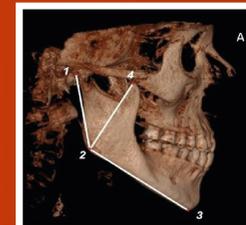




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Editor-in-Chief
İhsan Hubbezoğlu
Co-Editor-in-Chief
Burak Buldur



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Aims and Scope

Cumhuriyet Dental Journal (CDJ) is an international journal dedicated to the latest advancement of dentistry. The aim of this journal is to provide a platform for scientists and academicians all over the world to promote, share, and discuss various new issues and developments in different areas of dentistry.

CDJ publishes original research papers, reviews, and case reports within clinical dentistry, on all basic science aspects of structure, chemistry, developmental biology, physiology and pathology of relevant tissues, as well as on microbiology, biomaterials and the behavioral sciences as they relate to dentistry.



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e-mail: cdj@cumhuriyet.edu.tr Phone: +90 346 2191010 / 2730 (ext)

INDEXING



CUMHURİYET DENAL JOURNAL

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Case Report: Title, Abstract, Introduction, Case Report, Discussion, Conclusions, Acknowledgements, References, Tables and Figure Legends

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For various reasons, this kind of free and unrestricted online availability, which we will call open access, has so far been limited to small portions of the journal literature. But even in these limited collections, many different initiatives have shown that open access is economically feasible, that it gives readers extraordinary power to find and make use of relevant literature, and that it gives authors and their works vast and measurable new visibility, readership, and impact. To secure these benefits for all, we call on all interested institutions and individuals to help open up access to the rest of this literature and remove the barriers, especially the price barriers, that stand in the way. The more who join the effort to advance this cause, the sooner we will all enjoy the benefits of open access.

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While the peer-reviewed journal literature should be accessible online without cost to readers, it is not costless to produce. However, experiments show that the overall costs of providing open access to this literature are far lower than the costs of traditional forms of dissemination. With such an opportunity to save money and expand the scope of dissemination at the same time, there is today a strong incentive for professional associations, universities, libraries, foundations, and others to embrace open access as a means of advancing their missions. Achieving open access will require new cost recovery models and financing mechanisms, but the significantly lower overall cost of dissemination is a reason to be confident that the goal is attainable and not merely preferable or utopian.

To achieve open access to scholarly journal literature, we recommend two complementary strategies.

I. Self-Archiving: First, scholars need the tools and assistance to deposit their refereed journal articles in open electronic archives, a practice commonly called, self-archiving. When these archives conform to standards created by the Open Archives Initiative, then search engines and other tools can treat the separate archives as one. Users then need not know which archives exist or where they are located in order to find and make use of their contents.

II. Open-access Journals: Second, scholars need the means to launch a new generation of journals committed to open access, and to help existing journals that elect to make the transition to open access. Because journal articles should be disseminated as widely as possible, these new journals will no longer invoke copyright to restrict access to and use of the material they publish. Instead they will use copyright and other tools to ensure permanent open access to all the articles they publish. Because price is a barrier to access, these new journals will not charge subscription or access fees, and will turn to other methods for covering their expenses. There are many alternative sources of funds for this purpose, including the foundations and governments that fund research, the universities and laboratories that employ researchers, endowments set up by discipline or institution, friends of the cause of open access, profits from the sale of add-ons to the basic texts, funds freed up by the demise or cancellation of journals charging traditional subscription or access fees, or even contributions from the researchers themselves. There is no need to favor one of these solutions over the others for all disciplines or nations, and no need to stop looking for other.

Open access to peer-reviewed journal literature is the goal. Self-archiving (I.) and a new generation of open-access journals (II.) are the ways to attain this goal. They are not only direct and effective means to this end, they are within the reach of scholars themselves, immediately, and need not wait on changes brought about by markets or legislation. While we endorse the two strategies just outlined, we also encourage experimentation with further ways to make the transition from the present methods of dissemination to open access. Flexibility, experimentation, and adaptation to local circumstances are the best ways to assure that progress in diverse settings will be rapid, secure, and long-lived.

The Open Society Institute, the foundation network founded by philanthropist George Soros, is committed to providing initial help and funding to realize this goal. It will use its resources and influence to extend and promote institutional self-archiving, to launch new open-access journals, and to help an open-access journal system become economically self-sustaining. While the Open Society Institute's commitment and resources are substantial, this initiative is very much in need of other organizations to lend their effort and resources.

We invite governments, universities, libraries, journal editors, publishers, foundations, learned societies, professional associations, and individual scholars who share our vision to join us in the task of removing the barriers to open access and building a future in which research and education in every part of the world are that much more free to flourish. Submitting a paper to CDJ is free of charges. In addition, CDJ has not have article processing charges.

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The approval of the ethic committee, statement on the adherence to international guidelines mentioned above and that the patients' informed consent is obtained should be indicated in the "Materials and Methods" section and is required for case reports whenever data/media used could reveal identity of the patient. The declaration of the conflict of interest between authors, institutions, acknowledgement of any financial or material support, aid is mandatory for authors submitting manuscript and the statement should appear at the end of manuscript. Reviewers are required to report if any potential conflict of interest exists between reviewer and authors, institutions.

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TECHNOLOGY- BIG DATA- ARTIFICIAL INTELLIGENCE- COMMUNICATION AND COVID

 Marc Saadia¹

ORCID ID: 0000-0002-1128-4905

¹ Prado Sur 290, Lomas de Chapultepec, Mexico, 11000DF, Mexico

There is no doubt that technology has been and will continue to be advantageous and an unstoppable player that has invaded and improved our minute lives with good experiences, immediate results with answers, making our lives lazier, easier and more comfortable.

New generations are becoming more isolated and communication is jeopardized. This may separate us from what makes us human and may pave the way for humans to become expendable and replaceable. What we are seeing today, is just the beginning of what next decade revolutionary technological inventions is preparing for us and equals what humanity has produced since the invention of the wheel. The reason is that science and technology are growing exponentially.

We, as pediatric dentists thought that one of our major advantages that would always prevail in humans is our ability to connect with other people.

We were concerned with some telltales that did not look good. Young children boasting a tantrum when parents would take away the cell phones. Youngsters staying home connected digitally 8 to 9 hours every day. Young adults ordering food at home, eating and watching movies on demand alone. Parents ordering their supermarket items digitally and missing the opportunity use

their five senses. Not be able to see the variety of beautiful colors in the vegetable and fruit department, losing the possibility to smell all the fruits, touch them when picking them, hearing the different noises and tasting samples when they walk by the aisles.

Communication is becoming more and more virtual, human contact, social life, human interactions, physical presence, communication, bonding is slowly but irreversibly being lost.

With COVID and staying home, companies found that their employees could work from home. Self employed people could also operate at a distance. Dentists and physicians could dissociate themselves in many instances from the physical contact. They could with a phone and pictures, identify, collect, evaluate, implement information, diagnose, give indications, solutions and peace of mind with security for patient, parents and ourselves.

We are witnessing what new technology is undoing.

COMMUNICATION

No matter how fast technology advances, we cannot afford to lose out humanity.

A full paradigm shift will be necessary. Parents play an utmost part because their children

are the most vulnerable part of society and we are a key element to guide them.

We need to rediscover the power of communication, community, unity by being present and listening the “other”. Today our main asset that technology hopefully will never understand, is feeling, loving, bonding.

We know more about children than any other person, specialty or profession. We see them grow, we know their likes, they will even come for their dental checkups with their children.

As the world is turning colder, and communications among us are affected, we need

to show the patient that we genuinely care, because we do.

When we bond we enter a danger zone of “co-dependency” (in the good sense).

" Do we want to be keepers of the past to preserve the future?

" Can I care, still allowing the other to be separate?"

" Can I leave the comfortable me and enter the ever-changing other?

Are we ready to take on this challenge? If not, will most health specialties survive the XXI century?

The Editor's recommendation of this issue's article to readers

RELATIONSHIP BETWEEN GENDER, TEACHING EXPERIENCE, SUBJECT TAUGHT, AND TEACHER'S ATTITUDE AND KNOWLEDGE TOWARD DENTAL TRAUMA IN CHILDREN

I am pleased to inform you that I have chosen this article by Anggono *et al.*¹ as Editor's Choice for second issue of 2020.

Dental trauma is one of the most important problems among children in dental practice. Traumatic dental injuries are commonly seen at schools. Thus, teachers must be able to make emergency dental procedures. Within this rationale, assessing teacher's attitude and

knowledge about dental trauma in children is critical.

This article revealed that the attitude toward dental trauma in children was positive whereas, knowledge about dental trauma was insufficient among the teachers.

Happy readings in the second issue of 2020!

Burak Buldur

Co-Editor-in-Chief

REFERENCE:

1. Anggono J, Budiardjo S.B, Fauziah E. Relationship Between Gender, Teaching Experience, Subject Taught, and Teacher's Attitude and Knowledge Toward Dental Trauma in Children. *Cumhuriyet Dent J* 2020;23:2;88-95.



