difference was found between lasers after the application with different power settings (p<0.05). Post-hoc test revealed that mean temperature value of 2.75 W ErCr:Ysgg laser is significantly decreased compared to other power settings (p<0.05) (Table 4). While mean temperature values of 1 W diode laser and 4.5 W ErCr:Ysgg laser were significantly increased compared to 2.75 W ErCr:Ysgg laser (p<0.05), they were significantly decreased compared to 2 W diode laser, 5 W diode laser and 6 W ErCr:Ysgg laser (p<0.05). Mean temperature values of 2 W diode and 6 W ErCr:Ysgg lasers were significantly decreased compared to 5 W diode laser. Mean temperature values of 5 W diode laser was significantly increased compared to the mean values of remaining laser power parameters (p<0.05) (Table 5).

According to the MANOVA, the type of tissue and laser were found to have significant association with mean temperature difference on bone and soft tissue (F_{L*D}=19,91, p<0.05). Bonferroni correction test revealed that the application of diode laser generated significantly higher temperature difference on bone (4.21) and soft tissue (3.98) than the ErCr:Ysgg laser (1.44 for soft tissue and 2.28 for bone). Likewise, significant association was found between type of laser and power

Table 4. *The comparison of the mean temperature values before and after the application of diode and ErCr:Ysgg lasers with different power outputs*

Temperature	Power output	N	Mean	SD	F	p
Initial	Diode 1W (1)	20	37.32	0.70	0.87	0.50
	Diode 2W (2)	20	37.48	0.81		
	ErCr:Ysgg 2.75W (3)	20	37.33	0.90		
	ErCr:Ysgg 4.5W (4)	20	37.10	0.96		
	Diode 5W (5)	20	37.48	0.73		
	ErCr:Ysgg 6W (6)	20	37.08	0.96		
Post-application	Diode 1W (1)	20	38.74^{ad}	0.93	110.74	0.01
	Diode 2W (2)	20	40.44^{be}	1.06		
	ErCr:Ysgg 2,75W (3)	20	37.75^{ci}	0.89		
	ErCr:Ysgg 4,5W (4)	20	38.82^{af}	1.24		
	Diode 5W (5)	20	45.41^{g}	1.37		
	ErCr:Ysgg 6W (6)	20	40.53^{bh}	1.38		
Difference	Diode 1W (1)	20	1.42	0.70	148.40	0.01
	Diode 2W (2)	20	2.96	1.02		
	ErCr:Ysgg 2,75W (3)	20	0.42	0.54		
	ErCr:Ysgg 4,5W (4)	20	1.72	0.83		
	Diode 5W (5)	20	7.93	1.20		
	ErCr:Ysgg 6W (6)	20	3.45	1.33		

p<0.05 was determined as statistically significant. Mean values haring a superscript letter are not significantly different; SD: standard deviation

Table 5. *The comparison of the mean temperature values before and after diode and ErCr:Ysgg laser application with different power outputs*

Laser	Tissue	W	n	MD	SD	F_{L*D}	F_{L*W}	F_{L*D*W}
Diode	Bone	1	10	1.11^{c}	0.38	19.91	12.44	6.22
		2	10	3.51^{c}	0.38			
		5	10	8.03^{a}	1.41			
	Soft Tissue	1	10	1.73^{c}	0.81			
		2	10	2.41^{c}	1.17			
		5	10	7.82^{a}	1.00			
ErCr:Ysgg	Bone	2,75	10	0.35^{d}	0.30			
		4,5	10	2.05^{c}	0.71			
		6	10	4.45^{b}	1.02			
	Soft Tissue	2,75	10	0.49^{d}	0.71			
		4,5	10	1.39^{c}	0.84			
		6	10	2.45^{c}	0.70			

W: watt; n: sample size; MD: mean difference; SD: standard deviation *a>b>c>d. a-b-c-d indicates the different groups according to the pairwise comparison

output (F_{L*W}=12,44, p<0.05). Post-hoc analysis revealed that ErCr:Ysgg at 2.75 W (0.42) created significantly lower temperature difference on bone and soft tissue compared to the other power outputs (p<0.05). Diode laser at 5W (7.9) was found to have significantly higher temperature difference on bone and soft tissue compared to the other power outputs (p<0.05). The mean temperature difference on bone and soft tissue was ranking between diode laser at 5W(the highest), diode laser at 2W, ErCr:Ysgg laser at 6W, diode laser at 1W, Er,Cr:YSGG laser at 4.5W and Er,Cr:YSGG laser at 2.75W (the lowest), respectively.

The type of tissue, laser and power output were found to have significant association with mean temperature difference on bone and soft tissue (F_{L*D*W}=6.22, p<0.05) (Table 5). Post-hoc analysis revealed that diode laser at 5W and Er,Cr:YSGG laser at 6W created the highest temperature difference values among other groups. Diode laser at 5W created significantly higher temperature difference on bone (8.03) and soft tissue (7.82) compared to the ErCr:Ysgg laser at 6W (4.45 in bone and 2.45 in soft tissue). Furthermore, ErCr:Ysgg laser at 2.75W created the lowest temperature difference on both bone (0.35) and soft tissue (0.49) among the other groups.

Discussion

Soft tissue interventions with appropriate laser are beneficial compared to other surgical instruments such as scalpel and electrocautery (1, 5, 7). Diode lasers shows affinity to pigmented molecules in the affected tissue and mostly absorbed by haemoglobin, thus providing an advantage of suitability for soft tissue surgery (1, 8, 9). However, ErCr:Ysgg laser with 2780 nm wavelength is exceedingly absorbed by water and hydroxiapatite and can be safely used in both bone and soft tissues (2, 3, 9, 12).

Biological effects of thermal increase during the laser application have been reported both for diode and ErCr:Ysgg lasers (1, 7, 9). Cercadillo-Ibarguen *et al.* (10) reported that the microscopic extent of the thermal effect was lower after ErCr:Ysgg application compared to diode and CO_2 lasers in their study in which they used porcine mucosal membranes as experimental model. Furthermore, it was also suggested that ErCr:Ysgg laser incision was comparable to scalpel incision in the histological examination and thermal destruction caused by laser excision did not affect proper histological diagnosis when applied with distance to the examined pathology (10, 13). Similarly, Rizoiu *et al.* (14) suggested that soft tissue wound healing after ErCr:Ysgg laser application was comparable with scalpel incision in the histopathological examination. A temperature increase above 10°C is considered harmful for biological tissues and may provoke irreversible tissue damage. Geminiani *et al.* (15) reported that diode laser irradiation may increase the temperature above 10°C after 10 seconds application. However, in the study of Leja *et al.* (16) in which they investigated thermal changes of dental implants after laser application in vitro, it was reported that 810 nm and 980 nm diode lasers with 1 W power output did not increase the temperature to critical threshold of 10°C in 60 seconds application time. However, CO2 and Er:YAG lasers increased the temperature over the critical threshold.

In the current study, the highest temperature generation on soft and bone tissue was observed with 5 W diode laser. Similarly, Merigo *et al.* (7) also reported that the highest temperature elevation in deep soft tissue was observed after 5 W diode laser application and the lowest temperature elevation was observed after Er:YAG laser application. In the present study, ErCr:Ysgg laser was used with concomittant air-water spray similar to Er:YAG lasers and showed lower temperature change compared to diode laser. When using high energy outputs in lasers, concomittant cooling may be beneficial to reduce the accumulated heat on biological structures (17).

As an interesting outcome of the study, 6 W ErCr:Ysgg laser showed less heat generation compared to 5 W diode laser, as the increase in the heat production did not correlate with the increase in the power output of the laser. Similarly, 1 W diode laser generated higher levels of heat compared to 2.75W ErCr:Ysgg laser, indicating that heat generated with different laser types differed with different power outputs.

A previous study reported that ErCr:Ysgg laser was superior to diode laser with regards to the measurement of the damaged area adjacent to the laser incision (10). In the present study, following the application of both ErCr:Ysgg and diode lasers on soft and bone tissue, 2.75 W ErCr:Ysgg laser group showed the lowest heat generation among other ErCr:Ysgg and diode laser settings. Diode lasers should be used with special care due to their capability of penetrating deeper in the soft and bone tissues and causing irreversible damage (7, 15).

Conclusion

Present study revealed that diode laser (λ = 940 nm) with 5 W power output produced elevated levels of temperature leading to the thermal damage to soft and bone tissues and ErCr:Ysgg laser (λ = 2780 nm) with 2.75 W power generated low levels of heat compared to diode lasers. The findings of the current study support the idea that the use of ErCr:Ysgg laser with 2.75 W power may provide safer surgery regarding destructive effects due to temperature increase.

Ethics Committee Approval: Ethics committee approval was received for this study from the local Animal Research Ethics Commitee of the Akdeniz University (Antalya, Turkey) with approval number 556.

Peer-review: Externally peer-reviewed.

Author Contributions: AS designed the study. ÖD, ÖÖ and BK generated the data. MH and OND participated in gathering the data for the study. AS and AÖ analyzed the data. ÖD and ÖÖ wrote the majority of the original draft. AS, MH and AÖ helped writing the paper. OND and BK collected the raw data of the study. All authors approved the final version of the paper.

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Türkçe öz: *Diyot ve ErCr:Ysgg lazer uygulamalarını takiben sıcaklık artışının değerlendirilmesi: ex vivo çalışma. Amaç: Erbiyum, krom: itriyum-skandiyum-galyum-garnet (ErCr:Ysgg) lazerler oral cerrahi işlemlerde yaygın biçimde kullanılmakta olup, diyot lazerlere neredeyse alternatif olarak düşünülmektedir. Bu çalışmanın amacı, ErCr:Ysgg lazerler ve diyot lazerlerin kemik ve yumuşak dokuda meydana getirdikleri sıcaklık artışının bir hayvan modeli üzerinde karşılaştırılmasıdır. Gereç ve Yöntem: Otuz adet koyun mandibulası her biri kemik ve yumuşak doku içeren 120 eşit parçaya bölünmüştür. Yumuşak doku ve kemik üzerine ayrı ayrı olacak biçimde, 3 saniye süre ile 1, 2 ve 5 W çıkış güçlerinde galyum-aluminyum-arsenid (Ga-Al-As) diyot lazer (λ=940 nm) ve 2,75, 4,5 ve 6 W çıkış güçlerinde ErCr:Ysgg lazer (λ=2780 nm) uygulaması gerçekleştirilmiştir. Uygulama öncesi ve uygulamadan hemen sonraki ortalama sıcaklık değerleri karşılaştırılarak veriler analiz edilmiştir. Bulgular: En düşük ortalama sıcaklık değeri 2,75 W gücünde Er,Cr:YSGG lazer uygulamasında gözlenirken, 5 W gücünde diyot lazer uygulamasının en yüksek sıcaklık değerini oluşturduğu görülmüştür ($p<0,05$). Sonuç: ErCr:Ysgg lazerin (λ=2780 nm) 2,75 W güçte uygulanması, diyot lazerlerle kıyaslandığında daha düşük sıcaklık artışına neden olmakta ve sıcaklık artışının neden olabileceği yıkıcı etkiler bakımından, kemik ve yumuşak doku cerrahilerinde daha güvenli sonuçlar sağlayabileceği düşünülmektedir. Anahtar kelimeler: Diyot lazer; ErCr:Ysgg lazer; kemik; sıcaklık artışı; yumuşak doku.*

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Original research

Patients' perceptions and preferences of oral and maxillofacial surgeons in a university dental hospital

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İpek Kaçar

Purpose
There is a lack of information regarding the dental patient's point of view of oral and maxillofacial surgeons (OMFSs). The aim of this study was to evaluate the perceptions and preferences of a group of university dental hospital patients for OMFSs.

Materials and methods
This study was based on patients' self-assessment using a questionnaire. A total of 530 patients were enrolled for the study. The patients' preferences regarding the surgeons' age, gender, religion, race and experience were determined and compared statistically.

Results
A total of 506 questionnaires were considered as complete and used in the analysis. Female patients preferred female practitioner more than male patients did (p=0.002), but no significant difference was found between male and female patients regarding preference for the age (p=0.464), ethnicity (p=0.926) and religion (p=0.261) of the OMFS. The educational status of the patients did not have an effect on the gender preference for the OMFS (p=0.114); however, educational status significantly affected the preferences for the ethnicity and religion of the practitioners (p=0.001).

Conclusion
Today patient expectations and perceptions take place in post graduate education programs. In this study we determined a wide range of different factors for choosing an OMFS. The diversity of these factors may affect the quality of the health service and thus must be considered in determining the content of oral and maxillofacial curriculum.

Keywords: *Educational status; patient perception; patient preference; practitioner gender; oral and maxillofacial surgeon*

Introduction

Each medical practitioner has a unique personality profile depending on his/her birthplace, cultural status, and living conditions, as well as financial status. This variety of factors influences patients' demands and the service provided by the health care professionals; therefore, there appears to be a great challenge to deliver an effective and patient-sensitive health service. A few studies have focused on the demographic details of the medical and dental health care providers by considering patients' preferences and perceptions. Patients' preferences for their dentist mainly focus on the health care professionals' ability to communicate, express empathy, and manage pain. Other personal factors including gender, age, ethnicity, and attire may also influence the patient's perception (1-8). Besides, the physical environment of the dental office or quality of the

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service given (timeliness, communication, following recent scientific developments) may play a role in the patients' decision when choosing a dentist (1, 4, 5, 9). All of these aforementioned factors may affect the patient's decision or may be perceived as reflecting the 'quality of the dental service'. The quality of the health service is defined as a multifactorial concept including patient safety, patient experience, access and clinical effectiveness (1, 2, 5, 9). Standards and guidelines regarding health service quality focus on patient oriented, efficient and equitable facilities from which all patients can benefit.

Recent studies have mainly focused on the qualifications of general dentists; however, to our knowledge there is no previous research regarding the interaction between the patient and the oral and maxillofacial surgeons (OMFS). The aim of this study was therefore to evaluate patients' perceptions of and preferences of OMFS in a university dental hospital. The null hypothesis of the study is: OMFS related variables have no effect on the patients' perception and preferences.

Materials and methods

Sample selection

This study used a randomized, experimental, between-subjects design to assess patients' preferences and perception of OMFSs. Ethical approval was obtained from the İstanbul Medipol University ethical committee (No: 108400987-75). Between the years of 2015 and 2016, a total of 530 patients whom had been referred to oral and maxillofacial department of İstanbul Medipol University Dental Hospital with an initial treatment plan indicating the need for oral surgical procedures were randomly included in the study. Subjects who are illiterate, undergoing psychiatric/psychological therapy and/or psychiatric medication, under 18 years of age and unwilling to participate in the study were excluded.

Administration of the questionnaire

As our hospital is located in the center of Istanbul, it can be considered an urban academic dental center. Patients were randomly selected among those who had been given an appointment by the oral and maxillofacial surgery department but not yet seen an OMFS. Consenting patients completed the questionnaire in the waiting room prior to their first visit to our clinic in order to prevent bias that may result from the treatment outcome and from their impression of attendant OMFS. The multiple choice questionnaire consisted of three sections. The first part included basic demographic information about the participants; (i.e, age, gender, educational level). The second part included the presence of a dentist or an OMFS in the family, and any history of oral and maxillofacial surgical intervention. The third part consisted of questions and statements to assess the patients' preferences for, and perceptions of an OMFS such as gender, age, race, religion, professional experience and so on.