APICAL SEALING ABILITY OF DIFFERENT ENDODONTIC SEALERS USING GLUCOSE PENETRATION TEST: A STANDARDIZED METHODOLOGICAL APPROACH

ABSTRACT

Objectives: To compare the apical sealing ability of four endodontic sealers based on glucose penetration method and validate the uses of contralateral teeth to provide a well-balanced experimental group.

Materials and methods: One-hundred-and-twenty (sixty pair) extracted contralateral lower premolars were selected and undergone strict radiographic protocol. Root canal anatomy of each pair contralateral teeth was matched buccolingually and mesiodistally according to inclusion criteria (single canal, mature apical foramen, canal type, canal width, length, and curvature). Matched-pair contralateral teeth were then reevaluated using CBCT and divided into right and left sides (n=60, each side). Next, all canals were instrumented up to size 30, taper 0.06. Subsequently, teeth were subdivided into five groups for each side and obturated with single cone gutta-percha (GP) and various sealers: Group 1 - GP only (control); Group 2 - EndoRez; Group 3 - Sealapex; Group 4 - EndoSeal MTA and Group 5 - BioRoot RCS. All samples were placed in an incubator at 37°C, 100% humidity for 72 hours. Four matched-pair teeth from each group were then subjected to thermocycling for 100 cycles, 1000 cycles and 10000 cycles, respectively. After that, they were decoronated, coated with three layers of nail varnish, and used for glucose penetration test. The concentrations of glucose (mmol/L) were measured after 24 hours. Data analyzed using One-way ANOVA complemented by post hoc Dunnett T3 Test and Paired sample T-Test.

Results: EndoSeal MTA demonstrated statistically significant ($p < 0.05$) lowest glucose penetration followed by BioRoot RCS, Sealapex, EndoRez, and lastly control group. Apical sealing ability decreased as the number of thermocycles increased. No significant difference ($p > 0.05$) was found between matched-pair contralateral teeth.

Conclusions: Bioceramic sealers demonstrated better sealing ability than resin and calcium hydroxide sealers. Using matched-pair contralateral teeth provided a well-balanced experimental group.

Keywords: EndoRez, mineral trioxide aggregate, root canal filling materials, sealapex, tricalcium silicate.

*Galvin Sim Siang Lin¹
 Nik Rozainah Nik Abdul Ghani¹
 Tahir Yusuf Noorani¹
 Aimi Kamarudin²

ORCID IDs of the authors:

G.S.S.L. 0000-0002-3996-5470

N.R.N.A.G. 0000-0002-2195-3076

T.Y.N. 0000-0002-4661-7458

A.K. 0000-0002-3033-4701

¹ Conservative Dentistry Unit, School of Dental Sciences, Universiti Sains Malaysia, Health campus, 16150, Kubang Kerian, Kota Bharu, Kelantan, Malaysia.

² Paediatric Dentistry Unit, School of Dental Sciences, Universiti Sains Malaysia, Health campus, 16150, Kubang Kerian, Kota Bharu, Kelantan, Malaysia.

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INTRODUCTION

Endodontic treatment involves the removal of pulp and cleaning of the root canal system to preserve the tooth in the dental arch.¹ This treatment is reported to have a high success rate range between 86–98%.² Recently, great attention has shifted towards the seal of the root canal system, as adequate obturation of the prepared three-dimensional root canals is important in determining the long term success of endodontic treatment.^{3,4} With the use of gutta-percha and endodontic sealers, obturation allows hermetic seal of the canals, thus, prevents bacterial micro-leakage into the canals and provides good long-term prognosis.^{5,6}

In the past few decades, numerous endodontic sealers have been introduced and they are classified based on their main constituent, for instance, resin, calcium hydroxide, glass ionomer, and mineral trioxide aggregate (MTA) sealers.⁷ The introduction of adhesive dentistry concept allowed materials to bond and provide intimate contact with the dentine walls of the root canal.⁸ Bondable root canal sealer, such as methacrylate resin sealer can form a monoblock system within the root canal space which improves the seal and fracture resistance of the filled canals.^{8,9} Recently, bioceramics have become one of the most popular biomaterials used in endodontics after the clinical success of MTA.¹⁰⁻¹² BioRoot RCS, a tricalcium silicate-based material, is amongst the most recently introduced bioceramic based endodontic sealer in the market. Bioceramic sealer exhibit several advantages such as lower cytotoxicity, excellent antimicrobial activity due to its high pH value, promotes hard tissue formation and can form hydroxyapatite layer.¹³

Undeniably, the sealing ability of an endodontic sealer is still considered an important parameter to be evaluated, but this assessment has been despised due to the lack of standardization.⁷ There are a substantial number of studies among the literature that have claimed to evaluate the quality of seal of different endodontic sealers using an array of methods.^{5,7,14-17} However, there is still no clear answer on the appropriateness of these leakage methodologies with questionable

scientific significance. The reliability of leakage studies remains unclear and most of them are non-reproducible.¹⁸ Therefore, a well-controlled condition is needed for assessing and comparing the sealing ability of endodontic sealers.

Hence, the present study aimed to compare the sealing ability of resin, calcium hydroxide and bioceramic endodontic sealers to root dentinal walls of endodontically treated teeth after artificial ageing using glucose penetration method.¹⁹ Furthermore, the present study also aimed to validate the use of matched-pair contralateral teeth in providing a well-balanced experimental group for leakage study. The first null hypothesis tested was that there was no significant difference in terms of sealing ability among all four endodontic sealers. The second null hypothesis was there is no significant difference between the results of glucose penetration when comparing each matched-pair contralateral teeth used in this study.

MATERIALS AND METHODS

This was an *in-vitro* experimental study involving one-hundred and twenty (sixty pairs) human contralateral lower premolars recently extracted due to orthodontic reasons from patients of Asian origin and patients' age ranging from 20 to 40 years who attended dental clinics of Hospital Universiti Sains Malaysia. Ethical approval was obtained from the Human Research Ethics Committee USM (Ref. USM/JEPeM/18110691) on 10th January 2019. All teeth were inspected under Leica microscope (Leica Microsystem Imaging Solutions, Cambridge, UK) at a 20x magnification by two blinded examiners to ensure that they were free from fracture, abrasion, resorption defect, and root caries. The tooth length was measured using a metal ruler (CLR6, Hu-Friedy Mfg. Co. Inc., Chicago, USA) to include teeth with a total length of 21mm to 23mm and root length of 12mm to 14mm. Strict screening protocol with a digital radiographic examination (Planmeca Romexis®, Helsinki, Finland) was then carried out by matching the root canal anatomy of each pair contralateral teeth both buccolingually (BL) and mesiodistally (MD) to provide a consistent baseline. Only contralateral teeth with

single canal, mature apical foramen, Type 1 Vertucci's Classification, anatomical root canal width difference of ± 0.5 mm, canal length difference of ± 1 mm (measuring from the cemento-enamel-junction to apical foramen) and canal curvature difference (BL or MD) less than 25° were accepted for this study, whereas the remaining pairs of contralateral teeth were excluded. These step-by-step screening procedures were reevaluated again with three-dimensional (3D) radiographic analysis using Cone Beam Computer Tomography (CBCT) scan (Art 3D, Oy Ajat, Espoo, Finland) taken by a licensed radiologist and images taken were analyzed using Romexis 2.9.2 R software (PlanmecaRomexis®, Helsinki, Finland) to avoid selection mistake. Only sixty matched-pair contralateral lower premolars ($n=120$) were chosen after the selection process. Each matched pair contralateral teeth were then divided into the left side, α ($n=60$) and right side, β ($n=60$). They were numbered accordingly to ensure a well-controlled comparison for each matched-pair contralateral teeth. Soft tissue debris and calculus were removed using an ultrasonic scaler (Dentsply Sinora, Bensheim, Germany). Access cavities were then prepared using a diamond Endo-Access bur, 21mm, size 3 (A 0164, Dentsply Maillefer, Switzerland) and canal patency was checked using sizes 10 and 15 K-files (FlexOFiles; Dentsply Maillefer, Switzerland). Root canals were instrumented with NiTi rotary files (S5 Sendoline, Tillverkarvägen 6, SE-187 66 TÄBY, Sweden) up to the final size 30, 0.06 taper to the working length, 1 mm short from the radiographic apex. After that, canals were irrigated copiously using 2.5% sodium hypochlorite (Lenntech, Delfgauw, Netherlands) solution (NaOCl). Finally, 5ml of 17% ethylenediaminetetraacetic acid (EDTA) solution (Promega Corporation, Wisconsin, USA) was used to remove smear layer followed by another 5ml of normal saline solution (RMBIO, Missoula, Montana) as final irrigation to wash out remnants of EDTA in the root canals. The canals were dried with paper points size 30 (Dentsply, Maillefer, USA). Contralateral teeth were subdivided into five groups for each side and obturated with

matched gutta-percha size 30 taper 0.06 (Meta Dental Corp, Glendale, New York, US) using single cone technique and various endodontic sealers as below:

Group 1: Gutta-percha only without sealer (control)

Group 2: EndoRez (Ultradent Products, Inc., South Jordan, US)

Group 3: Sealapex (Kerr Corporation, Orange, California, US)

Group 4: EndoSeal MTA (Maruchi, Gangwon-do, South Korea)

Group 5: BioRoot RCS (Saint-Maur-des-Fossés Cedex, France)

The sealers were mixed according to manufacturers' instructions. Sealers were first coated around the canal walls using the matched gutta-percha point before placing gutta-percha into the canal. All canals were eventually obturated using single cone technique with matched gutta-percha point and respective sealers. Excess gutta-percha was cut off and access cavities were cleaned after obturation. The coronal accesses were then acid etched (Gel Etchant, Kerr Corporation, Orange, CA) for 10 seconds and bonding agent (OptiBond™ Universal, Kerr Corporation, Orange, CA) applied followed by light curing for 15 seconds and restored with microhybrid resin composite (Zmack, Italy) incrementally with adequate 40 seconds of light-curing using a pre-calibrated LED light-curing unit Elipar Free Light 2 (3M ESPE, St. Paul, MN, USA) with a light intensity of 800 mW/cm^2 . Final composite restorations were polished with composite polishing kits (PN 0310BB, Composite Polishing Kit CA, Shofu, CA, US). The teeth were then placed in an incubator (ICS200, Yamato Scientific Co., Ltd., Japan) at 37°C , 100% humidity for 72 hours to allow complete setting of the sealers. Four matched-pair teeth from each group both sides were randomly selected and subjected to 100 thermal cycles using a thermocycling machine (TS Series Liquid, Weiss Technik, North America) in sequential water baths of 5°C , 37°C and 55°C . The dwell time was set at 30 seconds with a

transfer time of 5 seconds. The same thermal cycle process was repeated accordingly with the other four matched-pair teeth from each group for 1000 thermal cycles. Lastly, the remaining four matched-pair teeth from each group were subjected to 10000 thermal cycles. Teeth were kept moist throughout the experiment by covering them with moist gauze.

Glucose Penetration Test

After thermal cycles, the teeth were decoronated at the cemento-enamel junction (CEJ) using a hard tissue cutter (EXAKT 312, EXAKT Technologies, Inc., Oklahoma City, US). Only sample in the control group (Group 1) were subdivided into positive (n=12) and negative (n=12) controls, each consisted of 6 matched-pair teeth.¹¹ All samples were coated with three layers of nail varnish leaving 1mm clear from the apical foramen and 1mm clear from the CEJ, except samples in the negative control group were entirely coated with three layers of nail varnish to prevent the glucose molecules from leaking out through lateral and other accessory canals which might affect the validity of the present study. Then, samples were set up as shown in Figure 1.

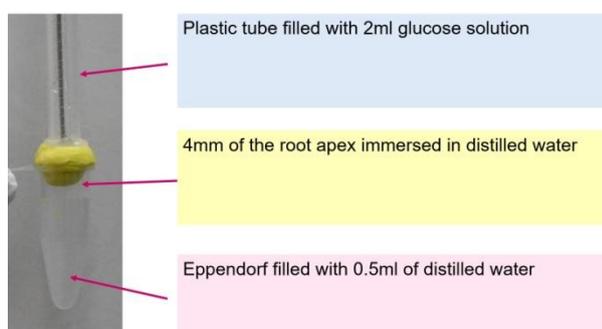


Figure 1: The set-up of experiment for glucose penetration test.

The samples were attached to the end of the plastic tube using sticky wax and glued to the opening of a 1.5ml Eppendorf (Eppendorf Asia Pacific Sdn. Bhd., Selangor, Malaysia). The Eppendorf was filled with 0.5ml of distilled water and only 4mm of the root apex was immersed into the distilled water. A 1mg/ml glucose solution (Standard glucose solution, Sigma Aldrich, USA) with a molecular weight of 180g/mol was used as

tracer in this study. 2ml of the glucose solution was injected into the plastic tube until it reached a height of 14cm to allow a hydrostatic pressure of 1.37kPa exerted on the gutta-percha and sealer. The samples were left for 24 hours in the same incubator at 37°C and 100% humidity to allow the glucose molecule to penetrate the root canals into the distilled water. The concentrations of glucose (mmol/L) in the distilled water were measured using glucose kit (Contour Plus, Ascensia Diabetes Care Holdings AG., Switzerland) and the data were recorded.

Statistical analysis

Data analysis for glucose penetration was carried out using SPSS version 24.0 for Windows (SPSS Inc., Chicago, IL, USA). One-way ANOVA complemented by post hoc Dunnett T3 Test were used for inter-group comparison with the significance level set at $p=0.05$. The differences in concentration of glucose penetration (mmol/L) for both matched pair contralateral teeth, α and β , were analyzed using Paired Samples T-test.

RESULTS

The results of glucose penetration are shown in Table 1. The samples used as controls behaved as expected in which the negative control groups showed no glucose penetration during the entire experiment, but positive controls exhibited the highest rate of glucose penetration leakage. A significant difference was noted ($p<0.05$) with the positive control in Group 1 showing the highest mean of glucose penetration, followed by EndoRez in Group 2, Sealapex in Group 3, BioRoot RCS in Group 5 and lastly EndoSeal MTA in Group 4 after 100, 1000 and 10000 thermocycles respectively. However, no significant difference was noted between Group 4 and Group 5 after 100, 1000 and 10000 thermocycles ($p=0.240$; 0.992; 0.979), respectively.

Table 1: Concentrations of glucose penetration (mmol/L) of different endodontic sealers using One-way ANOVA complemented by Dunnett T3 Test

Group	Type of Sealer	Mean (SD)	F(df)	p-value	Groups	Multiple Comparisons		
						Mean Diff.	Std. Err.	p-values
100 thermocycles								
1	Positive Control	5.40 (± 0.65)			1 vs 2	4.467	0.282	0.001*
					1 vs 3	4.917	0.269	0.001*
2	EndoRez	0.93 (± 0.24)			1 vs 4	5.335	0.265	0.001*
					1 vs 5	5.313	0.265	0.001*
3	Sealapex	0.48 (± 0.12)	313.17 (4, 25)	0.001*	2 vs 3	0.450	0.110	0.033*
					2 vs 4	0.868	0.099	0.002*
4	EndoSeal MTA	0.07 (± 0.01)			2 vs 5	0.847	0.099	0.003*
					3 vs 4	0.418	0.048	0.002*
5	BioRoot RCS	0.08 (± 0.01)			3 vs 5	0.397	0.048	0.003*
					4 vs 5	-0.022	0.005	0.240
1000 thermocycles								
1	Positive Control	8.55 (± 0.78)			1 vs 2	6.067	0.332	0.001*
					1 vs 3	7.133	0.324	0.001*
2	EndoRez	2.48 (± 0.23)			1 vs 4	7.950	0.323	0.001*
					1 vs 5	7.867	0.320	0.001*
3	Sealapex	1.42 (± 0.15)	470.15 (4, 25)	0.001*	2 vs 3	1.067	0.112	0.001*
					2 vs 4	1.883	0.111	0.001*
4	EndoSeal MTA	0.60 (± 0.14)			2 vs 5	1.800	0.099	0.001*
					3 vs 4	0.817	0.083	0.864
5	BioRoot RCS	0.61 (± 0.13)			3 vs 5	0.808	0.062	0.001*
					4 vs 5	-0.025	0.006	0.992
10000 thermocycles								
1	Positive Control	12.30 (± 0.38)			1 vs 2	6.133	0.180	0.001*
					1 vs 3	9.117	0.114	0.001*
2	EndoRez	6.17 (± 0.22)			1 vs 4	11.333	0.180	0.001*
					1 vs 5	11.233	0.171	0.001*
3	Sealapex	3.18 (± 0.18)	2221.25 (4, 25)	0.001*	2 vs 3	2.983	0.116	0.001*
					2 vs 4	5.200	0.125	0.001*
4	EndoSeal MTA	0.97 (± 0.22)			2 vs 5	5.100	0.111	0.001*
					3 vs 4	2.217	0.116	0.001*
5	BioRoot RCS	1.01 (± 0.16)			3 vs 5	2.117	0.110	0.121
					4 vs 5	-0.100	0.011	0.979

*Statistically significant

Results in Table 2 showed no significant difference ($p>0.05$) of glucose penetration when

comparing both matched-pair contralateral teeth, α and β .

Table 2: Concentration of glucose penetration (mmol/L) of different endodontic sealers in both matched-pair contralateral teeth, α and β , using the Paired Samples T-test.