Statistical analysis

The study's data were assessed using the IBM Statistical Package for the Social Sciences (SPSS) Statistics 22 program (IBM Corp.; Armonk, NY, USA). The compliance of the variables with a normal distribution was assessed using the Shapiro-Wilks test. Descriptive statistical methods (mean, standard deviation, frequency) were used and one-way Anova and Tukey's Honestly Significant Difference post-hoc test were employed in the comparison of the quantitative data and comparison of inter-group data. Student's t test was used to compare two groups showing a normal distribution. For the comparison of the qualitative data, the X^2 test, Fisher's exact test and Yates Continuity Correction were used. The level of $p<0.05$ was considered statistically significant.

Results

Twenty-four forms were excluded during the assessment procedure due to incomplete questionnaires or patients' voluntary withdrawal from the study. Consequently, 506 questionnaires were considered as complete and were used in the analysis. The age range of the patients varied from 18-75 years with a mean age of 33.7±12.38 years. One hundred and ninety-three patients were males (38.1%) whereas 313 were females (61.9%). The educational background of the patients was primary school graduate (n=128, 25.3%), high school graduate (n=158, 31.2%), university graduate (n=197, 38.9%), and postgraduate (n=23, 4.5%).

Eighty patients stated that there is a general dentist in their family (15.8%), an OMFS (n=15, 3%), both (n=31, 6.1%), none (n=344, 68%), or that had no idea (n=36, 7.1%). The majority of patients previously had an oral and maxillofacial surgical procedure (n=366, 72.3%), whereas others either had not had such an operation (n=129, 25.5%) or did not remember at that time (n=11, 2.2%) (Table 1).

Regarding the physical and professional features of an OMFS, 64 patients preferred a physically strong OMFS (12.6%), 419 (82.8%) preferred a staff OMFS (rather than a post graduate student), 169 (33.4%) preferred an OMFS that was suggested by the family or friends and 81 (16%) preferred a competent OMFS whereas 22 (4.3%) could not decide (Table 2).

Fifty patients (9.9%) preferred a female OMFS, 77 (15.2%) preferred a male OMFS, 357 (70.6%) stated they would accept both genders and 22 (4.3%) could not decide. When the patients' age preference for the OMFS was analyzed, 196 patients (38.7%) preferred an OMFS between 35-50 years of age, 71 (14%) preferred an OMFS between 25-35 years of age, 28 (5.5%) preferred an OMFS between 50-65 years of age and two (0.4%) preferred over 65 years of age, whereas 188 (37.2%) declared that the age of the practitioner did not matter and 21 (4.2%) were hesitant. Of all of the patients 341 (67.4%) indicated that the ethnic background of the OMFS did not matter, 140 (27.7%) indicated that being of the same ethnicity was an important factor in their preference for an OMFS, whereas 25 patients (4.9%) were hesitant. The religion of the OMFS was not indicated to be an important factor by some patients (n=328, 64.8%), whereas others preferred an OMFS of the same religion as their own (n=151, 29.8%) and the other patients were hesitant (n=27, 5.3%) (Table 3).

Table 1. *Demographic characteristics of the patients and their previous experience towards oral and maxillofacial surgery*

		n	%
Age (years)			
	14-19	45	8.9
	20-29	176	34.8
	30-39	133	26.3
	40-49	93	18.4
	Over 50	59	11.7
Gender			
	Male	193	38.1
	Female	313	61.9
Educational status			
	Primary school	128	25.3
	High school	158	31.2
	University	197	38.9
	Post graduate	23	4.5
Any general dentist/OMFS in the family or among friends?			
	General dentist	80	15.8
	OMFS	15	3
	Both	31	6.1
	None	344	68
	No idea	36	7.1
Previously OMF surgical procedure?			
	Yes	366	72.3
	No	129	25.5
	No idea	11	2.2

OMFS: oral and maxillofacial surgeons

Table 2. *Patients' preferences for oral and maxillofacial surgeons*

	n	%
Physically strong	64	12.6
A university staff	419	82.8
Suggested by the family or friends	169	33.4
Middle aged competent	81	16
No idea	22	4.3

There was a significant difference between male and female participants when considering the gender preference for an OMFS. Female patients preferred a female practitioner more than male patients did (p=0.002), but no significant difference was found between male and female patients considering the preference for the age (p=0.464), ethnicity (p=0.926) or religion (p=0.261) of the OMFS.

The educational status of the patients did not make a difference in the gender preference for the OMFS (p=0.114); however, their educational status did significantly affect preference for the ethnicity and religion of the practitioners (p<0.05). Primary school graduates significantly preferred an OMFS of the same ethnicity as their own (p=0.001). Furthermore, primary school graduates preferred an OMFS of the same religion as their own (p=0.001).

There was no significant difference between male and female patients in terms of age preference for the practitioner (p=0.464). Regarding educational status of the patients, there was not a significant difference between the education of the patients and the preference for the practitioner's age (p=0.07).

The gender of the patients did not significantly affect preference for a physically strong OMFS (p=0.698), a staff OMFS (p=0.158), or a competent OMFS (p=0.636); however, female patients preferred an OMFS that was suggested by the family or friends significantly more than males did (p=0.042). The education status of the patients did not significantly affect preference for a physically strong OMFS (p=0.813), or an OMFS that was suggested by the family and friends (p=0.176); however, the preference rate for a competent OMFS (p=0.003) or a staff OMFS (p=0.006) was significantly lower in primary school graduates than in the other school graduates.

Discussion

The factors affecting a patient's preference for a health care provider may influence their health needs, quality of life,

Table 3. *Demographic characteristics of an oral and maxillofacial surgeons preferred by the patients*

		n	%
Practitioner's gender			
	Female	50	9.9
	Male	77	15.2
	No difference	357	70.6
	Hesitant	22	4.3
Practitioner's age (years)			
	25-35	71	14.0
	35-50	196	38.7
	50-65	28	5.5
	Over 65	2	0.4
	No difference	188	37.2
	Hesitant	21	4.2
Practitioner's ethnicity			
	The same ethnicity	140	27.7
	No difference	341	67.4
	Hesitant	25	4.9
Practitioner's religion			
	The same religion	151	29.8
	No difference	328	64.8
	Hesitant	27	5.3

and satisfaction. The factors reported to be important when choosing a doctor are the doctor's competence, recommendation from someone well-known to the patient, quality of the service and interpersonal factors (1, 4, 10, 11). Patient perception and satisfaction are becoming more crucial when assessing the quality of the medical service provided. Patients take into account a variety of factors when choosing a health care provider (2, 12, 13). Patient satisfaction is multifactorial and the demographic features of the dental practitioner influence the preference and perception of the patients. Various factors have been investigated regarding patients' preference for doctors, including willingness to disclose information and discuss symptoms and general aspects of the doctor-patient relationship. Patients' tendency to prefer same-sex practitioners as well as their consideration of their ethnic background, communication skills, and experience are among the most emphasized points. Satisfaction and trust are interrelated concepts; the more the patient is satisfied, the more they trust the practitioner, thereby facilitating the performance of medical treatment and reducing anxiety (1, 2, 6-9, 12).

Ungureanu *et al.* (1) investigated factors affecting dental patients' choice of dentists. The most mentioned factors were the dentist's competence (22.22%), recommendation from someone known to the patient (20.56%) and quality of the service provided (19.72%). They applied their results to modify dental curricula in order to train dental students in a manner that will meet patients' expectations and increase patient satisfaction. The patient-doctor interaction should be emphasized during professional graduate and postgraduate education. In a study conducted among parents of orthodontic patients, the parents showed a positive preference for orthodontists to wear formal attire or scrubs, to have controlled hair, and to have a nametag, and preferred younger women and older men (14). In our study, we did not evaluate patients' preference for OMFS attire, but the physically strong appearance of the surgeon did not influence the patients' choice.

The same-gender preference for health care providers among modern societies is relevant in up to 35% of patients (15). In this study, female patients preferred a female OMFS more than male patients did, which is probably due to better communication skills or a closer relationship between women. Furthermore, the conservative and Islamic nature of the general population may cause the hesitation of the female patients for male doctors. A study by Smith *et al.* (16) confirmed our results that indicate that the preference for female dentists by the patients may be attributable to empathy skills and the more time they spend with their patients. An interesting point is that it may be only female patients in our study who preferred female practitioners more compared to males. The general belief regarding female practitioners is that, they have better personal and emotional skills and the patients may participate more in consultations with female doctors (2, 3, 10, 12, 16).

The practitioner's age may influence the patient's preference, but there is a lack of evidence. Patients may prefer middle-aged or older practitioners because they feel that medical experience increases with age (2, 5, 14). Another question that needs to be answered is whether patients prefer practitioners

who are a similar age to themselves. Two studies have reported that patients prefer younger dentists which may be related to their up-to-date knowledge and following of recent scientific developments (12, 17).

One important issue in both the medical and dental health service is the ethnic and religious disparities. In this study the ethnic background of the practitioner included belonging to a specific ethnic society or language differences. Research studies suggest that patients are more satisfied if they have the opportunity to choose their practitioner (6, 8, 10). There is evidence that patient-doctor racial concordance exists to some extent in medicine and dentistry. Geographical accessibility, using the same language or socioeconomic status may be factors that influence same race preference. In the literature, it is suggested that patients particularly females tend to prefer practitioners from the same ethnic group so that they can communicate better and feel more relaxed (4-6, 8). The language and cultural concordance between the patient and practitioner is reported to be more important than the gender of the practitioner (5). A practitioner with similar values can be more reliable for the patients. In this study we noticed that the educational status of the patients affected their search for concordance with practitioners in terms of their ethnic background and religious beliefs. The ethnic and religious concordances were more important among primary school graduates than other school graduates. This may be due to an increase in conservatism and traditionalism that is parallel with a decrease in the level of education. In a study by Abghari *et al.* (18) it was indicated that a possible correlation existed between the increasing education level of the patient and the quality of training of their orthopedic surgeon. We may assume that as the level of education increases, patients have more demands related to quality and prestige, but those who had a lower education seek for more conservative factors. Some research results suggest that empathy and communication skills are also important for some patients besides the race or gender of the practitioner (4, 19, 20). The cultural competency curricula in postgraduate oral and maxillofacial surgery education should be redesigned or initiated if it is not present, taking into account the outcome measures of cultural competency instructions and patients' expectations.

Shah and Ogden (21) have emphasized that patients no longer perceive practitioners only in terms of being health care providers, but also consider the practitioner's social characteristics when making decisions. Patients preferences for and perceptions of an OMFS may be important for establishing reliable and good patient-doctor relations. This trust-based relation may enhance the quality of the health service and help to meet the needs of the patients.

Conclusion

Oral and maxillofacial surgery comprises of a multidisciplinary approach that has both dental and medical components. In some countries it is included in only dental programs, whereas in some countries both dental and medical degrees are required. Both undergraduate and graduate medical education should focus on patients' perception and preferences to build reliable interaction between practitioners and patients. Communication skills gain importance in this aspect; therefore, dental education programs need to be re-evaluated accordingly.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Medipol University (No: 108400987-75).

Informed Consent: This is a questionaire study. The participants joined to the study on voluntary basis. We have ethical approval but we did not need informed consent from the patients.

Peer-review: Externally peer-reviewed.

Author Contributions: GG and ÇD designed the study and generated the data. İK gathered and analyzed the data. GG and ÇD wrote the paper. All authors have approved the final version of this paper.

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Türkçe öz: *Bir üniversite diş hastanesine başvuran hastaların oral ve maksillofasiyal cerrah algıları ve tercihleri. Amaç: Diş hekimliği hastalarının oral ve maksillofasiyal cerrahlar (OMFC) ile ilgili görüşlerine yönelik veri eksiktir. Bu çalışmanın amacı bir üniversite diş hastanesine başvuran bir grup hastanın OMFC ile ilgili algı ve tercihlerinin araştırılmasıdır. Gereç ve Yöntem: Bu çalışma özel hazırlanmış bir anket kullanılarak hastalardan elde edilen veriler üzerinde yapılmıştır. Toplam 530 hasta çalışmaya katıldı. Hastaların cerrahın yaşı, cinsiyeti, dini, etnik kökeni ve deneyimine yönelik tercihleri belirlenerek istatistiksel olarak karşılaştırıldı. Bulgular: Toplam 506 anket tam kabul edildi ve değerlendirmeye alındı. Kadın hastalar erkek hastalara göre daha fazla kadın cerrah tercih etmekteydi (p=0,002) ancak, kadın ve erkek hastalar arasında cerrahın yaşı (p=0,464), etnik kökeni (p=0,926) ve dinine (p=0,261) ilişkin anlamlı bir tercih farkı yoktu. Hastaların eğitim seviyesi ile cerrahı cinsiyetine göre tercih etmek arasında anlamlı bir ilişki yoktu (p=0,114); ancak hastaların eğitim seviyesinin, cerrahın etnik kökenini ve dinini dikkate alarak tercih etme açısından anlamlı etkisi bulundu (p=0,001). Sonuç: Günümüzde mezuniyet sonrası eğitim programlarında hastaların beklentilerine ve algılarına yer verilmektedir. Bu çalışmada hastaların OMFC seçiminde pek çok faktörün etkili olduğu görüldü. Bu faktörlerin değişkenliği verilen sağlık hizmetinin kalitesini etkileyebilmektedir ve bu nedenle oral ve maksillofasiyal cerrahi eğitim programının içeriği belirlenirken göz önünde bulundurulmalıdır. Anahtar kelimeler: Eğitim seviyesi; hasta tercihi; hasta algısı; hekim cinsiyeti; oral ve maksillofasiyal cerrah*

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Original research

The effect of *Hypericum Perforatum* on wound healing of oral mucosa in diabetic rats

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Purpose
This study aims to investigate the histopathological and biochemical effects of the topical application of *Hypericum perforatum* on the healing of surgical wounds created in the oral mucosa of rats with experimentally induced diabetes mellitus.

Materials and methods
The study was carried out on 48 adult male Wistar albino rats. The animals were divided into two main groups as control and study groups. Two main groups were divided into three subgroups according to the sacrification days. All rats were given streptozotocin 60 mg/kg, after 72 hours, and those having blood glucose levels above 200 mg/dL were included in the study. Mucosal defects were created in the palatal area of the rats. *H. perforatum* oil was applied topically twice a day to the wounds of the rats in the study group. Animal were sacrificed on the 3rd, 7th and 10th days and samples taken from the palatal wounds were examined histologically and biochemically.

Results
On the 7th day, ulceration, necrosis, epithelialization, polymorphonuclear leucocytes and hydroxyproline variables showed statistically significant differences ($p<0.05$). Ulceration, necrosis and polymorphonuclear leukocytes values were higher in the control group, whereas epithelialization and hydroxyproline values were found to be higher in the *H. perforatum* group. Among 10th-day groups, there was only a statistically significant difference between the values of hydroxyproline, whereas *H. perforatum*-treated group showed high hydroxyproline levels ($p<0.05$).

Conclusion
Topically applied *H. perforatum* did not create any difference on the 3rd day, but it has positively affected the wound healing on the 7th and 10th days in diabetic rats.

Keywords: *Diabetes mellitus; H. perforatum; oral mucosa; St. John's Wort; wound healing*

Introduction

Wound healing is characterized by the unification of epithelial cells, endothelial cells, inflammatory cells, platelets and fibroblasts, and the fulfilment of their normal functions in a specific sequence and order. Many people in the world are known to suffer from chronic wounds. There are many systemic and local factors affecting wound healing. Diabetes mellitus is a chronic metabolic disease with negative effects on wound healing. Diabetes has the effect of reducing wound tension and hydroxyproline levels. Problems encountered during wound healing in diabetic patients include decreased cellular infiltration, granulation tissue, angiogenesis and the amount and formation of collagens, resulting in increased infectious complications. Even though the causes of these problems seen in diabetes cannot be fully explained, hyperglycemia is generally held responsible for

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this situation. High blood glucose levels have been shown to inhibit cell proliferation and collagen production. Moreover, conditions such as the reduction of growth factors and fibroblast proliferation, apoptosis increase in wound tissue cells, infections caused by the decrease in chemotaxis and phagocytosis, have shown to be adverse effects of hyperglycemia on wound healing as well (1).

Plants have historically played an important role in the recovery of human health. *Hypericum perforatum*, a member of the Hypericaceae family, has been considered a valuable herbal medicine. This plant contains hyperforin, flavonoids and hypericin (2). Olive oil extract of *H. perforatum* has long been used both topically and orally as a homemade remedy to treat cuts, burns, depression, haemorrhoids, diabetes and gastrointestinal ulcers (3). Damlar *et al.* (4) reported that *H. perforatum* improve bone healing in defects filled with bovine-derived xenograft. The studies showed that *H. perforatum*, increased collagen deposition, shortened the duration of inflammation and stopped the migration of fibroblasts during wound healing (5).

The aim of this study to investigate the histopathological and biochemical effects of the topical application of *H. perforatum* on wound healing in the oral mucosa of experimentally induced diabetic rats. The null hypothesis tested in this study is that the topical application of *H. perforatum* does not have any histological and/or biochemical effect on the healing of surgical defects created in the palatal mucosa of diabetic rats.

Materials and methods

Animals

This study was conducted with conformity approval from the Animal Ethics Committee of Mustafa Kemal University (No: 2014017), regarding the ethical treatment of animals. 48 adult male Wistar albino rats were used, with an average age of 5 months and weight of 300–350 g. The experimental animals were given water ad libitum and standard rat chow, and they were kept in metal cages at room temperature, with a 12-hour light/dark cycle. Streptozotocin (STZ) was used to create experimentally induced diabetes. The dosage was calculated as 60 mg/kg, and a single dose of STZ was intraperitoneally injected into each rat. 72 hours after the STZ injection, blood glucose levels were determined from blood samples taken from the tail vein. The rats with blood glucose levels above 200 mg/dL were considered diabetic and those below this value were excluded from the study. Forty-eight rats were randomly divided into 2 groups of equal numbers as the study and control groups. *H. perforatum* oil was applied topically to rats in the study group two times daily, whereas the wound healing of rats in the control group was followed without any intervention. The animals were sacrificed on the 3rd, 7th and 10th day with cervical dislocation.

Surgical procedure

Prior to wound formation, the rats were anesthetized by intramuscular injection of 30 mg/kg ketamine-HCL and 5 mg/kg xylazine HCL. Next, 4-mm mucosal defects were created with round, stainless steel blades designed for punch biopsy in the palatal area of each rat (Figure 1). Following bleeding control, the wounds were left for secondary healing. The first day of formation of the wounds was recorded as day zero.

Preparation of traditional Hypericum perforatum oil

Natural *H. perforatum* flowers were collected, from Çamlıyayla, Mersin, dried and pulverized, and 50 g of *H. perforatum* was placed in a transparent glass jar containing 500 mL of olive oil. This jar was kept in the sunshine for 4 weeks during summer for 12 hours a day (3). It was observed that the red dye of the herb diffused into the olive oil. Gas chromatograph-mass spectrometer (GC-MS) and high-performance liquid chromatography (HPLC) (Shimadzu QP2010 Ultra, Kyoto, Japan) were used for analysis of the components of *Hypericum perforatum* oil extract. Results were compatible with previous studies (6, 7). Naftodiantron (0.06-0.4%), phenylpropanes (<1%), flavonoids (2-4%), proanthocyanidins (2-4%), phloroglucinols (0.2-4%) and biflavons represent the main components of this plant. The essential oils were listed as palmitic acid (17.61%), cyclopentadecanone, 2-hydroxy- (66.75%), heneicosane (2.27%) and cyclopropaneoctanoic acid (2.53%).

Histopathological evaluation

From all groups, samples of full thickness mucosa were collected from palate wound formations on the 3rd, 7th and 10th day, including robust tissue of 1 x 1 cm. The samples were fixed in 10% formalin solution for 48 hours and embedded in paraffin. The prepared slides were stained with hematoxylin-eosin and evaluated by light microscopy for monitoring inflammatory changes in the healing wounds and to examine the morphology. Histopathological analysis was performed at the Department of Pathology, Mustafa Kemal University by a single blinded examiner. Ulceration, necrosis and epithelialization variables were evaluated as present (1) and absent (0), whereas oedema, polymorphonuclear leukocytes (PNLs), mononuclear cells (MNLs), fibroblasts and vascularization were scored and evaluated as absent (0), low (1), moderate (2) or severe (3).

Figure 1. *Rat palatal wound after surgery.*

Biochemical examination

The hydroxyproline determination on the palate samples from the 3rd, 7th and 10th days was performed by partly modifying the method that was described by Woessner (8). In this method, chloramine T is neutralized with perchloric acid prior to reaction with p-dimethylaminobenzaldehyde (Ehrlich's reagent). The tissue samples were mixed with 1 mL buffered chloramine T reagent and incubated for 20 min at room temperature. Then, 1 mL perchloric acid was added and the mixture was incubated for a further 5 minutes at room temperature before addition of 1 mL Ehrlich's reagent. The mixture was heated for 20 minutes at 60ºC, cooled, and then absorbance was determined at 550 nm. The hydroxyproline concentration (1 mg/mL in 1 mM HCl) was determined.

Statistical analysis

IBM Statistical Package for the Social Sciences Statistics for Windows, version 20.0 (IBM Corp.; Armonk, NY, USA) was used for statistical analysis of the data. Mean, standard deviation, median and frequency were used for descriptive statistics. The consistency of the variables with normal distribution assumptions were tested with the Shapiro–Wilks test. In a general comparison of measurements of the groups with normal distribution, Student's t-test was used. The Mann–Whitney U test was used to compare abnormally distributed variables. Evaluation of categorical variables was performed with the chi-square test. Confidence interval was set to 95% and p values less than 0.05 were considered as statistically significant.

Results

The results of the statistical analysis showed that on the 3rd (Figure 2) and 10th days, no statistical difference between the two groups was found in any of the variables evaluated by histopathologic examination. On the 7th day, necrosis, epithelialization, ulceration and PNL parameters showed statistically significant differences ($p<0.05$) (Figure 3). It was found that the ulceration, necrosis and PNL values of these variables were higher in the control group, whereas the epithelialization value was found to be higher in the *H. perforatum*-administered group (Table 1).

On the 3rd day, no statistically significant difference between the two groups was found for the hydroxyproline level assessed by biochemical analysis ($p>0.05$). On the 7th and 10th days, the comparison between groups showed statistically significant differences ($p<0.05$). It was found that the amount of hydroxyproline was higher in the *H. perforatum*-administered group on the 7th and 10th days (Figure 4).

Discussion

H. perforatum, which is commonly used to accelerate the healing of burns and wounds, has been proven to have a positive effect on wound healing (5,9,10). In the literature, no studies were found about *H. perforatum* regarding its effect on wound healing in the oral mucosa in the presence of diabetes mellitus. The biologically active compounds in the structure of this plant can be listed as naftodiantron, phloroglucinol, flavonoids, biflavons, phenylpropane and proanthocyanidins. These active compounds were examined separately but; our study has investigated the traditional use of *H. perforatum* rather than the separate effects of these substances.

H. perforatum accelerates the migration of fibroblasts, shortens the period of inflammation, increases the collagen deposition and induces better epithelialization (5,11). However, in diabetes mellitus, studies showing the effect on wound healing are limited. Yadollah-Damavandi *et al.* (12) reported the number of fibroblast cells increased in diabetic rats that were treated with *H. perforatum* compared to the control group. Farsak *et al.* (13) claimed *H. perforatum* and olive oil decreased the oxidative stress in the diabetic tissue permitting better healing. In our study, topical application of *H. perforatum* positively affected the wound healing in experimentally induced diabetic rats on the 7th day. We determined a statistically significant decrease in the 7th-day *H. perforatum* group compared to the control group regarding necrosis and ulceration variables.

Rao *et al.* (14) compared the effectiveness of *H. perforatum* and Calendula plants in a study conducted on albino rats. At the end of the study, the group treated with *H. perforatum* completed epithelialization in 15 days, whereas this duration was 16.5 days in the group treated with Calendula. Lavagna *et al.* (10) investigated the effect of the combined use of Calendula and *H. perforatum* oils (30:70 ratio) in a study conducted on 24 female patients who had given birth by caesarean section. They found a significant reduction in wound surface area compared to the control group. Suntar *et al.* (5) reported in an in vivo study on wound healing that *H. perforatum* had positive effects on epithelialization. In the present study, the results obtained on the 7th day were statistically significant. Accordingly, the level of epithelialization in the topical *H. perforatum*-administered group was higher. Our results showed that the topical administration of *H. perforatum* increased epithelialization in experimentally induced diabetic rats.

Menegazzi *et al.* (15) reported that the methanol extract of *H. perforatum* has anti-inflammatory effects. Hammer *et al.* (16) have proven in vitro that flavonoids, such as hyperforin and pseudohypericin along with amentoflavone and quercetin, are active anti-inflammatory components of the plant. Hypericum species lead to faster and better wound healing through active antibacterial components, such as hyperforin and flavonoids (17). Flavonoids are antioxidant compounds that are effective in prevention of cell damage and inhibition of lipid peroxidation. These compounds allow a reduction in cell damage and an increase in vitality of collagen fibrils (18). It was found that aqueous suspensions of *H. perforatum* teas are antimicrobially effective against gram-positive bacteria, and especially against methicillin-resistant Staphylococcus aureus species (19). In the present study, we evaluated the number of PNLs and MNLs to examine the anti-inflammatory effects of *H. perforatum*. Our findings also showed that *H. perforatum* shortened the duration of inflammation and anti-inflammatory effects. On the 7th day, we detected lower levels of PNLs and MNLs in the *H. perforatum*-administered group. There was a statistically significant difference between the groups in terms of PNL levels. Therefore, we suggest that *H.*

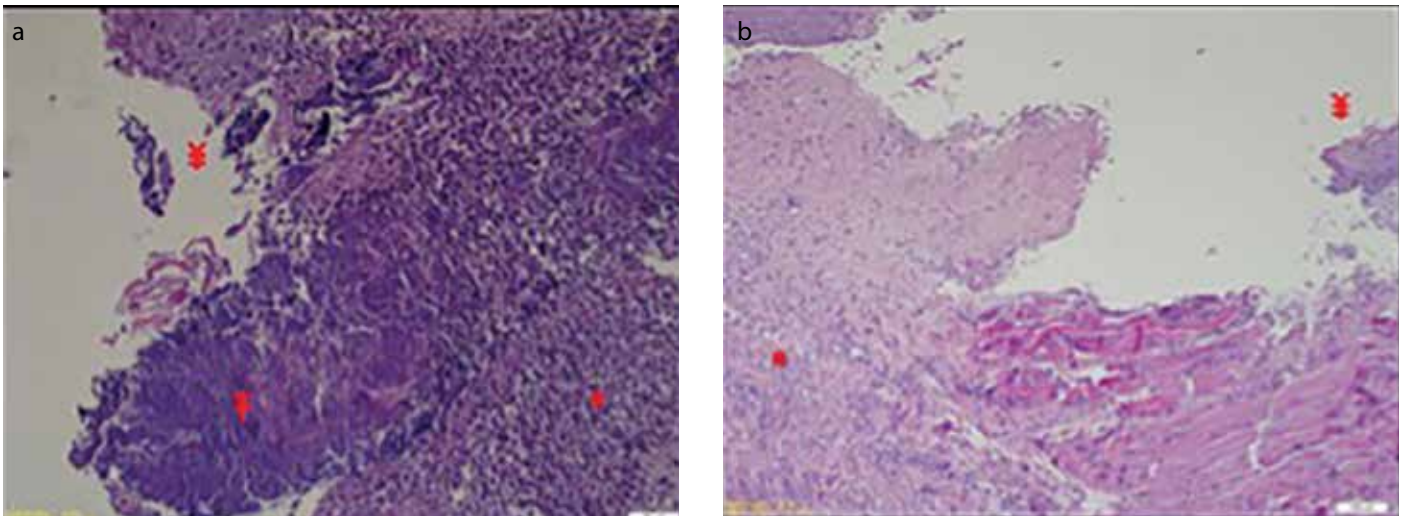

Figure 2. a, b. *Mucosal tissue samples taken at 3rd day post-surgery. H. perforatum group (a) and control group (b). ulceration (¥), bacterium colonization (₸) on the surface and leukocytes dominant, severely mixed inflammation (*) (H&E stain X200 and X100).*

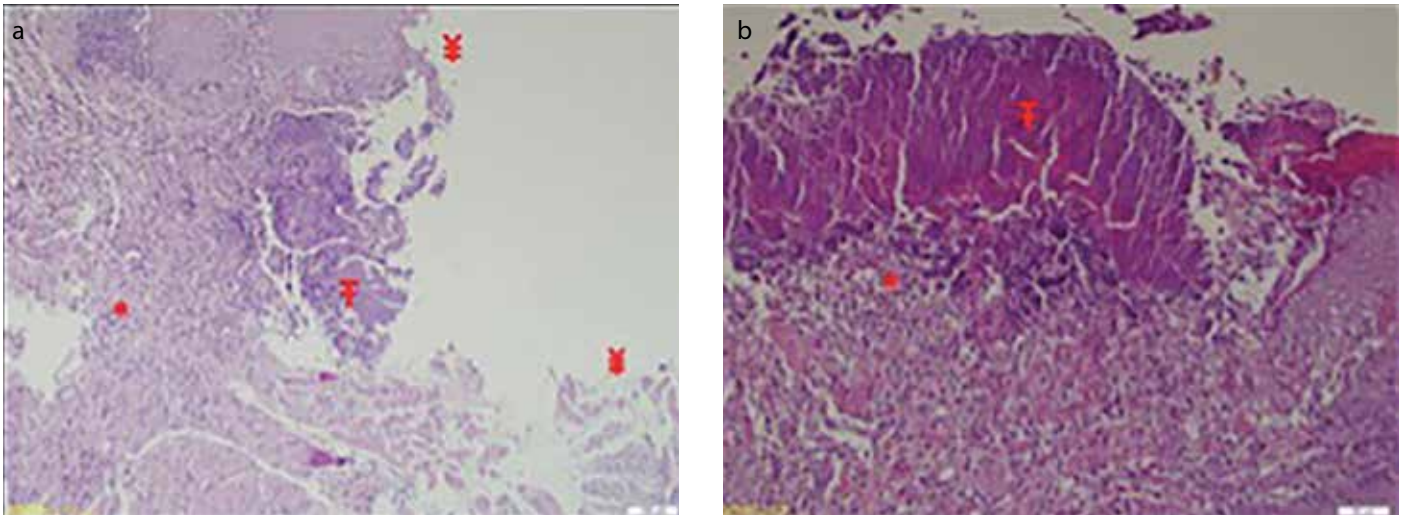

Figure 3. a, b. *Mucosal tissue of the 7th day H. perforatum group (a) and control group (b). ulceration (¥), bacterium colonization (₸) and medium-density, mixed type cell inflammation (*) is present. (H&E stain X100 and X200).*