Group	Type of Sealer	Mean (SD)		Mean diff.	Std. Error	p-values
		Left Tooth, α	Right Tooth, β			
100 Thermocycles						
1	Positive Control	5.57 (± 0.81)	5.23 (± 0.57)	0.333	0.187	0.214
2	EndoRez	1.00 (± 0.26)	0.87 (± 0.25)	0.133	0.067	0.184
3	Sealapex	0.47 (± 0.15)	0.50 (± 0.10)	0.033	0.031	0.423
4	EndoSeal MTA	0.07 (± 0.02)	0.06 (± 0.01)	0.040	0.031	0.321
5	BioRoot RCS	0.09 (± 0.01)	0.08 (± 0.01)	0.007	0.003	0.284
1000 Thermocycles						
1	Positive Control	8.43 (± 1.11)	8.37 (± 0.45)	0.367	0.376	0.432
2	EndoRez	2.57 (± 0.21)	2.40 (± 0.26)	0.167	0.033	0.183
3	Sealapex	1.43 (± 0.15)	1.40 (± 0.17)	0.233	0.088	0.118
4	EndoSeal MTA	0.60 (± 0.20)	0.60 (± 0.10)	0.200	0.058	0.892
5	BioRoot RCS	0.59 (± 0.15)	0.63 (± 0.06)	0.067	0.013	0.423
10000 Thermocycles						
1	Positive Control	12.33 (± 0.45)	12.27 (± 0.41)	0.067	0.145	0.691
2	EndoRez	6.17 (± 0.31)	6.37 (± 0.12)	0.333	0.133	0.130
3	Sealapex	3.10 (± 0.20)	3.17 (± 0.15)	0.301	0.058	0.350
4	EndoSeal MTA	0.91 (± 0.10)	1.01 (± 0.31)	0.267	0.033	0.508
5	BioRoot RCS	1.03 (± 0.21)	1.00 (± 0.10)	0.133	0.067	0.184

*Statistically significant

DISCUSSION

The first null hypothesis was rejected because a significant difference was found between the sealing ability of endodontic sealers. In the current study, methacrylate resin-based sealer, EndoRez demonstrated the poorest sealing ability which is in agreement with other findings.²⁰⁻²² EndoRez, a second-generation bondable sealer is able to flow into accessory canals and dentinal tubules to promote the formation of resin tag for retention, but it was reported to exhibit low bond strength to the dentinal wall which could be one of the reasons of its poor seal.^{8, 23} Another factor that attributed to its poor sealing ability is the intrinsic volumetric shrinkage and interfacial stress during polymerization that causes gap formation between the sealer material and dentine wall.²⁴ Additionally, the C-factor in a root canal is extremely high, which causes the sealer material to debond from dentine walls and causes microleakage due to improper seal.²⁵ Sealapex, a calcium hydroxide-based sealer, in the present study showed slightly better sealing ability than the resin sealer. Sealapex can form chemical bond between isobutyl salicylate found in the material itself and calcium in the tooth structure that leads to better sealing and adaptation to root canal walls.²⁶ However, in the present study, Sealapex demonstrated poorer sealing ability than the other two bioceramic sealers which is in contradiction with several studies.¹⁴⁻¹⁶ The difference in the results could probably be due to the methodological design of different studies.

Bioceramic sealers have recently gained attention in the field of endodontics since they can form an apatite layer, allowing intrafibrillar apatite deposition.^{13,27} This promotes the formation of a tag-like structure which plugs along with the dentine bonding interface, thus, creating a strong mineral infiltration zone resulting in a better seal.²⁸ Although bioceramic sealers (EndoSeal MTA and BioRoot RCS) in the present study demonstrated excellent sealing ability which is in agreement with the other authors²⁹⁻³¹, but several studies found that there is no significant difference when comparing the sealing ability of bioceramic sealers with resin and calcium

hydroxide based sealers.³¹⁻³³ A recent study also reported that BioRoot RCS demonstrated a higher percentage of voids as compared to the conventional epoxy resin sealer, AH Plus.⁷ Information regarding sealing ability of BioRoot RCS is still scarce and controversial in the literature, therefore, more studies need to be done on the sealing ability of this new bioceramic sealer to provide a better comparison.

Based on the results of the present study, all sealers showed a decrease in sealing ability as the number of thermocycles increased. Thermocycling process was used to simulate and accelerate the physiological ageing of materials in clinical setting.³⁴ Thermal tests tend to stress the bond between the materials by causing continuous expansion and contraction, thus, resulting in crack propagation and gap formation.³⁵ However, the use of thermocycling for endodontic sealers remains controversial. Nevertheless, even though the root is embedded in the bone, due to the thermophysical properties of a tooth^{36,37}, extreme temperatures experienced by the crown will be transferred to the root as well.³⁸ Besides, a few other studies also reported the use of thermal tests on materials placed in the root canals.^{39,40}

Numerous *in-vitro* studies have been carried out to evaluate the sealing ability of endodontic sealers using different techniques such as dye leakage, bacterial culture, glucose penetration, and fluid filtration methods.^{5,14-16} Glucose penetration method was used in the present study due to the small glucose molecular size which resembles bacterial toxic products, high sensitivity, and it provides a more precise quantitative measurement with fewer operator errors.^{19,41,42} Dye leakage study is no longer undertaken largely because the assessment of dye penetration using longitudinal tooth sectioning method ended up with dye dissolution problems and a lower probability of cutting through the deepest part of the dye leakage due to the random selection of cutting axis.⁴³ Although the bacterial leakage method closely approximates the real clinical situation⁵, but due to the antibacterial property of endodontic sealers^{8,13}, this method might affect the results of a leakage study. A negative control group is crucial

in sealing ability test because it can enhance the internal validity of such study by ensuring that a proper baseline of glucose penetration has been achieved. Without a negative control group, it is difficult to hypothesize that the glucose penetration value will start from 0 mmol/L which causes the results obtained to be not reliable.

Unfortunately, most laboratory leakage models are poorly designed and not well-controlled with several confounding factors that reduce the reliability of the results. One of the major factors is most leakage studies used nonpaired extracted teeth with extremely large anatomical root canal variation.¹⁸ Utilizing well-balanced groups with matching canals is still scarce in leakage studies. Hence, the present study used contralateral teeth from the same individual and matched the root canal anatomy of these teeth with strict screening procedures to reduce the bias of different root canal morphology on the results and provide better comparability. To increase the validity and quality of this research work from a previous similar study⁷, the present study took patients' age and ethnic origin factors into account since these factors might partially affect the root canal anatomy of contralateral teeth.^{18,44,45} Apart from that, results from this study revealed no significant difference in the concentration of glucose penetration when comparing each pair of contralateral teeth. This showed a high level of sensitivity and valid outcomes, thus, creating more concrete evidence to support the reliability of the present methodology. So, the second null hypothesis was accepted.

Additionally, results obtained in *in-vitro* studies might not be appropriate to be directly extrapolated to clinical situations due to the lack of simulated periodontal ligament and the absence of other clinical parameters. However, this study provided a reproducible outcome that can be used for future comparison with various endodontic sealers. Therefore, *in-vivo* studies and clinical trials need to be done to provide more reliable and valid outcomes.

CONCLUSIONS

Within the limitations of this study, it can be concluded that bioceramic sealers demonstrated

excellent sealing ability, especially after ageing as compared to resin and calcium hydroxide based sealers. The sealing ability of endodontic sealers decreased as the number of thermocycles increased. Glucose penetration test using matched-pair contralateral teeth after strict radiographic examination provided a well-balanced experimental group.

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CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest in this study.

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RELATIONSHIP BETWEEN GENDER, TEACHING EXPERIENCE, SUBJECT TAUGHT, AND TEACHER'S ATTITUDE AND KNOWLEDGE TOWARD DENTAL TRAUMA IN CHILDREN

ABSTRACT

Objectives: The purpose of this study was to analyze the relationship between gender, teaching experience, and subject taught by the primary school teacher and the attitude as well as knowledge of the teacher about dental trauma in the Indonesian population.

Materials and Methods: Ninety teachers from 14 public elementary schools in Central Jakarta were randomly chosen to fill in a questionnaire. The data were analyzed using the Kendall rank correlation.

Results: A significant relationship (weak negative linear correlation) between teaching experience and teacher's attitude ($p < 0.05$) was observed. No significant correlation between attitude and knowledge and the other variables were noted.

Conclusions: A significant relationship between teaching experience and the teacher's attitude toward dental trauma in children was observed. In general, the attitude toward dental trauma in children was positive whereas, knowledge about dental trauma was insufficient among the teachers.

Keywords: Attitude, knowledge, teacher, dental, trauma.

 Jessica Anggono¹
 Sarworini B. Budiardjo²
 *Eva Fauziah³

ORCID IDs of the authors:

J.A. 0000-0001-5034-8636

S.B.B.0000-0002-2506-6191

E.F. 0000-0002-8785-5874

¹ Pediatric Dentistry Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia.

² Professor Dr., Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia.

³ Lecturer Dr., Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia.

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INTRODUCTION

Trauma to the permanent teeth occurs most commonly found in children at school.¹⁻³ Children usually spend a minimum 6 h per day at school; one out of fourteen students experience a dental injury in school every year.^{3,4} The clinical manifestations may be vary from simple enamel fracture to a complex dental avulsion.¹ Treatment strategies such as prompt treatment and proper first aid management are vital to increasing the prognosis of the traumatized tooth.³ Delayed replatantion and improper management can dramatically decrease the prognosis of an avulsed tooth leading to tooth loss.¹ The teacher is the guardian of the child at school and is responsible for the dental trauma cases that occur in the school environment.^{2,3,5} However, there is a lack of knowledge about first aid dental trauma management among primary school teachers.^{2,6-8}

Attitude is the tendency of a reaction or response to an object, situation, and people. Knowledge is a result of knowing and it is gained after the individual senses a particular object. Knowledge is an important domain because it is the basis of the behavioral performance. Several internal and external factors, such as the physiological aspect, occupation, and personal experience determine the attitude and knowledge of an individual. Physiological attributes (such as gender); the working environment (such as the subject taught by the teacher), and the teaching experience can, directly and indirectly, contribute to the teacher's attitude and knowledge directly and indirectly.⁹ Significant correlations between teachers' knowledge towards dental trauma and gender, teaching experience, and the subject taught have been reported previously.^{4,10}

Questionnaire-based studies evaluating the attitude and knowledge toward dental trauma have been reported in many countries.^{6,11-14} The results of the questionnaires can be used as education guidelines based on the population studied.¹⁵ Currently, teachers' attitude and knowledge toward dental trauma in children has been evaluated in the Middle East, India, Iran, Brazil, and other countries.^{4,16-18} However, there is no specific questionnaire to assess the teacher's

attitude and knowledge about dental trauma in children in Indonesia.

The purpose of this study was to analyze the relationship between gender, teaching experience, subject taught by the primary school teacher and the teacher's attitude and knowledge toward dental trauma in the Indonesian population.

MATERIALS AND METHODS

Ethical approval

This cross-sectional study was approved by the Research Ethics Committee at the Faculty of Dentistry, University of Indonesia (21/Ethical Approval/FKGUI/III/2019), Indonesia.

Subjects

The inclusion criteria were public primary school teachers in Central Jakarta who had not filled any questionnaire regarding dental trauma in children previously and were prepared to participate in this study. The exclusion criteria were homeschooling teachers, special education teachers, and teachers who move or transfer schools in one year.

Questionnaire

A self-administered questionnaire consisting of various sections on demographic data, attitude, and knowledge regarding first aid for dental trauma in children was used in this study. The questionnaire was modified from previous studies based on discussions in the expert panel and translated to the Indonesian language.^{1,2,19} The section on demographic data consisted of questions on the type of school, the gender, age, education, teaching experience, position, first aid training experience, and dental trauma self-first aid training experience of the teacher, other sources of information about dental trauma, and whether the teacher had witnessed or experienced dental trauma with information about where it had occurred. There were 10 Likert scale questions regarding dental trauma in children in the attitude section with the score ranging from 1 for the wrong answer to 4 for the right answer. The total range of the score was 10 to 40. The scores for attitude were classified as follows: poor (10-19), fair (20-30), and good (31-40). The knowledge section consisted of nine questions on dental trauma; a score of 0 was given for an incorrect answer and 2 for a correct answer. In cases where

there was more than 1 correct answer, a score of 0 was given for an incorrect answer, 1 for a correct answer, and 2 if they got more than one answer correct. The total range of the scores was 0–18. The scores for the knowledge section were classified as follows: poor (0–6), fair (7–13), and good (14–18).

Sample Size Calculation

The minimum sample needed from the correlation formula with $z\alpha=5\%$ (type I error of 5%), $z\beta=20\%$ (type II error of power 80%), $r=0.3$ (value of minimum correlation) was 85. Based on consecutive random sampling, a total of 90 primary school teachers participated in this study from April to May 2019.

Statistical analysis

SPSS (Statistical Package for Social Studies) version 23.0 (IBM Corporation, Chicago, IL, USA) was used for data entry, descriptive statistics, and data analysis.

The filled questionnaires were statistically analyzed using Cronbach's alpha to measure the internal consistency.

Kendall's correlation test was used to analyze the correlation between gender, teaching experience, and school subject to teacher's attitude and knowledge toward dental trauma in children. A p-value <0.05 was considered significant.

RESULTS

Ninety primary school teachers met the inclusive criteria and participated in this study. The questionnaire was found to be reliable with a Cronbach's alpha value of 0.766 in the attitude section and 0.715 in the knowledge section.

Among the teachers, 37 (41.1%) were males and 53 (58.9%) were females; 20 teachers (22.2%) had below 5 years of experience and 70 had over 5 years of experience (77.8%). Furthermore, there were 77 non-sport teachers (85.6%) and 13 sports teachers (14.4%); 36 teachers (40%) were below 35 years of age, 24 (26.7%) were between 36–45 years old, and 30 (33.3%) were above 45 years of age. Nine teachers (10%) had diploma degrees, 79 (87.8%)

had bachelor degrees, and 2 (2.2%) had master degrees. Among the 90 teachers, 26 (28.9%) had attended a first aid course, and only 3 (3.3%) had attended a first aid course for dental trauma.

One teacher (1.1%) demonstrated a poor attitude, 37 (41.1%) showed a moderate attitude, and 52 teachers (57.8%) presented with a positive attitude toward dental trauma in children. The median score in the attitude section was 31.5 with a minimum score of 19 and a maximum score of 40. With regard to knowledge about dental trauma, 55 teachers (61.1%) demonstrated poor knowledge, 34 (37.8%) had fair knowledge, and 1 teacher (1.1%) showed good knowledge. The median score was 6 out of 18, which was classified as poor. The minimum score in the knowledge section was 1 and the maximum score was 17.

Approximately 59 teachers (65.6%) remarked that teachers are responsible for the provision of emergency care to children who experience dental trauma at school and 81 (90%) agreed that dental avulsion needs prompt treatment. Regarding the effect of time on long term prognosis, 70 teachers (77.8%) believed time has an important role and 71 teachers (78.9%) agreed that dental trauma is an emergency situation. About 85 teachers (94.4%) remarked that the teacher must inform the parents about the incidence of dental trauma in the school. Furthermore, 72 teachers (80%) encouraged the use of a mouthguard in all contact sports and 74 teachers (82.2%) agreed that dental trauma emergency management must be an educational priority for teachers. Seventy-five teachers (83.3%) believed that they could provide better assistance in traumatic dental scenarios if they were provided with some short pertinent educational experiences. Fifty-four teachers (60%) agreed that emergency management of dental trauma is thoroughly professional and requires special education and training, whereas 59 teachers (65.5%) believed that teacher intervention in dental injuries that occur in the school may play a key role in the survival of the tooth.

Among the 90 teachers, only 33 (36.7%) knew that the damaged tooth was permanent.

Most teachers (86.7%) would investigate as to whether the child had received a tetanus vaccine. Only 5 teachers (5.6%) knew how to store the tooth immediately after the trauma, whereas 35 teachers (38.9%) were aware of the best time to place the avulsed tooth back in the mouth.

The result of the Kendall correlation test between gender and attitude is shown in Table 1, and that between gender and knowledge in Table 2. No significant correlation between gender and both attitude and knowledge toward dental trauma ($p < 0.05$) was observed.

Table 1. Correlation between gender and attitude toward Trauma

Variable	Attitude			Total	p	r
	Poor	Fair	Good			
Males	0 (0%)	13 (35.1%)	24 (64.9%)	37 (100%)	0.235	-0.125
Females	1 (1.9%)	24 (45.3%)	28 (52.8%)	53 (100%)		

Kendall Corellation Test, significant = $p < 0.05$

Table 2. Correlation between gender and knowledge about dental trauma

Variable	Knowledge			Total	p	r
	Poor	Fair	Good			
Males	21 (56.8%)	15 (40.5%)	1 (2.7%)	37 (100%)	0.427	-0.084
Females	34 (64.2%)	19 (35.8%)	0 (0%)	53 (100%)		

Kendall Corellation Test, significant = $p < 0.05$

The results of Kendall's correlation tests between teaching experience and attitude and knowledge are shown in Tables 3 and 4, respectively. There was a significant correlation between teaching experience and attitude toward dental trauma ($p <$

0.05) with a weak linear negative correlation. No significant correlation between teaching experience and knowledge toward dental trauma ($p < 0.05$) was observed, although the correlation was weakly positive.

Table 3. Correlation between teaching experience and attitude towards dental trauma

Variable	Attitude			Total	p	r
	Poor	Fair	Good			
< 5 years	0 (0%)	4 (20%)	16 (80%)	20 (100%)	0.022*	-0.241
≥5 years	1 (1.4%)	33 (47.1%)	36 (51.5%)	70 (100%)		

Kendall Correlation Test, *significant = $p < 0.05$

Table 4. Correlation between teaching experience and knowledge about dental trauma

Variable	Knowledge			Total	p	r
	Poor	Fair	Good			
< 5 years	14 (70%)	6 (30%)	0 (0%)	20 (100%)	0.342	0.1
≥5 years	41 (58.6%)	28 (40%)	1 (1.4%)	70 (100%)		

Kendall correlation test, *significance = $p < 0.05$

The results of the Kendall correlation test between school subject and attitude toward trauma was $p = 0.129$ with $r = 0.160$. (Table 5)

Table 5. Correlation between subject taught and attitude toward dental trauma

Variable	Attitude			Total	p	r
	Poor	Fair	Good			
Non-sport	1 (1.3%)	34 (44.2%)	42 (54.5%)	77 (100%)	0.129	0.160
Sport	0 (0%)	3 (23.1%)	10 (76.9%)	13 (100%)		

Kendall Correlation Test, significant = $p < 0.05$

While the result between school subject and attitude toward trauma was $p = 0.503$ with $r = -0.071$. (Table 6) There was no significant correlation statistically ($p < 0.05$) between school

subject neither to attitude nor knowledge toward trauma with positive weak linear correlation to attitude and negative weak linear correlation to knowledge.