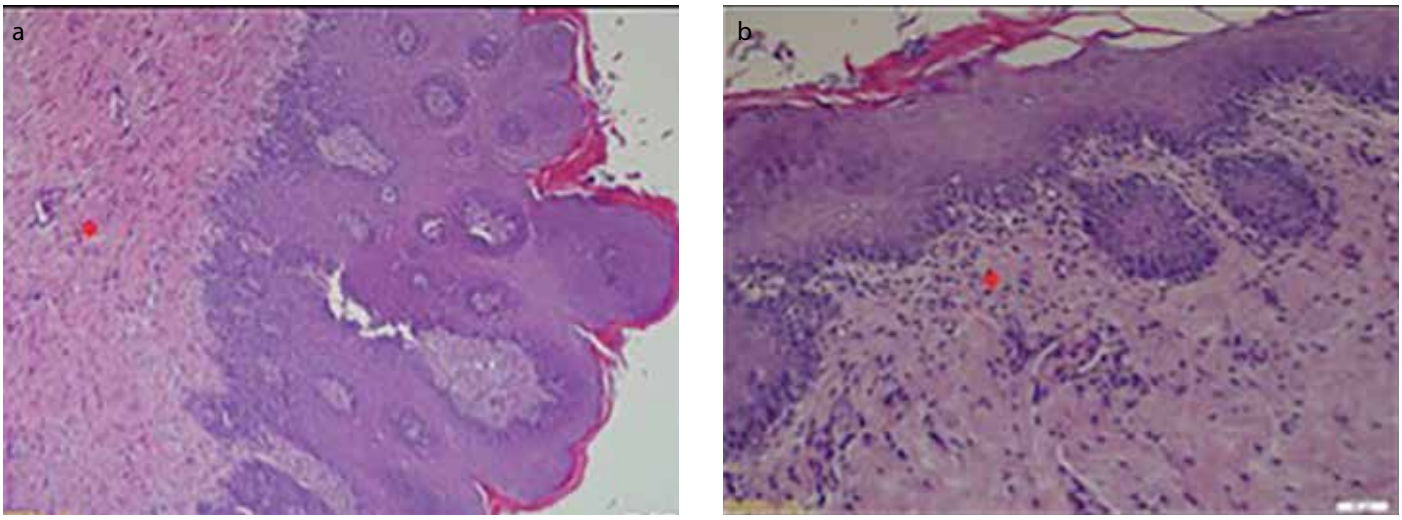

Figure 4. a, b. *Mucosal tissue of the 10th day H. perforatum group (a) and control group (b). Underneath the epithelium, chronic inflammation and inflammatory granulation tissue (*) is present (Hematoxylin-Eosin stain X100 and X200).*

perforatum has anti-inflammatory effects on wounds in the presence of diabetes mellitus, as well as on normal wounds.

Delayed wound healing in diabetic organisms may be due to insufficient formation of new blood vessels. In previous studies, hyperglycaemia occurring during diabetes has been shown to have adverse effects on the vascularisation (20). However, a consensus has not been reached regarding the effects of *H. perforatum*. Castro *et al.* (9) reported that the

Table 1. *The results of statistical analysis of histopathological and biochemical variables*

	3rd day			7th day			10th day		
	HP group (n=8)	**Control group (n=8)**	**p**	**HP group (n=8)**	**Control group (n=7)**	**p**	**HP group (n=8)**	**Control group (n=7)**	**p**
Ulceration	0.750±.462	0.875±.353	0.519	0.375±.517	0.857±.377	0.049*	0.00±.000	0.14±.377	0.205
Necrosis	0.500±.534	0.500±.534	1.000	0.125±.353	0.714±.487	0.016*	0.00±.000	0.00±.000	1.000
Epithelialization	0.250±.462	0.125±.353	0.519	0.625±.517	0.142±.377	0.049*	1.00±.000	0.85±.377	0.205
Edema	1.250±.462	1.625±.517	0.143	1.250±.462	1.142±.377	0.617	1.25±.462	1.42±.534	0.480
PNL	1.625±1.187	1.250±.707	0.397	0.875±1.125	2.142±1.069	0.039*	0.62±.744	0.28±.487	0.350
MNL	2.000±.000	2.000±.000	1.000	1.625±.517	2.000±1.154	0.293	1.37±.517	1.57±.534	0.462
Fibroblasts	2.375±.517	2.500±.534	0.626	2.625±.517	2.857±.377	0.327	2.50±.534	2.4286±.534	0.789
Vascularization	2.250±.462	2.500±.534	0.317	2.500±.534	2.714±.487	0.414	2.37±.517	2.2857±.487	0.724
Hydroxyproline	18.70±.766	18.49±.937	0.205	25.25±.854	19.87±.967	0.001*	28.17±1.249	20.19±.829	0.001*

*p<0.05; HP: *Hypericum perforatum;* PNL: polymorphonuclear leukocytes; MNL: mononuclear cells

vascularisation was more frequent in their *H. perforatum*-administered group and it was observed that the formation of blood vessels was more extensive. Schempp *et al.* (21) reported that hyperforin, which is an active substances in *H. perforatum*, is an inhibitor of angiogenesis. Suntar *et al.* (5) observed in their study that *H. perforatum* had no effect on new blood vessel formation or proliferation of fibroblasts. They reported that the positive effect of *H. perforatum* on wound healing is caused by fibroblast migration and collagen deposition. We have found in our study that topical administration of *H. perforatum* has no effect on vascularisation.

Collagen synthesis, which is one of the most important events in wound healing, slows down and decreases in diabetic organisms (22). Öztürk *et al.* (23) suggested that the positive effect of *H. perforatum* on wound healing is caused by fibroblastic activity and increased collagen synthesis, in an in vitro study on embryonic chicken fibroblasts. Castro *et al.* (9) administered *H. perforatum* and Arnica montana on skin wounds in a study conducted on Wistar rats. The results indicated that this administration had a positive effect on the formation of new tissue compared to the control group, and the number of formed blood vessels along with the percentage of mature collagen fibres was higher. In our study, the difference between hydroxyproline values among the 7th-day and 10th-day groups was statistically significant. However, the fibroblast level, which is one of the parameters of histopathological examination, did not support this conclusion. With respect to the number of fibroblasts, there was no significant difference between the groups on the 3rd, 7th and 10th days. Biochemically, based on the fact that hydroxyproline determination is an objective method, we can say that topical *H. perforatum* administration mitigates the decrease seen in collagen synthesis for diabetic organisms, and affects wound healing positively with the occurring increase.

In diabetes mellitus, in which wound healing is adversely affected, the problems observed in wound healing can be listed as decreased cellular infiltration, angiogenesis, granulation tissue, collagen amount and organization, along with increased infectious complications (1). Although the causes of this problem seen in diabetes cannot be fully explained, hyperglycaemia is generally held responsible. Arokiyaraj *et al.* (24) reported that the oral use of ethyl acetate extract of *H. perforatum* has anti-hyperglycaemic activity in rats, which were made diabetic with STZ. Husain *et al.* (25) reported in an animal study conducted on type 2 diabetes that a daily oral intake of 300 mg/kg of *H. perforatum* had the same effect as a daily intake of 10 mg/kg of glibenclamide, which is a potent hypoglycaemic agent. Can *et al.* (26) reported in their study, which investigated the effect of *H. perforatum* on blood glucose levels, that the plant has antidiabetic effects and this is caused by the flavonoids found in it. In a similar study, Kamalakkannan *et al.* (27) found that orally administered flavonoids had anti-hyperglycaemic and antioxidant effects on rats that were made diabetic with STZ. In terms of response to wound healing, it is observable that the topical and oral use of the same drug can give different results (28). As we used topical administration in our study, in which we saw the positive effects of *H. perforatum* oil for wound healing on the oral mucosa of diabetic rats, we believe that further studies should be conducted to be able to conclude that the positive effect on wound healing as mentioned in the literature is a result of the anti-hyperglycaemic effect, which occurs systematically.

Conclusion

Within the limitations of this experimental study, it can be concluded that the topical application of *H. perforatum* demonstrated positive effects on the healing of surgical defects created in the palatal mucosa of diabetic rats at 7th and 10th days.

Ethics Committee Approval: Ethics committee approval was received for this study from the Animal Ethics Committee of Mustafa Kemal University (No: 2014017).

Peer-review: Externally peer-reviewed.

Author Contributions: AA, CA designed the study. HG and OO generated the data. AA and İD gathered the data. MHA analyzed the data. AA and İD wrote the majority of the original draft. AA and CA collected the raw data of the study. All authors approved the final version of the paper.

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Türkçe öz: *Diyabetik sıçanlarda kantaronun (Hypericum perforatum) ağız mukozasındaki yara iyileşmesine etkisi. Amaç: Bu çalışmanın amacı H. perforatum'un (kantaron) topikal olarak uygulanmasının, deneysel olarak diyabet oluşturulmuş sıçanlarda ağız mukozasındaki yara iyileşmesine etkilerinin histopatolojik ve biyokimyasal olarak araştırılmasıdır. Gereç ve Yöntem: Çalışmada 48 adet, erkek, 5 aylık Wistar Albino rat kullanıldı. Deney hayvanları çalışma ve kontrol grubu olmak üzere, 24 sıçandan oluşan 2 ana gruba ayrıldı. Ana gruplar hayvanların sakrifiye edileceği günlere göre üç alt gruba ayrıldı. Tüm ratlara 60 mg/kg dozunda streptozotosin verildi. Yetmiş iki saat sonra yapılan ölçümlerde kan glikoz seviyesi 200 mg/dL'nin üzerinde olanlar çalışmaya dahil edildi. Sıçanlarda damak bölgesinde mukozal defektler oluşturuldu. Çalışma grubunda yaraya kantaron yağı, topikal olarak günde iki defa uygulandı. Kontrol grubundaki sıçanların yara bölgelerine herhangi bir uygulama yapılmadı. Sakrifiye edilen tüm ratlarda damakta oluşturulan yaralardan 3., 7. ve 10. günlerde örnekler alındı. Alınan örnekler histopatolojik ve biyokimyasal olarak incelendi. Bulgular: Yapılan istatistiksel analiz sonucunda 3. gün grupları arasında anlamlı bir fark görülmedi. 7. günde ise nekroz, polimorfonükleer lökosit, epitelizasyon, ülserasyon, ve hidroksipirolin değişkenlerinde istatistiksel olarak anlamlı farklılık bulundu ($p<0,05$). Bu değişkenlerden nekroz, ülserasyon ve polimorfonükleer lökosit değerlerinin kontrol grubunda yüksek olduğu; epitelizasyon ve hidroksipirolin değerlerinin ise kantaron uygulanan çalışma grubunda daha yüksek olduğu tespit edildi ($p<0,05$). Onuncu gün gruplar arasında sadece hidroksipirolin değerleri arasında anlamlı bir fark tespit edilmiş olup, kantaron uygulanan grupta hidroksipirolin seviyesinin yüksek olduğu görüldü ($p<0,05$). Sonuç: Bu çalışma deneysel olarak diyabet oluşturulmuş sıçanlarda topikal olarak uygulanan Hypericum perforatum'un 3. günde herhangi bir farklılık oluşturmasa bile 7. ve 10. günlerde yara iyileşmesini olumlu yönde etkilediğini göstermiştir. Anahtar kelimeler: Diyabet; Hypericum perforatum; kantaron; ağız mukozası; yara iyileşmesi*

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Original research

Clinical evaluation of dental enamel defects and oral findings in coeliac children

Purpose
To examine dental hard and soft tissue changes of coeliac children in order to increase the awareness of the pediatric dentists in prediagnosis of especially undiagnosed coeliac disease.

Materials and methods
Sixty children, 28 (46.7%) boys and 32 (53.3%) girls whose ages were between 6 to 16 years were included in the present study. Thirty children who had undergone endoscopy and diagnosed with the coeliac disease in the Şişli Hamidiye Etfal Hospital, İstanbul, Turkey, formed the study group. Also, thirty children clinically suspected of having the coeliac disease with the same gastrointestinal complaints had undergone endoscopy and proven not coeliac were chosen as the control group. Oral examination involved assessment of dentition and specific and unspecific dental enamel defects. Also, soft tissue lesions, clinical delay of the dental eruption, salivary flow rate, pH, and buffering capacity were examined.

Results
Twenty coeliac patients had enamel defects, however none in the control subjects. In the coeliac group, all enamel defects were diagnosed in permanent teeth and as specific in all children. Grade I dental enamel defects found mainly in the incisors. The clinical delayed eruption was observed in 10 (33.3%) of 30 coeliac children and none of the children in the control group. While the level of DMFT/S numbers and stimulated salivary flow rate were found significantly lower in the coeliac group, pH was found significantly higher.

Conclusion
Oral cavity may be involved in coeliac disease and pediatric dentists can play an important role in the early diagnosis of the coeliac disease.

Keywords: *Caries; coeliac disease; dental enamel defects; dental eruption; recurrent aphthous stomatitis*

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Introduction

Coeliac disease is a systemic immune-mediated primary small bowel disease characterized by inflammation in the small intestine mucosa and submucosa, often accompanied by malabsorption, which results in hypersensitivity to gluten found in cereal and cereal products. Coeliac disease is sometimes called gluten-sensitive enteropathy or celiac sprue. Clinical findings have improved with the removal of gluten from the diet (1, 2). Gluten is an insoluble protein found especially in wheat, barley, oats, and rye and reacts with alcohol, resulting in a molecule called gliadin. Although the mechanism of gliadin damage to the small intestinal mucosa is not fully understood, environmental and immunological factors in genetically susceptible individuals initiate disease (1, 3). Typical clinical features of the coeliac disease include malabsorption, chronic diarrhea, abdominal pain, and

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weight loss. However, many cases are asymptomatic and do not show gastroenterological symptoms (4, 5). The oral cavity which is the entrance of the gastrointestinal tract is also affected in individuals with the coeliac disease, it can be easily examined and has a great prospect for early detection of coeliac disease. Dental enamel defects, recurrent aphthous stomatitis (RAS), dermatitis herpetiformis, Sjögren's syndrome and oral lichen planus have been reported in patients with coeliac disease (4-6). Dental enamel defects were first reported by Aine (7). Enamel defects are due to genetic factors that cause hypocalcemia-induced or glutamine-dependent specific immunological response. In addition, enamel hypoplasia may also occur due to malnutrition and vitamin D and A deficiency (8-10). The association of oral lesions with coeliac disease is controversial but it is thought to be the indirect effect of malabsorption (11). Based on this previous information, the null hypothesis of this study is that the dental enamel defects, oral diseases, and mouth dryness are not common in children with coeliac disease when compared to children having similar gastrointestinal complaints but not having coeliac disease.

Materials and methods

Study groups

All parents of the patients gave informed written consent for the participation of their children in the study, all study protocols were also approved by the Marmara University, Institute of Health Sciences Non-invasive Clinical Research Studies Ethics Committee (26.11.2013-1). Sixty children, 28 (46.7%) boys and 32 (53.3%) girls with ages between 6-16 years (mean age=12.76 ±3.08 years) from January 1, 2014 to December 31, 2014, living in İstanbul, Turkey during that entire time period were included in this study. Thirty children had undergone endoscopy diagnosed with the coeliac disease who attended to the gastroenterology unit of the Şişli Hamidiye Etfal Hospital, İstanbul, Turkey, formed the study group. Also, thirty children clinically suspected of having the coeliac disease with the same gastrointestinal complaints had undergone endoscopy and proven not coeliac were chosen as the control group.