Table 6. Correlation between subject taught and knowledge of dental trauma

Variable	Knowledge			Total	p	r
	Poor	Fair	Good			
Non-sport	46 (59.7%)	30 (39%)	1 (1.3%)	77 (100%)	0.503	-0.071
Sport	9 (69.2%)	4 (30.8%)	0 (0%)	13 (100%)		

Kendall Correlation Test, significant = $p < 0.05$

DISCUSSION

This cross-sectional study was conducted by consecutive random sampling in 14 out of 195 public elementary schools in Central Jakarta. There were 90 primary school teachers participated in this study. The questionnaire was translated into the Indonesian version^{1,20,21}; the adaptation process of a questionnaire includes the translation phase, synthesis phase, review phase by the expertise, and a confirmation from the original author. In the present study, data were collected using a modified questionnaire based on previous studies and an expert panel discussion. The questionnaire copyright was confirmed by the original author through email. In some cases, a modification may be needed during the translation phase because of some words that are not compatible with other languages.²² A neutral option or a safe answer such as “Neither agree nor disagree” used in a previous study can be deleted to avoid a biased result in the research.²¹ In the present study, the Cronbach alpha was applied to obtain internal consistency in the questionnaire, which was tested on 30 teachers in the pilot study.²² The results showed a value of 0.766 in the attitude section and 0.715 in the knowledge section that indicates acceptable reliability.

The gender proportion was higher in females based on the demographic data. From all of the participants, the proportion of teachers with below 5 years teaching experienced was relatively lower than teachers with over 5 years teaching experienced. Based on the public primary school curriculum in 2013 from Ministry of Education and Culture of Indonesia, there were class teacher, religion teacher, art and culture teacher, and sport teacher. The class teacher covers mathematic, natural science, social science, Indonesian language, and civics. Therefore, the number of sports teachers was dramatically lower than non-sport teacher as in this study respectively.

Good knowledge and attitude results in good oral behavior.²³ The median attitude score of the school teachers with regard to dental trauma in children was 31.5 of 40, which was considered as positive in this study. This result was consistent to the teachers' attitude toward dental trauma in children in Singapore and Iran.^{1,15}

The median score of teachers' attitudes in the current study was 31.5 of 40, which was considered as good. The teacher is responsible for providing first aid dental trauma management to the child in the school.^{2,3,5} Approximately 94.4% of the teachers agreed to actively contact the

parents when a child encountered dental trauma.^{2,6} Most teachers (78.9%) were aware that dental trauma is an emergency situation, 90% of them were prepared to treat an avulsed tooth, and 77.8% acknowledged that time had an important role in increasing the prognosis of the avulsed tooth. Delayed replantation and improper treatment might dramatically decrease the long term prognosis, especially in the case of a dental avulsion.¹

A significant correlation between the duration of teaching experience and the teacher's attitude toward dental trauma in children at school was noted in the study. The result of positive attitude was seen more in the younger age group consistent to findings in the United Arab Emirates.¹⁰ On the other hand, no significant correlations were observed between teachers' attitude and both gender as well as the subject taught as reported in Iran.^{1,17}

The median score in the knowledge section was 6 out of 18 and was classified as poor in this study. The minimum score was 1 and the maximum score was 17. Bayram *et al.*²⁴ reported a lack of knowledge in public and private primary school teachers regarding dental trauma in school. The majority of the teachers (63.3%) in this study demonstrated a lack of knowledge as to whether the involved tooth was a permanent or a deciduous tooth. This proportion was relatively higher than that reported in Iran and the United Arab Emirates.^{1,10} Knowledge regarding the order of tooth eruption is inadequate among primary school teachers and could lead to a decrease in the prognosis of the avulsed tooth.^{10,25} Only 35 teachers (38.9%) answered correctly about the best time to reimplant an avulsed tooth. This was lower than that reported in a study conducted in Saudi Arabia, wherein 52.2% of the participants answered that the best time to replace an avulsed tooth was as soon as possible.⁴ In general, there was a lack of knowledge among primary school teachers toward dental trauma in school.

Alsadhan *et al.*⁴ reported there is no significant correlation between gender and knowledge was noted as in this study. However, Pithon *et al.*²⁶ found female teachers had better

knowledge about dental trauma in children due to the intimate and intensive contact with kids during outdoor activities and their role as a mother.

No significant correlation between teaching experience and the teacher's knowledge about dental trauma in children was observed in this study (Table 4). This not in accordance with Alsadhan *et al.* where a group of teachers with 21–30 years of experience demonstrated better knowledge than those with less than 20 years of experience. Teachers with higher amounts of teaching experience may be more exposed to cases of dental trauma in the school.⁴ However, some Al Jundi *et al.*⁷ and Pithon *et al.*²⁶ have reported no correlation between teaching experience and primary school teacher's knowledge of dental trauma in children. Neither the length of the teaching experience nor the attendance of a dental trauma training course was associated with the knowledge of the teacher in a previous study.¹⁸

A sports teacher has an important role in providing first aid treatment because a child spends 40% of their active time in school and is generally involved in sports and games. The prevalence of trauma in school is about 60% and is mainly caused by sports-related accidents.^{2,5,19,36} No significant correlation between the subject taught and the teacher's knowledge was noted (Table 6). This is in accordance with Raouf *et al.*¹, Kaul *et al.*¹⁶, and Pithon *et al.*²⁶ although a sports teacher is expected to have better knowledge than a non-sports teacher.

Only 3 (3.3%) out of 90 teachers in this study had been trained for first-aid management of dental trauma. This result clearly seen since the Ministry of Health in Indonesia only includes first aid management of toothache due to caries in the school dental care curriculum. Similar results have been reported in the United States of America, Saudi Arabia, Europe, and United Arab Emirates^{2,4,19,27}, and may be due to the lack of education about dental trauma. The conventional first aid management training was inadequate to fulfill the teacher's knowledge.¹⁰ On the other hand, the teachers' awareness about including first aid management in the teaching curriculum was

quite high (82.2%) in this study. Therefore, further training regarding the management of dental trauma in children should be provided to the teachers.

A larger sample might be needed to represent the whole population in the future. Only three variables were tested in this study. However, this is the first study to use a questionnaire as a tool to assess the teachers' attitude and knowledge toward dental trauma in Indonesia.

CONCLUSIONS

There was a significant relationship between teachers' attitude toward dental trauma in children and teaching experience; on the other hand, no significant relationships with the other variables were observed. In general, the teachers demonstrated a positive attitude toward dental trauma in children, whereas knowledge about dental was insufficient in this study population. First aid management for dental trauma in children should be included in the UKGS. Moreover, the results of this research could be used as a reference for future guidelines based on the Indonesian population.

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CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest in this research.

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LACK OF ASSOCIATION BETWEEN ENAMEL FORMATION GENE VARIANTS AND DENTAL CARIES IN ADULTS

ABSTRACT

Objectives: Studies report that gene polymorphisms associated with mineralization may change the structure of enamel and create a predisposition for developing dental caries. The aim of the study was to evaluate the *VDR* and *TFIP11* gene variants in adults with caries experience and to investigate their interactions with the environmental factors.

Materials and Methods: A total of 160 individuals at the age of 24-40 years were included in the study and they were assigned to two groups according to decayed-missing-filled teeth index (DMFT); namely the low caries experience (LCE, DMFT \leq 4) and high caries experience (HCE, DMFT $>$ 9.13). DNA was isolated from buccal swab samples to genotype the *VDR* (TaqI; rs731236) and *TFIP11* (rs5997096) gene variants. The real-time PCR was used for genotyping. The frequency of tooth brushing, carbohydrate intake, smoking, and the dental plaques were evaluated as environmental risk factors.

Results: Between the caries groups and the distribution of the genotypes and alleles of the *VDR* rs731236 and *TFIP11* rs5997096 gene variants were not statistically different. There was also no significant difference when homozygous, heterozygous, dominant, and recessive models were evaluated for the two variants. The frequency of tooth brushing was significantly higher in the LCE group. According to the regression analysis; the amount of plaque explained the high caries experience at a rate of 51.4%.

Conclusions: The study findings indicated that common variants in the *VDR* and *TFIP11* genes were not associated with high caries experiences in Turkish adults.

Key words: Dental caries, enamel, genes, vitamin D receptor.

 *Gül Yıldız Telatar¹
 Faruk Saydam²

ORCID IDs of the authors:

G.Y.T. 0000-0001-5137-9282

F.S. 0000-0003-2358-8719

¹ Department of Restorative Dentistry, Recep Tayyip Erdogan University, Faculty of Dentistry, Rize, Turkey.

² Department of Medical Biology and Genetic, Recep Tayyip Erdogan University, Faculty of Medicine Rize, Turkey.

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INTRODUCTION

Dental caries, one of the most common chronic diseases in the world, occurs as a consequence of a series of pathological events starting from the fermentation of carbohydrates by cariogenic bacteria in the dental plaque leading to formation of acid, which gradually converts the organic-inorganic molecules of the dental hard tissues to soluble forms, breaking down their chemical bonds.¹

Researchers have so far evaluated the caries risk by addressing environmental factors including diet, bacteria, oral hygiene habits, dental plaque, and saliva alone or in combination.^{2,3} Additionally; innate defense mechanisms are other important factors in the formation and progression of dental caries. Especially, soluble mediators contained in saliva include many antimicrobial molecules such as statherin, proline-rich proteins, cystatins, and histatins.⁴ Saliva has also antioxidant system which prevents dental caries by influencing oral bacteria when inflammation beginning.⁵ Previous studies have shown significantly higher levels of total antioxidant capacity in dental caries.^{6,7} Furthermore, it has been shown that good oral hygiene and toothbrushing decrease salivary oxidative stresses.⁸

The studies in the literature have underlined that the assessment of individual environmental factors alone does not explain the formation of dental caries.^{9,10} Because the caries risk is not the same for individuals exposed to the same environmental risk factors, it has been suggested that genetic factors are other players in the etiology of caries.¹¹

It is reported that there may be a relationship between the susceptibility to caries and the genes encoding the proteins involved in enamel and dentin formation.¹²⁻¹⁵ The following proteins have been investigated so far; including amelogenin, enamelin, tuftelin, tuftelin interactive protein, ameloblastin, and kallikrein involved in enamel mineralization; sialophosphoprotein involved in dentin formation, and the vitamin D receptor (VDR) involved in both enamel and dentin mineralization.^{9-11,16}

Vitamin D regulates the balance between the calcium and phosphate ions, playing a vital role in making the teeth stronger.^{17,18} The deficiency of vitamin D compromises the immune system defenses against oral pathogens in periodontitis and untreated dental caries.¹⁷ The biologically active form of vitamin D; 1,25 (OH)₂D₃, is activated only after binding to the VDR encoded by the VDR gene.¹⁸ Although numerous polymorphisms of the VDR gene have been reported on the chromosome 12q13.11 of the human genome, only the nucleotide polymorphisms ApaI, FokI, Cdx2, and TaqI have been investigated in regards to tooth decay. TaqI (A>G, rs731236) single nucleotide polymorphism (SNP) is located in the region of intron 8/exon 9 of the vitamin D receptor gene (VDR), close to the 3' terminus of the gene, and does not determine structural modification in the receptor. Nevertheless, most researchers suggest that it is related to mRNA stability. Previous studies on Chinese adults, Chinese young adults, Turkish children, and Czech children investigated the relationship between the TaqI polymorphism and dental caries, reporting contradicting findings.¹⁷⁻²⁰ In the literature, there are no studies investigating the relationship between the TaqI polymorphism and dental caries in Turkish adults.

Genetic variations influencing the development of enamel, which is the most mineralized tissue in the human body, have been investigated in genetic studies in association with dental caries. These studies have reached a consensus on a common hypothesis saying that the mineralization-related gene polymorphisms change the enamel structure, creating a predisposition to dental caries.^{14,15,21-25}

The tuftelin-interacting protein 11 (TFIP11) is localized in the 22q12.1 region, playing an important role in the formation and mineralization of the enamel by interacting with the tuftelin protein.²⁰ The TFIP11 rs5997096 (C>T) single nucleotide polymorphism is the most common intron variant in populations. The studies in the literature investigating the relationship between TFIP11 and dental caries in different age groups and populations report variable findings.^{12,13,24,26,27}

In this study, the null hypothesis is that the variants in the mineralization-related VDR and TFIP11 genes in adults elevate the risk of caries in combination with the effects of the environmental factors including gender, tooth brushing frequency, carbohydrate intake, and smoking. Therefore, the aim of the study is to evaluate the interactions between the caries-experience-related environmental factors and the variants of the VDR and TFIP11 genes involved in enamel formation in adults.

MATERIALS AND METHODS

Study population and oral examination

The study was registered at clinicaltrials.gov with registration No. NCT04124718. A total of 160 adults (86 women, 74 men) at the age range from 24 to 40 years and living in the Northeast of Turkey were included in the study. Individuals with neurological, mental, systemic, and genetic diseases and individuals with regular medicine intake were excluded from the study. The study was approved by the the Clinical Research Ethics Committee of Recep Tayyip Erdogan University School of Medicine (ID: 2019/204).

In the clinical evaluation, the patients were examined for the presence of caries and dental plaque by an investigator (GYT) using dental unit light, mouth mirror, and probe. According to WHO criteria; clinically visible cavitated lesions, softened enamel surfaces caught by the dental probe, radiolucent areas invading the dentine starting from the enamel-dentin border were documented as 'caries lesions'. Whitish-brown discolorations not caught by the exploring probe were not considered a caries lesion.²⁸

To determine the risk groups based on past caries experiences; DMFT indices were calculated based on the WHO criteria after summing up the teeth count with following features, including decay (D), extracted teeth due to caries (M), and filled (F) teeth in the mouth of each study participant. According to these calculations; 80 individuals with DMFT indices of >13.9 and previous HCE were included in the experimental group and 80 individuals with DMFT indices of ≤4 and previous LCE were included in the control group (Table 1).

Table 1. Distributions of gender and age among adult in the HCE and LCE groups.

Characteristic	Total N = 160	High caries experience HCE N=80	Low caries experience LCE N=80	p value
Gender n (%) ^a				
Female	86 (53.8)	47 (54.7)	39 (45.3)	0.267
Male	74 (46.2)	33 (44.6)	41 (55.4)	
Age in years, median (IQR) ^b	33 (17.0)	35 (14.0)	29.5 (15.0)	0.005

^aχ² test

^bMann-Whitney test.

HCE, Adult with dental caries experience, DMFT>13.9.

P < 0.05, statistically significant difference between HCE and LCE.

P > 0.05, no statistically significant difference between HCE and LCE.

LCE, Adult with dental caries experience, DMFT≤4.

A standard questionnaire form, comprising items about the frequency of tooth brushing, carbohydrate intake, and smoking was administered to all study participants to evaluate the environmental risk factors for caries. The Silness & Loe plaque index was used for determining the amount of dental plaque.²⁹

Collection of Samples and DNA Isolation

Samples were obtained from the buccal mucosa using swabs which are the material of the Swab Collection and DNA Preservation System

(Norgen Biotek Corp., Ontario, Canada). The components of this system allow DNA samples to be stored at room temperature for over 2 years. The samples were stored at room temperature in the preservative solution until the DNA isolation step. Genomic DNA was isolated with Genomic DNA Isolation Kit (Norgen Biotek Corp. Ontario, Canada). The purity and concentration of the isolated DNA samples were analyzed using a fluorescent dye on a fluorometer (Denovix QFX Fluorometer, Denovix Inc., DE, USA). The

purified DNA samples were then stored at -20 until further use.

Genotyping

Genotyping of VDR rs731236 and TFIP11 rs5997096 gene variants were performed using the TaqMan® SNP Genotyping Assays (C__2404008_10 for VDR rs731236, C__29903745_10 for TFIP11). The reactions were carried out in LightCycler® 480 Instrument II (Roche Diagnostics, Basel, Switzerland), a real-time PCR system. A standard PCR reaction mixture was prepared, and the same mixture was used for both candidate genes. The cycling conditions were as follows: an initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min.

Statistical analyses

The statistical analysis was processed with SPSS Statistics 23.0 (IBM, Chicago, IL, USA). For data that were not normally distributed, as shown by a Kolmogorov-Smirnov test, Mann-Whitney U test was used to compare age differences. The determination of the deviations from the Hardy-Weinberg Equilibrium and the differences between genetic models and the calculation of odds ratios in genetic models were carried out in the FINNETI program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl.21>). Binary logistic regression analysis was used to evaluate the effects of genetic and environmental factors on caries experience. Power analysis was performed with respect to the cross-sectional study design. In all tests, the level of significance was set at $P < 0.05$.

RESULTS

As the age and DMFT scores were not normally distributed, median with interquartile range (IQR) was used to determine between group differences (Table 1). The median age was 33.0 (IQR = 17.0, minimum = 25.0, maximum = 44.0). Significant differences in age were observed between the low and high caries experience groups (Mann-Whitney U test, $P = 0.005$). Median DMFT scores

of 15.0 (IQR = 5.0) and 2.0 (IQR = 3.0) were observed in the high and low caries experience groups, respectively.

The graph indicating the genotype results of the VDR TaqI; rs731236 gene variant obtained from the LightCycler® 480 Instrument II are representatively shown in Figure 1(A-B).