Inclusion criteria

The study group consisted of children whose ages were between 6 to 16, whose caregivers consented and endoscopically proven to have coeliac disease. The control group consisted of children having the same complaints with the coeliac group, willing to give consent, and endoscopically proven not to have coeliac disease (12, 13).

Exclusion criteria

Exclusion criteria for the study and control groups were not having a definite diagnosis about the presence or absence of coeliac disease. Children whose first permanent incisors and molars were not yet totally erupted and children with fixed orthodontic treatment were also removed from the study. Furthermore, coeliac children, who previously followed a gluten-free diet for a period of one year or more, were excluded from the study (14).

Gastric examination

Endoscopy was performed with a gastro-duodenoscope (Olympus® GIF-XP 150N, Tokyo, Japan) to children attending the gastroenterology unit of the Şişli Hamidiye Etfal Hospital. Biopsy forceps were sterilized and endoscopes were fully disinfected before and after each examination. Diagnosis of coeliac disease was based on European Society for Paediatric Gastroenterology, Hepatology, and Nutrition criteria (15).

Demographic questionnaire

A detailed medical history was taken from all children. Questions about use of medication, comorbidities for dental enamel defects (fluoride exposure, premature birth, fall on the front teeth, diabetes mellitus, long period of high fever, icterus, antibiotic use), history of coeliac disease among family members, frequency of daily toothbrushing (none or irregular, 1 or more per day), sugar intake (none or several times per week, at least once a day), education status of mothers and fathers (primary school, high school) and socioeconomic status of the family (low income< 3000₺, high income>3000₺) were asked to parents of children participating in this study. The questionnaire was validated statistically and created according to previous studies (14, 16-18).

Oral examination

All children were clinically examined in order to assess their dental status. They have all brushed their teeth before the examination. The clinical measurements were recorded by one examiner. Oral examinations were done before endoscopy procedure without knowing whether the children were coeliac or not. The teeth were air dried using the portable dental equipment and examined with the help of a disposable mirror for the presence of dental defects (19). Both specific and unspecific defects were screened on the buccal surfaces of primary and permanent teeth. Enamel defects were classified as unspecific if only one tooth was affected on one side of the dentition. Specific defects had to be symmetrical, involving the same teeth in both hemiarches. Classification of specific enamel defects were evaluated according to Aine was shown in Table 1 (20). Also, oral examination involved assessment of dentition involving the number of teeth and carious teeth. Dental caries were diagnosed at the tooth surface level according to the WHO criteria (21). To determine the DMFT/dmft indices, the total numbers of decayed, missing, and filled teeth were calculated. Soft tissue lesions (presence of RAS, geographical tongue, angular cheilitis, atrophic glossitis) and clinical delay of the dental eruption were also examined (14, 22, 23). Oral mucosal surfaces including tongue, lips, palate and their mucosa were observed (24). RAS was detected as recurrent, round, small ulcers with circumscribed margins, erythematous halos and yellow or gray floors (25). Minor RAS lesions are round ulcers less than 10 mm in diameter, major lesions are clinically similar to the minor but are larger than 10 mm in diameter and more persistent (26).

Table 1. *Classification of systematic and chronological enamel defects, according to Aine (12, 20)*

Classification	Enamel Defect
Grade 0	No defect
Grade I	Defect in colour of enamel. Single or multiple cream, yellow or brown opacities with clearly defined or diffuse margins; in addition a part or the entire surface of enamel is without glaze.
Grade II	Slight structural defects. Enamel surface rough, filled with horizontal grooves or shallow pits, light opacities and discolorations may be found; in addition a part or the entire surface of enamel is without glaze.
Grade III	Evident structural defects. A part or the entire surface of enamel rough and filled with deep horizontal grooves that vary in width or have large vertical pits; large opacities of different colours or strong discolorations may appear in combination.
Grade IV	Severe structural defects. The shape of the tooth has changed: the tips of cusps are sharp-pointed and/or the incisal edges are unevenly thinned and rough; the thinning of the enamel material is easily detectable and the margins of the lesions are well defined; the lesion may be strongly discolored.

Table 2. *Grading of enamel defects in coeliac group, according to classification of Aine (20)*

Systematic Defect Grades	n	(%)
No	10	33.3
I	14	46.6
II	6	20.0
III	0	0.0
IV	0	0.0
Total	30	100.0

Figure 1. *Grade II enamel defects: rough enamel surface with patchy symmetric opacities and discolouration.*

Figure 2. *Grade I enamel defects: multiple white and cream opacities with clearly defined margins.*

Evaluation of salivary flow rate, pH, and buffering capacity

After chewing paraffin wax gums, the children were requested to spit for 5 minutes in order to detect stimulated salivary flow rates. Salivary pH was measured with the pH meter (Thermo Scientific™ Orion™ 3-Star Benchtop pH Meter, Thermo Fisher Scientific Inc. Waltham, MA, USA) and the buffering capacity was measured using Ericsson method from all subjects participated in this study (27, 28). All the tests in the study were carried out by a specialist.

Statistical analysis

IBM Statistical Package for the Social Sciences Statistics 22 program (IBM Corp.; Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA) was used for statistical analysis. The normal distribution of the variables was evaluated by the Shapiro-Wilk test. Student t test was used for the comparison of two groups with normal distribution, and Mann-Whitney-U test was used for the comparison of two groups with non-normal distribution. Chi-square test, Fisher's Exact Chi-square test, and Pearson Chi-square tests were used for the comparison of categorical data. Significance was assessed at $p<0.05$ level. Sample size was calculated with power analysis prior to the study. With 80% targeted power and 0.05 confidence level, using the previous literature, 28 individuals had to be enrolled in each group. To ensure the statistical power, 30 children were included in each group.

Results

In total, 60 subjects were enrolled in this study were between 6 to 16 years of age. Twenty-five (41.6%) children were in mixed dentition and 35 (58.3%) children were in permanent dentition. Fifty percent of coeliac children (15/30) were typical, 46.7% of them were atypical and the rest of them (3.3%) were asymptomatic and the mean diagnosis age of the disease was 7.23±4.32 years. Four (13.3%) children had a

family member suffering from the same disease. There were no differences between coeliac and control groups in mean age, gender distribution, height, body mass and the presence of comorbidities. Twenty coeliac children had enamel defects while the control subjects had none. In the coeliac group, all enamel defects were diagnosed as specific and located on the permanent teeth. The most frequently seen dental enamel defects among coeliac children were in Grade I. Grade I was found in 14 (46.6%) and Grade II was found in 6 (20%) of coeliac patients (Table 2), (Figure 1, 2).

Grade III and IV were not observed in the current study. Enamel defects were found mainly in the incisors. The location and frequency of the specific enamel defects are given in Table 3.

The clinical delayed eruption was observed in 10 (33.3%) coeliac children. Delay of the eruption was found none of the children without the coeliac disease. The difference was statistically significant ($p<0.05$). The overall prevalence of RAS was 5 (16.6%) in control group and none in the coeliac group. The difference was not significant between groups ($p>0.05$). While the level of DMFT/S numbers and stimulated salivary flow rate was found significantly lower in the study group, pH was found significantly higher. The consumption of sugar in the control group was more than the coeliac group ($p<0.05$). The level of dmft/s numbers and the mean buffering capacity scores did not differ significantly between study and control groups. Oral findings among coeliac and control groups are shown in Table 4.

The frequency of daily tooth brushing did not differ significantly between coeliac and control groups. The sugar consumption frequency of 16 (53.3%) children in the control group were at least once a day, however, it was found in 5 (16.7%) children in the coeliac group. The influence of family income did not significantly contribute to the study and control groups. Mother's and father's education levels of the coeliac group were found higher than the control group.

Discussion

Coeliac disease is a common disorder affecting both children and adults. As many people with the disease do not present gastrointestinal symptoms, delays in diagnosis are very common and cause malignancies (29). In our report, we evaluated the prevalence of dental enamel defects, RAS, some oral and demographic parameters in patients with diagnosed coeliac diseases, and compared the results with subjects without coeliac disease. Mean age of diagnosis was 7.23±4.32 years in this study. Aguirre *et al.* (30) diagnosed the coeliac disease in the first 2 years of life in 64% of all the cases which was earlier than our study. Mina *et al.* (25) did not observe dental defects in coeliac children who had been diagnosed as coeliac at around 1 year old. Early introduction of gluten-free diet might have prevented the disturbances of dental enamel mineralization. Even though coeliac children had gluten-free diet immediately after diagnosis, late diagnose might have led to disturbances in the permanent dentition. Acar *et al.* (31) detected the mean diagnosis age as 9.5 years in coeliac patients with enamel defects and 7.8 years in coeliac patients without enamel defects.

Although our study did not show any enamel defects in the control group and unspecific defects in the coeliac group, the greater number of systematic enamel defects in coeliac children demonstrated that enamel hypoplasia was more frequent in coeliac children than the control group. The enamel defects in the present study were generally symmetrical and mostly seen in anterior teeth. Similar observations were reported in previous studies (5, 7, 12, 17, 19, 24, 32- 36) and only some studies (6, 37) contradicted the present findings. Similarly, Acar *et al.* (31) demonstrated enamel defects in 14 (40%) of 35 coeliac patients, while 21 of the coeliac patients did not have any defect. Also, none of the subjects in the control group had enamel defects. This finding showed that the dental enamel defects occurred significantly more often in coeliac patients.

Table 3. *Location of systematic enamel defects in coeliac group*

Location of enamel defects	n	%
Incisors	17	53.4%
Incisors&canines	2	6.7%
Molars	1	3.3%
Incisors&molars	1	3.3%
No defects	10	33.3%
Total	30	100%

Table 4. *Oral findings in coeliac and control groups*

	Study group (n=30)					Control group (n=30)					
	Mean	SD	Med	Min	Max	Mean	SD	Med	Min	Max	p
DMFT	4.48	3.67	4.00	0.00	12.00	6.77	4.43	6.50	0.00	20.00	0.035
DMFS	6.20	6.74	4.00	0.00	23.00	8.96	8.75	7.00	0.00	45.00	0.043
dmft	2.84	1.99	2.00	0.00	6.00	2.33	2.83	1.00	0.00	8.00	0.295
dmfs	5.76	5.19	5.00	0.00	18.00	4.08	5.59	1.50	0.00	15.00	0.147
Salivary flow fate	3.65	2.08	3.50	0.50	9.50	7.46	3.13	6.50	3.50	20.00	<0.001
Buffering capacity	5.99	0.55	6.10	4.62	6.73	5.87	0.44	5.99	4.74	6.55	0.228
Saliva pH	7.99	0.46	7.97	6.91	8.85	7.34	0.25	7.32	6.95	7.97	<0.001

p<0.05 significant difference between groups

SD: standard deviation; DMFT/S: decayed, missing and filled permanent teeth/surfaces; dmft/s: decayed, missing, and filled primary teeth/surfaces

According to Aine's classification; Grade I was found in 14 (46.6%) and Grade II was found in 6 (20%) of 30 coeliac children. Grade III and IV were not observed in the current study. The findings of our study was found to be in accordance with those of Aguirre *et al.* (30) and Avşar *et al.* (17). In the study of Costacurta *et al.* (16) 80% of enamel defects were classified as Grade I, 15% Grade II, 3% Grade III, and 2% Grade IV. Cheng *et al.* (38) reported that dental enamel defects of children distributed as 14% Grade I, 53% Grade II, 19% Grade III and 11% Grade IV. Campisi *et al.* (5) reported dental enamel defects as 87% Grade I, 11% Grade II and 4% Grade IV. In the study of Aine *et al.* (7) 30% of coeliac children had grade III-IV defects. Differences in the severity and diagnosis age of coeliac disease, time to start and compliance to gluten-free diet, type of population studied might be responsible for the different results in the studies. Enamel defects were found mainly in the incisors (53.4%) also they were symmetric and chronologic in the current study. According to Aine (20); the central incisors are always affected in children with coeliac disease. Aguirre *et al.* (30), Costacurta *et al.* (16), Wierink *et al.* (12) and Cantekin *et al.* (34) also determined enamel defects mainly in the anterior teeth. The exact mechanism of development of dental enamel defects in coeliac disease is still not clear. The central incisors are the first dental elements where the mineralization process begin and affected through an influence on dental mineralization during odontogenesis. In coeliac children malabsorption due to enteropathy determines an alteration of phospho-calcium metabolism and cause hypocalcemia (37, 39).

The clinical delayed eruption was observed in 10 (33.3%) out of 30 coeliac children in the present study. Delay of the eruption was found none of the children in the control group. This findings was consistent with those Costacurta *et al.* (16) Campisi *et al.* (5) but not in accordance with Mina *et al.* (25).

In the previous studies (6, 16, 24, 26, 31, 34, 38) RAS was found to be more frequent in coeliac patients. On the contrary in the present study; RAS frequency was found to be higher in the control group rather than coeliac children but the difference was not statistically significant. Sedghizadeh *et al.* (40) reported that there were no significant differences between coeliac patients and healthy controls in the prevalence of RAS and they referred coeliac disease as a 'risk indicator' and not a 'risk factor' for RAS. Yaşar *et al.* (41) concluded that there is no apparent etiological link between RAS and coeliac disease and that screening RAS for coeliac disease has little clinical value. Conflicting datas have been published on the real frequency of RAS in coeliac patients and there were few datas on the effect of a gluten-free diet on RAS in coeliac patients. It must be remembered that RAS can also be associated with other inflammatory bowel diseases and consequently the association cannot be considered specific (26). Also, patients in this study might not present RAS at the time of oral examination, this did not mean that they did not suffer from RAS at any other times before. Families or children might not notice or remember whether they had RAS before clinical examination.

The relationship of caries and coeliac disease was the other aspect of this research. Amongst the coeliac group, the level of DMFT/S numbers was found to be lower than the control group. This was in agreement with the studies of Aguirre *et al.* (30), Farmakis *et al.* (32), Priovolou *et al.* (33) and Cantekin *et al.* (34), on the other hand; not in agreement with Costacurta *et al.* (16), Avsar *et al.* (17), Acar *et al.* (31), and Bramanti *et al.* (14). In the study of Shteyer *et al.* (36) no significant difference was reported among coeliac group and control group in mean DMFT/dmft scores although there was a tendency toward a higher DMFT/dmft scores in the control group which was consistent with the present study. Mina *et al.* (25) reported no statistical differences in the mean DMFT or dmft scores of coeliac children and control children. Páez *et al.* (19) investigated children with complete deciduous dentition and found higher numbers of caries in the control subjects. In contrast, dmft/s numbers did not differ between coeliac and control groups in the present study in which 25 (41.6%) children were in mixed dentition and 35 (58.3%) children were in permanent dentition. Our results were similar to the studies of Cantekin *et al.* (34) and Acar *et al.* (31).

Patients with the coeliac disease more frequently suffer from Sjögren's syndrome than do healthy controls (43). In the present study, stimulated salivary flow rate of coeliac children was found lower than the control group as previous studies (13, 24, 34, 42). However, in another pilot study including 30 coeliac patients and 30 healthy age and sex matched controls, no differences in saliva secretion rate was found (43). Moreover, pH was found to be higher amongst the coeliac group and the level of buffering capacity did not differ between groups in the current study. In the study of Shteyer *et al.* (36) pH and buffering capacity were not different between coeliac and control groups. Acar *et al.* (31) demonstrated that the salivary pH, salivary flow rate, and buffering capacity were also similar in coeliac and control groups. In another study (25) buffering capacity and flow rate revealed no statistically significant differences.

The differences in toothbrushing habits such as frequency of daily tooth brushing between the coeliac group and control group were not statistically significant in our study as in the study of Avşar *et al.* (17). Also, daily sugar intake of the coeliac group was found lower than those of without coeliac. Sugar contains gliadin that coeliac patients do not want to consume (19). This result strongly supported the assumption that lower DMFT/S numbers might be related to low cariogenic dietary habits of the coeliac group. The reason for no significant difference in the mean number of DMFS/dmfs values between groups in the study of Acar *et al.* (31) might be related to similar daily sugar exposures of coeliac and healthy groups. The influence of family income did not significantly contribute to the coeliac and control groups. Mother's and father's education levels of the coeliac group were found higher than the control group. In the study of Avşar *et al.* (17) socioeconomic status and education levels of the parents between the coeliac group and control group were not statistically significant.

Control group of our study consisted of children who had gastrointestinal complaints and proved not coeliac endoscopically. Besides clinical examination biopsy procedures had also been performed to these children in order to examine the type of the gastrointestinal problem had increased the reliability of our study. Because previous studies had

shown that the incidence of undetected coeliac disease was very high and the ratio between diagnosed and undiagnosed patients even 1:7 (44). The limitation of our study is the lack of investigation of specific antigens which increases the risk for enamel defects (45). Further studies must be done in order to elucidate the genetic relationship between the coeliac disease and enamel defects. Also, more extensive population-based studies are needed in order the demonstrate the oral effects of the coeliac disease.

Conclusion

The changes in the oral cavity can be involved in coeliac disease and pediatric dentists therefore play an important role in the early diagnosis of the disease. As coeliac children may have various developmental disabilities in the dentition, they must be examined by pediatric dentists at least 2 to 3 times per year.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Marmara University, Institute of Health Sciences (26.11.2013-1).

Informed Consent: Written informed consent was obtained from patients' parents who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: All authors designed the study and generated the data. All authors participated in gathering the data for the study. DAB and AY analyzed the data. DAB and AY wrote the majority of the original draft. All authors participated in writing the paper. All authors had access to all of the raw data of the study. All authors approved the final version of the paper.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Türkçe öz: *Çölyak hastalığı olan çocuklarda diş mine defektlerinin ve oral bulguların değerlendirilmesi. Amaç: Çölyak hastalığı olan çocukların diş sert ve yumuşak doku değişikliklerini inceleyerek özellikle tanı konulmamış çölyak hastalığının ön tanısında çocuk diş hekimlerinin farkındalığını arttırmaktır. Gereç ve Yöntem: Çalışmaya yaşları 6-16 arasında değişen, 28 (%46,7) erkek ve 32 (%53,3) kız toplam 60 çocuk dâhil edilmiştir. Çalışma grubunu, Şişli Hamidiye Etfal Hastanesi, gastroenteroloji bölümüne başvuran ve endoskopi sonucuna göre çölyak hastalığı teşhisi konulmuş 30 çocuk oluşturmuştur. Kontrol grubunu ise klinik olarak aynı gastrointestinal şikayetlere sahip olan, çölyak hastalığından şüphelenilenerek endoskopi yapılmış ve çölyak hastalığı olmadığı kanıtlanmış 30 çocuk oluşturmuştur. Ağız içi muayenede, dişlenme dönemi, spesifik ve spesifik olmayan diş mine defektleri değerlendirilmiştir. Ayrıca, yumuşak doku lezyonları, diş sürme gecikmesi varlığı, tükürük akış hızı, pH ve tamponlama kapasitesi değerleri incelenmiştir. Bulgular: Yirmi çölyak hastası çocukta diş mine defekti saptanırken, kontrol grubunda saptanmamıştır. Çölyak grubunda tüm diş mine defektleri spesifik tipte ve daimi dişlerde tespit edilmiştir. Birinci derecede olan diş mine defektleri çoğunlukla kesici dişlerde görülmüştür. Sürme gecikmesi 30 çölyak hastası çocuğunun 10'unda (%33,3) gözlenmiş ve kontrol grubundaki hiçbir çocukta gözlenmemiştir. Çölyak grubunda DMFT/S değerleri ve uyarılmış tükürük akış hızı düzeyleri kontrol grubundan anlamlı olarak daha düşük, pH değeri anlamlı olarak daha yüksek bulunmuştur. Sonuç: Çölyak hastalığında ağız boşluğu etkilenebilir ve çocuk diş hekimleri bu hastalığın ön tanısında önemli bir rol oynayabilir. Anahtar kelimeler: Çürük; çölyak hastalığı; diş mine defekti; diş sürmesi; tekrarlayan aftöz stomatit.*

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Original research

Evaluation of salivary tumour necrosis factor–alpha in patients with recurrent aphthous stomatitis

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Purpose
Present study was undertaken to evaluate and compare the salivary levels of tumour necrosis factor alpha (TNF-α) in subjects with RAS, traumatic ulcers (TUs) in the oral mucosa and in healthy controls.

Materials and Methods
Present study involved 90 participants of which 30 subjects were diagnosed with RAS, 30 subjects with TUs and 30 healthy controls grouped as group 1, group 2 and group 3 respectively. Unstimulated saliva was collected from the subjects through 'Spit Technique' and the estimation of TNF-α was done by enzyme linked immunosorbent assay. The data collected was statistically analysed.

Results
Salivary level of TNF-α was significantly higher in RAS patients than in patients with TUs and healthy controls. Difference between the Salivary TNF-α level in our study groups were statistically significant ($p<0.001$).

Conclusion
Present study suggests that saliva is a convenient and ideal medium for the detection of TNF-α. Statistically significant difference in the level of salivary TNF-α between the RAS and TUs subjects as well as controls suggests the significant contribution of TNF-α in pathogenesis of RAS.

Keywords: *Recurrent aphthous stomatitis; tumour necrosis factor-alpha; saliva; oral mucosa; traumatic ulcer*

Introduction

Recurrent aphthous stomatitis (RAS) is a common oral mucosal disorder characterized by recurrent ulcers (1, 2). These ulcers are either single or multiple, small, round or ovoid, with well circumscribed margins and erythematous haloes. RAS first appears in childhood or adolescence (3). The prevalence of RAS ranges from 5% to 60% based on the ethnic and socioeconomic background (4). RAS is one of the least understood oral diseases due to which management of these common lesions have posed a challenge to the general and specialty dental practitioners (5, 6). RAS results in considerable pain leading to difficulty in eating, speech and swallowing (3). RAS affects therefore the quality of life. There is no particular curative treatment available for RAS because of diverse precipitating factors for recurrent episodes (5). Various causative factors such as genetic tendency, immunologic basis, nutritional deficiency, emotional stress, hematologic and hormonal disturbances, local injury, microbial agents and other influences have been suggested in previous studies (5, 7). There is no definitive explanation for the pathogenesis of RAS (8). The epithelial cell death and ulceration in RAS are the results of cell-mediated

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immune response, mainly T-lymphocyte activation which leads to the formation of cytokines such as tumour necrosis factor-α (TNF-α) (9). Among the cytokines implicated in the development of new lesions in RAS patients, TNF-α is considered to be one of the most important. Several studies have reported about the possible role of TNF-α in the active phase of the disease (8, 10-13). Currently the management of RAS is symptomatic due to its unclear aetiology. Effective treatment for RAS is possible only by discovering the underlying factors (14). Previous studies focusing on genetic as well as immunologic background showed inconsistent results. Limited research on Indian population with this regard indicated the need of performing such study in this region. Present study evaluated the salivary levels of TNF-α in RAS patients of in and around Mangalore and compared the salivary levels of tumour necrosis factor alpha (TNF-α) in subjects with RAS, TUs in the oral mucosa and in healthy controls. The null hypothesis is that there are no differences among any variable.

Materials and methods

Study population

The study involved patients visiting a private dental hospital in Mangalore, India between 2015 and 2017. Institutional Ethical clearance was obtained on 3.10.2014. The project was funded by the NITTE University vide Research Project Number NURG/STF/05/11-2014. Study objectives and procedures were explained to the subjects and informed consent was obtained from all the participants. Detailed case history recording and clinical examination was carried out by trained professionals. Patients diagnosed with RAS, traumatic ulcer and healthy controls were recruited for the study. Subjects with history of medication, subjects with systemic diseases were excluded from the study.

Study groups

Selected patients were grouped as follows: Group 1: 30 patients diagnosed with recurrent apthous stomatitis, Group 2: 30 patients diagnosed with traumatic ulcers in the oral mucosa, Group 3: 30 healthy control subjects.

Saliva collection

Unstimulated saliva was collected from the subjects through 'Spit Technique'. The subjects were instructed to spit into a sterile graduated container every minute for 8-10 minutes. The collected sample was centrifuged at 3000 rpm for 10 minutes and the supernatant collected was stored at -20°C. The estimation of salivary tumour necrosis factor-α was done by commercially available enzyme linked immunosorbent assay (ELISA) (Hu TNFα Us Elisa Kit, Thermo Fisher Scientific, Waltham, MA USA) at the central research laboratory of the university as per the procedure by Beutler *et al.* (15).

Statistical analysis

The data collected was analysed using IBM Statistical Package for the Social Sciences Statistics, Version 22 (SPSS IBM Corp.; Armonk, NY, USA). Descriptive data were presented in the form of mean and standard deviation. One Way analysis of variance (ANOVA) test was used to compare TNF-α values between multiple study groups. Comparison of age between the study groups was also done by using ANOVA. Pairwise comparison of age between the study groups was done with post-hoc Tukey's HSD test. Distribution of study participants according to gender evaluated by chi-square test. Confidence interval was set to 95% and $p<0.05$ was considered as statistically significant.

Results

The present study involved 3 groups. Group 1 involved 30 patients clinically diagnosed with recurrent aphthous stomatitis. The subjects were between 12 to 54 years of age and the mean age was 29.30 years. The study group 2 involved 30 patients diagnosed with traumatic ulcers of oral mucosa. The subjects were between 22 to 66 years of age and the mean age of the study group was 38.67 years. The group 3 involved 30 healthy controls. These subjects were between the ages of 18 to 63 with the mean age of 32.53. Data is shown in Table 1.

In the group 1 there were 14 males (46.7%) and 16 females (53.3%). Among group 2 subjects 17 (56.7%) were males and 13 (43.3%) were females. Group 3 involved 12 (40.0%) males and 18 (60.0%) females. Details shown in Figure 1. Comparison of salivary TNF-α level between the study groups was done. Difference between the salivary TNF-α level in our study groups were statistically significant ($p<0.001$). Details given in Table 2 and Figure 2.

Study group 1 included 30 RAS patients, out of which 26 subjects were diagnosed with minor RAS and 4 subjects were diagnosed with major RAS. Among the RAS patient group no subjects were diagnosed with herpetiform RAS. Comparison of type of RAS with salivary level of TNF-α is shown in Table 3. Correlation analysis between the age and TNF-α variables in each study group was shown in Table 4.

Discussion

Our study subjects were between 12 to 54 years of age and the mean age was 29.30 years in group 1. RAS was predominant in adults in the present sample. Female predominance was noticed in our study which is in accordance with previous work (16-18). Prevalence of RAS is lower in this region compared to other populations which was showed in our previous study 1.9% (16). Accurate aetiology of RAS still remains unknown (13). Controversy exists among different authors about the pathogenesis of RAS (8). Various factors predispose for its occurrence. Genetic background, stress, anxiety, food allergens, local trauma, smoking cessation, menstrual cycle, chemicals and microbes were identified as predisposing factors (5, 7, 19). A Cochrane analysis mentioned that bacterial or viral aetiology was unlikely (20). Local and systemic factors lead to the targeting of oral mucosal cells by lymphocytes,

Table 1. *Comparison of age between the study groups*

				ANOVA	
	n	**Mean**	**SD**	**F**	**p**
Group 1	30	29.30	10.70		
Group 2	30	38.67	12.95	5.03	0.009*
Group 3	30	32.53	11.08		

*p<0.05 statistically significant; ANOVA: analysis of variance; SD: standard deviation

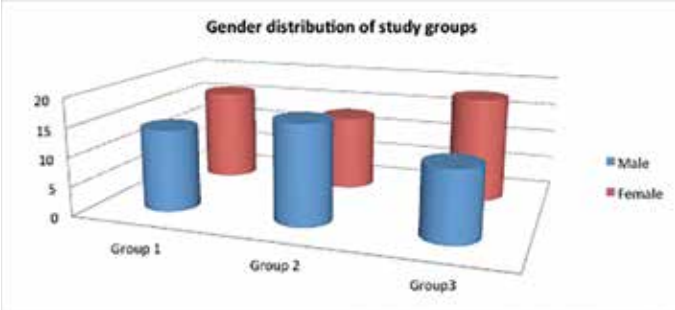

Figure 1. *Gender distribution of study groups.*

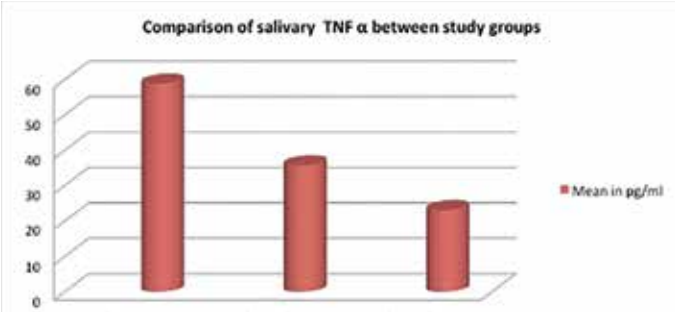

Figure 2. *Comparison of salivary TNF-α between study groups.*

Table 2. *Comparison of Salivary TNF-α level between the study groups*

				ANOVA	
	n	**Mean (pg/mL)**	**SD (pq/mL)**	**F**	**p**
Group 1	30	58.82	15.24		
Group 2	30	35.76	11.11	73.10	<0.001*
Group 3	30	23.09	6.95		

*p<0.05 statistically significant; ANOVA: analysis of variance; SD: standard deviation

Table 3. *Comparison of the type of RAS with Salivary level of TNF-α*

Type of RAS	**Number of patients**	**Mean TNF-α in pg/mL**
Minor	26	57.71
Major	4	66.04

monocytes and neutrophils. This leads to the oral mucosal cell destruction, and accumulation of acute inflammatory mediators followed by the development of an aphthous ulcer (11). The process is initiated by antigenic stimulation of the mucosal keratinocytes which leads to stimulation of T-lymphocytes, cytokine release as well as migration of other lymphocytes, neutrophils and Langerhans cells. Cytotoxic trigger causes ulceration of the mucosa. At present, TNF α is considered as the most significant cytokine implied in the development of new RAS lesions (21-23).

Table 4. *Correlation between age and TNF-α in each study group*

Group	**Age**	**TNF-α in pg/mL**
Group 1	R	0.33
	p	0.08 (NS)
Group 2	R	0.17
	p	0.37 (NS)
Group 3	R	0.27
	p	0.15 (NS)

NS: not significant; R: Pearson correlation test

Even though the exact role of TNF in the aetiology of RAS has not been determined yet, its possible contribution was explained by the facts such as high levels of TNF in oral ulcerations and high efficacy of anti-TNF drugs like levamisole, thalidomide or pentoxifylline in the treatment of RAS (8). In the present study we evaluated salivary TNF-α in patients with RAS in our region. Several studies have reported an increase in salivary and serological TNF-α, especially during the active phase of the disease (10, 11). However specific cause of this increase has not been established. Most of the studies have compared salivary TNF-α in RAS patients and healthy controls (8, 11). As existing studies shown the role of TNF-α role in the ulceration of mucosa, in the present study we investigated salivary TNF-α in patients with traumatic ulcer and compared with that of controls and RAS. We observed that salivary TNF-α levels was lower in patients with TUs compared to RAS patients, indicating the significant role of TNF-α in RAS than in TUs.

One study used biopsy samples to compare the TNF-α and its cellular distribution in RAS and in induced oral Tus (14). Present study results were in accordance with their findings. We examined the salivary TNF-α and statistically significant difference was noticed in the study groups. This result underlines the importance of this cytokine in the development of RAS. Our study results were consistent with Sun *et al.* (12) study. Present study used saliva samples since obtaining saliva is easy and non invasive. Patient acceptance of saliva sample collection is much higher when compared to obtaining serum or biopsy samples. We found higher salivary TNF-α in RAS patients compared to TUs and controls. Previous studies have shown significantly higher serum levels of TNF- α in patients with of major, minor or herpetiform RAU (14). In our study equal distribution among the types of RAS was not present, hence analysis of TNF- α in various clinical types of RAS was not carried out. Inflammation and metabolism of free radicals in RAS patients and healthy controls was evaluated by Avci *et al.* (23). They investigated TNF-α, interleukin-2 (IL-2), IL-10, and IL-12 using ELISA and emphasized their importance in the occurrence of RAS. Similar pattern was observed in our study, however we only evaluated TNF-α.

The use of TNF- α inhibitors or rituximab in ulcerative oral conditions was reviewed by previous researchers. They also

analysed the potential benefits, adverse effect, principles of use and future developments. They concluded that TNF- α inhibitors such as infliximab can be effective in RAS and indicated in patients with severe refractory disease (24). Pentoxifylline, a methylxanthine derivative with immune modulatory and anti-inflammatory properties, is beneficial in the treatment of RAS. This effect was reported to be due to the inhibition on the production of TNF- α and other inflammatory cytokines (11). However, the use of these drugs is restricted due to their side effects. New formulations which provide TNF- α inhibition with less side effects still need to be evaluated.

Conclusion

Detection of higher level of TNF- α in RAS compared to TUs and controls indicates the role of TNF- α in the pathogenesis of RAS. This suggests the significant contribution of TNF- α in the pathogenesis of RAS which can be detected by using saliva as a convenient medium. Thus, future management of RAS should be directed towards newer treatment modalities for better outcome.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of A B Shetty Memorial Institute of Dental Sciences dated 30-09-2014 Cert No. ABSM/EC/114/2014.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: SH designed the study. SH, VA, SB, SK, HU and AM generated the data. SH gathered the data for the study. SH analyzed the data. SH wrote the majority of the original draft. SH, VA, SB, SK, HU and AM contributed towards writing the paper. SH, VA and SB analyzed the raw data of the study. All authors approved the final version of the paper.

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Türkçe öz: *Rekurrent aftöz stomatit hastalarında tümör nekrotizan faktör alfa seviyelerinin değerlendirilmesi. Amaç: Bu çalışmanın amacı ağız mukozasında rekurrent aftöz stomatitis (RAS), travmatik ülser (TÜ) lezyonları olan ve olmayan sağlıklı bireylerde tükürükteki tümör nekrotizan faktör alfa (TNF-α) seviyelerini karşılaştırmaktır. Gereç ve Yöntem: Bu çalışma; RAS saptanan 30 (Grup 1), TU saptanan 30 (Grup 2) ve 30 sağlıklı kontrollerden (Grup 3) oluşan üç gruba ayrılan 90 hasta üzerinde gerçekleştirilmiştir. Bireylerden tükürme tekniği ile uyarılmamış tükürük örnekleri alınmıştır. Örneklerin içerisindeki TNF-α miktarı enzyme linked immunosorbent assay yöntemi ile belirlenmiş ve istatistiksel olarak karşılaştırılmıştır. Bulgular: RAS saptanan bireylerin tükürük örneklerindeki TNF-α miktarının TU ve kontrol grubunda saptanan değerlerden istatistiksel olarak anlamlı derecede yüksek olduğu bulunmuştur. Çalışma grupları arasında da istatistiksel olarak anlamlı farklılık saptanmıştır (p<0,001). Sonuç: Bu çalışma, tükürük örnekleri alınmasının TNF-α seviyelerinin belirlenmesinde uygun bir yöntem olduğunu göstermektedir. RAS, TU ve kontrol grupları arasında saptanan istatistiksel olarak anlamlı farklılıklar TNF-α'nın RAS gelişiminde önemli bir rol üstlendiğini düşündürmektedir. Anahtar kelimeler: Rekurrent aftöz stomatit; tümör nekrotizan faktör alfa; tükürük; ağız mukozası; travmatik ülser*

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Original research

The prevelance of mesiodens in a group of non-syndromic Turkish children: a radiographic study

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Purpose
The aim of the present study is to determine the prevelance and clinical status of mesiodens in a group of non-syndromic Turkish children, with an analysis of the associated clinical-eruptive complications.

Materials and Methods
This study sample consists of 58142 pediatric patients'. Standard equipment and films were used in the suspected patients. The examination of all radiographs was performed under standard conditions by two pediatric dentists with over 10 years experience. Age, gender, number of mesiodens, morphology and clinical status were recorded in forms.

Results
A total of 83 mesiodentes were diagnosed in 59 children with ages ranging from 6-14 years. The prevelance of mesiodens was estimated as 0.1%. Males were more frequently affected than females in the ratio of 2.3:1. Of the 83 mesiodentes, 48.2% were conical, 31.3% were tuberculate and 20.5% were incisor like, 22.9% were inverted, and 68.7% were fully impacted. The number of mesiodens was one in 36 cases (61.0%), two in 22 cases (37.3%) and three in one case (1.7%). The mean age at the time of diagnosis of the mesiodens was 9.5 years. The main complication associated with the mesiodens was displacement or rotation of the permanent teeth (73.3%).

Conclusion
This study presents 0.1% prevelance of mesiodens in a group of Turkish children sample. The majority of the mesiodentes were unilateral located in the premaxillary region, were conical shaped, and remained unerupted. The mean age at the time of diagnosis of the mesiodens in this study was 9.5 years, with this period being later than the eruption time of the maxillary central incisor.

Keywords: *Mesiodens; pediatric population; radiographic study; prevelance; complication*

Introduction

The most common type of supernumerary tooth which can appear in the maxillary midline area is defined as mesiodens (1). The reported prevalence in the permanent dentition ranges between 0.1-3.8% whereas, in the primary dentition the range is between 0.03-1.9% (2-5). Supernumerary teeth are estimated to occur in the maxilla more frequently than in the mandible (6). The mesiodens is the most frequent type of supernumerary tooth and accounts 80% of all (7-9). Mesiodens may occur either as single or multiple (8).

Mesiodentes can cause a variety of problems, including; retention of the primary tooth, delay or prevent of eruption of central incisors and can lead to ectopic eruption, tooth displacement, central incisor rotation , abnormal root development, dilacerations in the developing roots,

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root resorption and loss of tooth vitality, crowding, spacing of the anterior teeth, dentigerous cyst formation, follicular cysts, eruption of the mesiodentes into the nasal cavity as well as other alterations requiring surgical or orthodontic intervention (10-12).

The etiology of mesiodens remains unclear; however, various theories have been suggested regarding the presence of supernumerary teeth. Supernumerary teeth have also been attributed to atavism. The first theory of atavism - referring to having more teeth - is widely rejected. For splitting of the dental follicle (the theory of dichotomy), some factors such as trauma or evolutionary mutations, can cause accidental follicle division into two or more fragments. The hyperactivity of the dental lamina and the combination of genetic and environmental factors may be considered as the most acceptable etiologic factors in the development of mesiodens (13-15). Familial occurrence of mesiodens is reported to involve more than one sibling, or one generation (16, 17).

Mesiodens may also occur in association with syndromes like; cleidocranial dysostosis, Gardner's syndrome, especially cleft lip and palate, Down's syndrome (18, 19).

However, the appearance of a mesiodens can occur in non-syndromic individuals. Positive family history is one of the predisposing factors and this condition might be found as an isolated finding (20).

The objective of the present study was to examine the prevelance and clinical status of mesiodens, with an analysis of the associated clinical-eruptive complications. The null hypothesis of the study is the appearance of a mesiodens don't occur in non-syndromic individuals and there is no associated mesiodens complications.

Materials and Methods

The study was based on the evaluation of 58142 pediatric patients who attended the Istanbul University Faculty of Dentistry, Department of Pedodontics between September 2013 and December 2015. Ethical committee approval was obtained from the ethical committee of Istanbul University Faculty of Dentistry (Ref.Number 158; 2016/44). All patients and/or parents signed a letter of consent giving permission to use data for research purposes after related radiographs were taken.

Inclusion criteria

Only patients who accepted the use of their data for research purposes and visited the faculty for: treatment of caries, gingival conditions, tooth fracture, malocclusion or routine dental check-ups during the specified period and had no history of any previous extraction or tooth loss due to trauma, were included in the study.

Exclusion criteria

Patients without adequate documentation or patients who had any associated developmental anomalies, missing teeth adjacent to the mesiodens and with poor quality radiographs were excluded.

Radiographic examination

Standard equipment (Kodak 8000; Tropphy, etx) and films (Kodak) were used. Radiographic examination of the premaxilla was based on intraoral periapical (anterior region, +40°) and panoramic radiographs (kVp 65-68; mA range varies between 2-3,2 for infants; 5-6.5 for adolescents) for all children. Some of the cases were supplemented with occlusal radiographs (Kodak film ultra-speed). The examination of all radiographs was performed under standard conditions (on standard light boxes) by two pediatric dentists with over 10 years experience. All discrepancies were solved by consensus and agreement.

Diagnosis and recording of the mesiodentes

The presence of a supernumerary tooth or tooth bud between two central incisors, or of unilateral or bilateral teeth in the midline of the maxilla was noted as mesiodentes on radiographs. Age, gender, number of mesiodentes, morphology (conical, tuberculate, supplemental, other), clinical status (erupted, impacted) were recorded in forms.

Statistical analysis

The variables were analyzed using Statistical Package for the Social Sciences 12 (IBM Corp.; 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA). The Pearson chi-square test was used to analyse sex differences. A p value of < 0.05 was considered statistically significant.

Results

Results showed that among the total 58142 children screened (male-28733; female- 29409), 0.1% had mesiodentes. A total of 83 mesiodentes were diagnosed in 59 (18 girls, 41 boys) children from 58142 samples from a pediatric Turkish population with ages ranging from 6 to 14 years. No statistical significance was found between genders.

Age, gender, number of mesiodentes, morphology (conical, tuberculate, supplemental, other), clinical status (erupted, impacted) of the cases are presented in Table 1.

Forty one (49.4%) mesiodentes presence were detected in age group of 6-9 years, and 42 (50.6%) in age group of 10-14 years. Of the 59 children, 36 children (61.0%) had one mesiodens (Figure 1), 22 children (37.3%) had two mesiodentes (Figure 2) and 1 child (1.7%) had three mesiodentes.

Among the 83 mesiodentes, the conical shape was the most common type accounting for 48.2%, followed by tuberculate in 31.3% followed by incisor-like in 20.5% (Figure 3).

Of the 83 mesiodentes, 68.7% were fully impacted and 31.3% were either partially or completely erupted. Among 83 mesiodentes, 77.1% were located in the vertical direction, followed by invertion with 22.9%. The main complication associated with the mesiodentes was displacement or rotation of permanent teeth seen in 44 patients (73.3%) followed by the delayed eruption of permanent central incisors (24 patients, 40.0%), delayed or abnormal root development of permanent central incisors (7 patients, 11.6%). Six patients were asymptomatic.

Table 1. *The percentage distribution of mesiodentes according to the age, gender, number, morphology, clinical status*

Age of subjects	Ratio of boys to girls	Number of mesiodens	Morphology	Clinical status
		One (61 %)	Conical (48.2%)	Impacted (68.7%)
6-14 years	2.3:1	Two (37.3%)	Tuberculate (31.3%)	Erupted (31.3%)
		Three (1.7 %)	Incisor like (20.5%)	(partialy/completely)

Figure 1. *Panoramic radiograph of the case with one mesiodens.*

Figure 2. *Panoramic radiograph of the case with two mesiodentes.*

Figure 3. *Photograph of the case with an incisor-like mesiodens.*

Discussion

In this study, the complete records of 58142 pediatric patients who presented mixed or permanent dentition were assessed. A total of 83 mesiodentes were diagnosed in 59 children (average of 1.4 mesiodentes per child), corresponding to 0.1% prevalence in the overall sample. This prevalence was less than that described in studies by Hurlen and Humerfelt (21) (1.4%) and Salcido-García *et al.* (22) (1.6%), Patil *et al.* (3) (1.4%) and was very close to the mean frequency observed in the prevalence values presented in Çolak *et al.* (2) (0.13%). The null hypothesis of the study was not supported.

Supernumerary teeth affect both dentitions equally, but mesiodens are the most frequently observed dental anomaly in permanent dentition (20) and it was more common among males. In the present study, there was a male to female ratio of 2.3:1, in the 59 patients with mesiodens. This ratio was 2.8:1 in the 200 patients (3 -84 years old) in Asaumi *et al.* (1) study. Kim and Lee (7) examined 40 children, whose ages ranged from 4 to 26 years, also found that males were affected approximately four times as frequently as females.

The mean age at the time of diagnosis of the mesiodens in this study was 9.5 years, but of 59 cases, 32 (54.2%) were discovered at 9-11 years. This period is later than the eruption time of the maxillary central incisors. When the delay of eruption and malposition of the maxillary central incisors or supernumerary teeth, congenitally missing teeth were seen, the radiographic examination was performed as a screening aid. Usually, mesiodens are discovered when adjacent teeth are displaced or have delayed eruption. Furthermore, during a routine radiographic check-up, an unerupted mesiodens without significant effect on the adjacent teeth may be examined (15, 23). Most mesiodientes are discovered with radiographic evaluation in the eruption period.

Although, mesiodentes may be single or multiple, multiple supernumerary teeth are rare in individuals with no other associated diseases or syndromes (24). A single mesiodens was found in 61% of the sample, while the remaining 37.3% presented two and 1.7% had three mesiodentes in this study. These findings were similar to the findings of Asaumi *et al.* (1), Gunduz *et al.* (25), Kim and Lee (7), and Huang *et al.* (26), who recorded one mesiodens in most of the reported cases.

Among 83 mesiodentes, 57 (68.7%) were impacted and 26 (31.3%) had erupted in the oral cavity. With regard to the direction of the crown, a mesiodens is most often in an upright position, but it can be found in an inverted or even in a horizontal position (4, 25-27). In the present study of the 83 mesiodentes, 64 (77.1%) were in a normal direction and 19 (22.9%) in an inverted direction against the axis of the tooth. Roychoudhury *et al.* (28) have reported seeing inverted impacted mesiodens in 62.5% of impacted mesiodentes, yet such a relationship was not observed in our study.

A mesiodens is often unique (1, 4, 7, 25, 26) and different in shape and size (16), but may vary in morphology, from a small rudimentary conical shape (4, 7, 22, 25, 28), to a complex form with several tubercles. In the current study, the crown shape was mainly conical (48.2% of cases) and this was in accordance with Giacontti *et al.* (29), Seddon *et al.* (30) and Kim and Lee (7).

The main complications associated with mesiodentes in this study were: displacement or rotation of permanent incisors (74.6%), delayed eruption of permanent incisors (42.4%),

delayed or abnormal root development of associated permanent teeth (11.8%). Similar findings have been previously reported (7, 25, 27). Von Arx (31) reported the retention and malposition of the adjacent permanent incisors in 34.5% of 113 mesiodentes.

The treatment of mesiodentes should be planned, after consideration of all clinical and radiological findings. Management always depends on the type of supernumerary teeth, its position in relation to other teeth, and its effects on adjacent teeth. Extraction is not always the preferred treatment. Surgical removal should be avoided if unerupted supernumerary teeth remain asymptomatic and are sometimes best left and kept under observation (24). Late or delayed removal of supernumerary teeth is recommended in order to prevent damage to tooth buds and/or adjacent teeth, decrease the surgical burden for a child, and to avoid repetitive surgery. However, periodic follow-up is necessary (32). Most recommendations for early and late removal of supernumerary teeth are anecdotal rather than evidence based (33).

This report shows the need for early and correct diagnosis of mesiodentes, which requires an individualized treatment plan. Unilateral persistence of a deciduous incisor, failure of eruption or ectopic eruption of a permanent incisor, a wide diastema, or rotation of erupted permanent incisors should alert the clinician to the possible presence of supernumerary teeth (31) and indicate appropriate radiographic investigation. Whatever the management approach, early diagnosis is critical. An appropriate treatment plan should emphasize prevention and include regular clinical and radiographic monitoring and, if possible on eruption.

Conclusion

This study showed a 0.1% prevelance of mesiodens in a group of Turkish children sample. The majority of the mesiodentes were unilateral located in the premaxillary region, were conical shaped, and remained unerupted. In this study the main complication associated with the mesiodens was displacement or rotation of permanent teeth (73.3%). The mean age at the time of diagnosis of the mesiodens in this study was 9.5 years, and this period is later than the eruption time of the maxillary central incisor. On appropriate diagnosis early intervention is required in the form of surgical or orthodontic treatment and combination in order to minimize unwanted side effects to the developing dentition in children.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University Faculty of Dentistry (Ref.Number 158; 2016/44).

Informed Consent: Written and verbal informed consent was obtained from patients/patients' parents who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: GAr and GAk designed the study. GAr and GAk generated the data. APE gathered the data. ODO analyzed the data. GAr wrote the majority of the original draft. APE participated in writing the paper. All authors approved thefinal version of the paper.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Türkçe öz: *Sendromu olmayan bir grup Türk çocuğunda mezyodens prevelansı: bir radyografik çalışma. Amaç: Bu çalışmanın amacı, sendromu olmayan bir grup Türk çocuğunda mezyodens sıklığını ve klinik durumunu ilgili klinik sürme komplikasyonlarıyla ilişkilendirerek incelemektir. Gereç ve Yöntem: Bu çalışma, 58142 çocuk hastanın panaromik radyografilerinin değerlendirilmesiyle gerçekleştirilmiştir. Çalışmada, standart gereç ve filmler kullanılmıştır. Tüm radyografiler, standart koşullar altında, 10 yıl üstü deneyime sahip iki pedodontist tarafından değerlendirilmiştir. Yaş, cinsiyet, mezyodens sayısı, morfoloji ve klinik durum formlara kayıt edilmiştir. Bulgular: Yaşları 6-14 arasında değişen 59 hastada 83 mezyodens tespit edilmiştir. Mezyodens prevelansı %0,1 olarak saptanmıştır. Kadınlar, erkeklerden 2.3:1 oranında daha fazla etkilenmiştir. Seksen üç mezyodensin; %48,2'sinin konik, %31,3'ünün tüberküllü, %20,5'inin kesici diş görünümünde, %22,9'unun enverte ve %68,7'sinin ise tamamen gömük olduğu belirlenmiştir. Otuz altı olguda bir (%61,0), 22 olguda iki (%37,3) ve bir olguda 3 (%1,7) mezyodens varlığı izlenmiştir. Mezyodens tanısının yapıldığı ortalama yaş 9,5 yıl olarak belirlenmiştir. Mezyodens ile ilişkili en önemli komplikasyonun, kalıcı dişlerin yer değiştirmesi veya rotasyonu (%73,3) olduğu saptanmıştır. Sonuç: Bu çalışma, bir grup Türk çocuğunda mezyodens prevelansını %0,1 olarak vermektedir. Mezyodenslerin çoğunluğunun premaxiller bölgede tek taraflı, konik şekilli ve sürmemiş olduğu belirlenmiştir. Bu çalışmada, mezyodens tanısının konduğu yaş ortalaması 9,5 yaş olup, bu periyot üst birinci kesici dişin sürme zamanından sonradır. Anahtar kelimeler: Mezyodens; çocuk popülasyonu; radyografik çalışma; prevelans; komplikasyon*

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Case report

Unusual radiographic images of radiopaque contrast media incidentally observed in intracranial region: two case reports

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The oil-based contrast medium has extremely slow clearance rate from cerebrospinal fluid. The medium known as myodil or pantopaque or iopenydylate was firstly introduced in 1944 to be used in myelography, cisternography and ventriculography. It was commonly used until 1980s but was later replaced by water-soluble mediums in 1990s because of its complication and sequelae. Although rare, images of the remnants may still be encountered on radiograms since its remnants may be seen after six decades. In this article, incidental radiopaque images in panoramic radiography and cone-beam computed tomography (CBCT) were presented in two patients whose myelography was taken before herniated discs' operation. Unusual incidental radiopacities in intracranial region were observed on panoramic radiography image of a male and CBCT image of a female, both of whom underwent myelography more than 30 years ago. Dentomaxillofacial radiologists should be aware of this radiographic appearance, should be able to differentiate it from possible pathologies.

Keywords: *Long-term adverse effect; contrast media; panoramic radiography; cone-beam computed tomography; diagnostic imaging*

Introduction

The oil-based contrast medium has extremely slow clearance rate from cerebrospinal fluid. The medium known as myodil or pantopaque or iopenydylate was firstly introduced in 1944 by Ramsey *et al.* (1) and was widely used as synchronous by Steinhausen *et al.* (2) for myelography. This oil-based contrast medium was commonly used until 1980s for myelography, cisternography and ventriculography because of its ideal physical and radiographic features (3, 4). However, the myodil was replaced by water-soluble medium in 1990s due to its several complications and sequelae such as arachnoidids, chronic irritation, arachnoid adhesions and nerve damage depending on extremely slow clearance rate from cerebrospinal fluid (5-7). The remnants of the oil-based contrast medium may be seen after six decades (8). Although rare, images of the remnants may be still encountered on skull radiograms in recent dental practice. In the literature, it has been reported that the remnants of contrast media were observed on brain computed tomography and lumbar and thoracic magnetic resonance images. However, there are limited number of published cases in conventional skull radiograms (4, 9, 10).

The aim of this article is to present two cases with intracranial oil-based contrast medium-related radioopacities incidentally discovered on panoramic radiography and cone-beam computed tomography (CBCT) who had underwent myelography for herniated discs more than 3 decades ago.

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Case reports

Case 1

An 81-year-old man was referred to our clinic for prosthetic rehabilitation. Written informed consent was obtained from the patient. Medical history revealed that he had undergone herniated disc surgery in 1979. There were no other noteworthy findings. Patient claimed that he had had an injection in the lumbar region and a radiograph had been taken before the procedure. Multiple small rounded radiopacities of the millimetric dimensions were observed in the bilateral zygomatic bone region of his panoramic radiography image (Figure 1). Panoramic image was taken by using a Orthophos XG unit (Orthophos XG, Sirona Dental Systems GmbH D-64625, Bensheim, Germany) operated at 90 kVp, 12 mA and 14 seconds exposure time. It was thought that the radiopacities may be related with myelography which was performed 38 years ago. No further examination was performed since the patient was asymptomatic.

Case 2

A 73-year-old woman was referred to our clinic for dental-implant supported prosthetic rehabilitation. Written informed consent was obtained from the patient. Medical history revealed that she had undergone herniated discs' surgery in 1982 and, also, she complained about unilateral headache in the left side and backache since then. Additionally, she was using antihypertensive and antidiabetic drugs. CBCT image were obtained before implant planning. CBCT images were obtained by using a Promax 3D unit (Planmeca Oy, Helsinki, Finland) operated at 90 kVp and 12 mA with a voxel size of 0.16 mm, exposure time of 13.8 seconds, 0.4 mm slice thickness and field of view (face); 200x170 cm. Multiple small rounded radiopacities in the millimetric dimension were observed in intracranial region including sella turcica, left temporal bone and orbita (Figure 2). She was advised to visit a neurology specialist for head and back symptoms but patient refused to do so.

Figure 1. *Multiple small rounded radiopacities (the areas with yellow-marked) on panoramic radiography image in bilateral zygomatic bone regions of case 1.*

Discussion

The remnants of oil-based contrast medium in the spine may cause numerous complications including lumbar arachnoidid, spinal cord compression, nerve damage, low-back pain, weakness in the lower limbs and sensory changes. The intracranial remnants may lead to chronic arachnoidid, headache, backache, hydrocephaly, meningitis, imbalance and vertigo (4-10). It has been explained that the slow clearance rate and prolonged presence of the remnants can cause irritation (4-10).

Myodil remnants presented in the published reports have been observed mostly in the lumbar and thoracic regions, but intracranial remnants are relatively rare (4-11). The intracranial remnants on conventional skull radiograms have been observed in only three published papers (4, 9, 10). In those reports, residual myodil has been shown on Waters' view (4), brain ventriculogram (9), and skull radiograms (10) of the male patients. According to best of our knowledge, there is no published case in panoramic radiography or CBCT. In this report, intracranial radiopacities were observed on panoramic radiography image of a male and CBCT image of a female patient, both of whom had underwent myelography more than 30 years ago.

The typical radiographic appearance of these materials is usually multiple dots or droplet-like radiopaque areas in the intracranial region. These findings may be encountered in daily practice because of prolonged presence of the remnants and may be falsely interpreted as lipomas, hemorrhages and hemangiomas on conventional radiographs, computed tomography and magnetic resonance images (11). However, it has been reported that this condition can be easily diagnosed together with patient history and the presence of radiopacities on plain radiographs or computed tomography images (11-13). In the present cases, multiple small rounded radiopacities were observed on panoramic radiography and CBCT images. Both patients were easily diagnosed based on

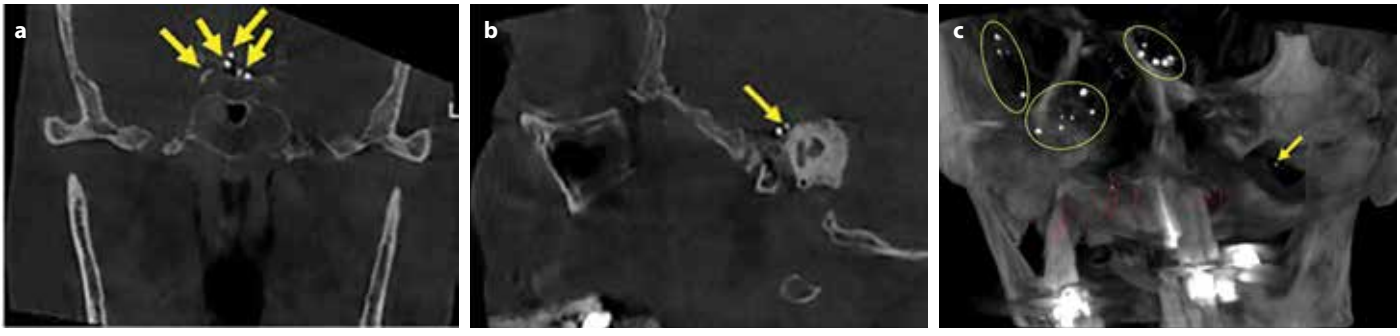

Figure 2. a-c. *Multiple small rounded radiopacities (yellow circles and arrows) can be seen in the intracranial region of case 2 (a) coronal CBCT section of sella turcica region, (b) sagittal CBCT section of left temporal bone, (c) 3-dimensional reconstruction image.*

their medical history as well as by their clinic and radiographic examinations by dentomaxillofacial radiologists.

Complications of pantopaque have been previously investigated in animal studies, clinical and case reports. Mild or severe headache, meningitis, vertigo, imbalance, arachnoidids, hydrocephalus, hypersensitivity have been reported in patients who have intracranial remnants of myodil (4, 9-11). Although some authors believed that the remnants of pantopaque in the intracranial subarachnoid space should be removed (10), symptomatic treatment and/or periodic follow-up are usually accepted approaches by several authors in minor symptomatic and/or asymptomatic cases (4, 8, 9, 14). Accordingly, in the present cases, no further examinations were carried out in the asymptomatic patient (Case 1), but the patient who complained from headache and backache (Case 2) was advised to visit a neurologist.

Conclusion

Although the use of oil-based media has been left in 1990s, its intracranial remnants on radiographic images may be still encountered in daily practice. Especially dentomaxillofacial radiologists should be aware of its radiographic appearance. The clinicians should be able to differentiate from possible pathologies by evaluating rare findings in routinely used imaging modalities.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: İP and ÖÜ designed the study. BÇ and UP generated and gathered the data. İP, BÇ, UP and ÖÜ analyzed the data. İP and ÖÜ wrote the majority of the original draft. All authors approved the final version of the paper.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Türkçe öz: *İntrakraniyal bölgede tesadüfen izlenen nadir radyoopak kontrast madde görüntüleri: iki olgu bildirisi. Yağ-bazlı kontrast maddeler serebrospinal sıvıdan çok yavaş emilirler. Myodil, pantopaq veya iyofenidilat olarak bilinen bu maddeler ilk defa 1944 yılında miyelografi, sisternografi ve ventrikülografide kullanılması için tanımlanmıştır. Bu maddeler 1980'lere kadar yaygın olarak kullanılmıştır, fakat komplikasyonlar ve sekelleri nedeniyle 1990'larda yerini su bazlı kontrast maddelere bırakmışlardır. Nadir görülmekle birlikte, maddelerin artıklarının altı dekat sonra bile kalabilmesi nedeniyle, günümüzde hala artıkların görüntüleri ile karşılaşılabilmektedir. Bu makalede bel fıtığı operasyonu öncesi miyelografi yapılmış iki hastanın panoramik radyografi ve konik-ışınlı bilgisayarlı tomografi (KIBT) görüntülerinde tesadüfi olarak izlenen radyoopak görüntüler sunulmuştur. İntrakraniyal bölgede nadir olarak karşılaşılan bu tesadüfi bulgular, otuz yıldan fazla süre önce miyelografi yapılmış bir erkek hastanın panoramik radyografi görüntüsünde ve bir kadın hastanın KIBT görüntüsünde izlenmiştir. Dentomaksillofasiyal radyologlar bu görüntülerden haberdar olmalı ve görüntüleri olası patolojilerden ayırt edebilmelidir. Anahtar kelimeler: Uzun süreli yan etki; kontrast madde; panoramik radyografi; konik-ışınlı bilgisayarlı tomografi, diagnostik görüntüleme*

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