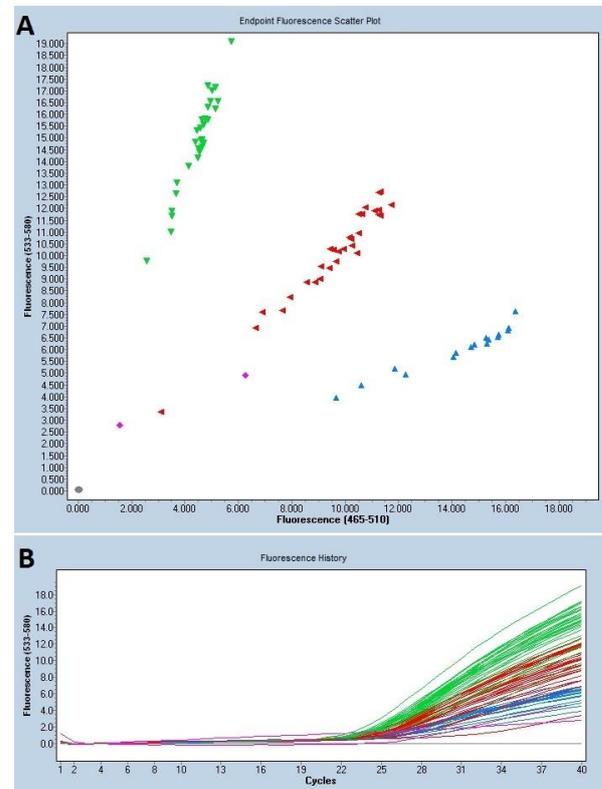


Figure 1. Typical results for the VDR rs731236 gene variant, using the LightCycler® 480 II Instrument. (A) A fragment of the human VDR gene was amplified using the 2X Taqman Universal PCR Master mix and subjected to endpoint analysis. Scatter plots consistently revealed the wild-type (AA), mutant (GG) and heterozygote (AG) variants. The green small triangles show the AA genotypes, the blue small triangles show the GG genotypes and the red small triangles show the AG genotypes. The pink small squares show samples without distinctive fluorescent light that need to be analyzed again, while the gray round shows the negative control. (B) Amplification curves of the VDR gene fragments amplified from each sample were shown.

The instrument also graphed for the TFIP11 rs5997096, giving us the results of genotyping. The Pearson Chi-Square test was used between the genotype frequencies of the VDR rs731236 and TFIP11 rs5997096 gene variants of the groups to test for deviations from the Hardy-Weinberg Equilibrium (HWE). The frequencies of both polymorphisms did not deviate from this equilibrium ($p > 0.05$, Table 2 and Table 3).

Table 2. Frequencies and odds values of alleles and genotypes for the VDR rs731236 in HCE and LCE groups.

Group	Allele		P	OR (95 % CI)	Genotype			HWE	P
	A n (%)	G n (%)			AA n (%)	AG n (%)	GG n (%)		
LCE (n=80)	97 (60.6)	63 (39.4)	0.30	1.2 (0.8-1.96)	30 (37.5)	37 (46.25)	13 (16.25)	0.77	0.32
HCE (n=80)	88 (55)	72 (45)			27 (33.75)	34 (42.5)	19 (23.75)		
Test for association OR (95% CI) (Risk allele G)									
Heterozygous AA vs. AG		Homozygous AA vs. GG		Dominant AA vs. AG+GG		Recessive AA+AG vs. GG			
1.02 (0.5-2.05)		1.6 (0.6-3.9)		1.17 (0.6-2.25)		0.6 (0.2-1.36)			
P=0.95		P=0.27		P=0.62		P=0.23			

OR: Odds Ratio

CI: Confidence Interval

HWE: Hardy-Weinberg Equilibrium

Table 3. Frequencies and odds value of alleles and genotypes for the TFIP11 rs5997096 in HCE and LCE groups

Group	Allele		P	OR (95 % CI)	Genotype			HWE	P
	C n (%)	T n (%)			CC n (%)	CT n (%)	TT n (%)		
LCE (n=80)	79 (49.3)	81 (50.7)	0.91	1.02 (0.6-1.58)	18 (22.5)	43 (53.75)	19 (23.75)	0.50	0.91
HCE (n=80)	78 (48.75)	82 (51.25)			20 (25)	38 (47.5)	22 (27.5)		
Test for association OR (95% CI) (Risk allele T)									
Heterozygous CC vs. CT		Homozygous CC vs. TT		Dominant CC vs. CT+TT		Recessive CC+CT vs. TT			
0.79 (0.36-1.72)		1.04 (0.4-2.5)		0.87 (0.42-1.8)		0.82 (0.4-1.67)			
P=0.56		P=0.92		P=0.71		P=0.58			

OR: Odds Ratio

CI: Confidence Interval

HWE: Hardy-Weinberg Equilibrium

The comparisons of the genotype and allele frequencies of the VDR rs731236 gene variant between the HCE and LCE groups were shown in Table 2. Analysis of different genetic models including dominant (AA vs. AG+GG), recessive (AA+AG vs. GG), and co-dominant (AA vs. AG, AA vs. GG) was done using Chi-square test. The genotype frequencies of the AG and GG genotypes versus the ancestral genotype (AA) were not statistically significantly different between the HCE and LCE groups (P=0.95 and P=0.27, respectively). Similarly, the allele frequencies were not statistically significantly different between these two groups (P=0.30). As shown in Table 2, no evidence of significant association was found in any genetic model.

Table 3 shows the comparisons of the genotype and allele frequencies of the TFIP11 rs5997096 gene variant between the high and low caries experience groups.

TT homozygous polymorphic genotype of the TFIP11 gene was found at frequencies of 23.75% and 27.5% in the LCE and HCE groups, respectively. There were no statistically significant differences in the TFIP11 genotype and allele distributions between the LCE and HCE groups (P=0.91, P=0.91). Analysis of different genetic models including dominant (CC vs. CT + TT), recessive (CC + CT vs. TT), and co-dominant (CC vs. CT, CC vs. TT) was done using Chi-square test. As shown in Table 3, no

statistical difference was found in any of the genetic models.

Based on the scores received by the study participants, the statistical comparisons of the frequency distributions of the environmental caries risk factors in the LCE and HCE groups are

shown in Table 4. The amount of dental plaque was significantly larger in the HCE group compared to the LCE group (2 test; p=0.000). The tooth brushing frequency was significantly higher in the LCE group compared to the HCE group (2 test; p=0.042).

Table 4: Comparison of environmental factors between Low caries experience (LCE) and High caries experience (HCE) groups

	LCE	HCE	Total	p value
Dental plaque				
PI<1.0	54 (68)	6 (7)	60 (38)	
PI 1.1-2.0	25 (31)	59 (74)	84 (52)	0.000
PI>2	1 (1)	15 (19)	16 (10)	
Toothbrushing frequency				
>twice a day	12 (15)	8 (10)	20 (12)	
Twice a day	35 (43)	21 (26)	56 (35)	0.042
Once a day	22 (28)	35 (44)	57 (36)	
<once a day	11 (14)	16 (20)	27 (17)	
Carbohydrate intake				
Less than 1/day	15 (19)	11 (13)	26 (16)	
1 or 2/day	46 (58)	37 (45)	83 (52)	
3 or 4/day	13 (16)	19 (22)	32 (20)	0.151
5 or more/day	6 (7)	13 (20)	19 (12)	
Smoking				
no	59 (74)	64 (80)	123 (77)	
yes	21 (26)	16 (20)	37 (23)	0.454

Values are presented as n (%) of subjects. P values based on χ^2 test, P < 0.05.

The binary logistic regression analysis was used for evaluating the effects of genetic and environmental factors on caries experience (Table 5). The participants with larger plaque

accumulation were significantly at higher risk of experiencing dental caries as compared to their counterparts.

Table 5. Binary logistic regression analyse showing odds ratio (OR) and 95% confidence interval for caries experience

Independent variables	OR (%95 CI)	p
Dental plaque	21.345 (7.722- 58.996)	0.000
Carbohydrate intake	1.198 (0.734 – 1.956)	0.470
Toothbrushing frequency	0.915 (0.554 – 1.511)	0.728
Age	1.004 (0.945 – 1.067)	0.902
Gender	0.428 (0.175 – 1.042)	0.062
Smoking	0.889 (0.324 – 2.440)	0.820
VDR	1.159 (0.654 – 2.054)	0.614
TFIP11	0.780 (0.431- 1.410)	0.411

Coding: Caries experience (low = 0, high = 1), age (25-35 = 1, 35-44 = 2); gender (male=1, female = 2); VDR (AA=1, AG=2, GG=3); TFIP11 (CC=1, CT=2, TT=3)

R²=0.514

DISCUSSION

It has been shown that environmental factors including oral hygiene, diet, and bacteria, as well as the individual genetic differences, are associated with the caries risk.^{11,22,30} Therefore, the influences of enamel formation-related VDR and TFIP11 gene polymorphisms and the gene-environment interactions on the caries experience were investigated in this study.

In many epidemiological studies, past caries experiences have been reported as important risk indicators for developing new caries in the future.^{31,32} Several studies^{18,19,22,26,33-35} evaluating the caries risk have described the caries risk groups based on past caries experiences. According to the World Health Organization data, the DMFT index should be less than 5 to be considered the low caries risk; whereas the DMFT index should be higher than 13.9 to be considered the high caries risk.³⁶ Therefore, the risk groups of the individuals included in the study were determined based on their past caries experiences.

It has been reported that VDR gene polymorphisms are important factors for the normal enamel formation²⁰ and that the variations in this gene lead to inherited phenotypes of enamel malformations.³⁷ Hypothesis of the present study says that the TaqI polymorphism of the VDR gene affects enamel formation, leading to a higher caries experience in adults. The study findings did not indicate differences in the allele and genotype frequency distributions of the TaqI polymorphism of the VDR gene between the individuals with low and high caries experiences. This result was similar to those previously reported by the Kong *et al.*¹⁷ study on 0-4-year-old Chinese children, Yu *et al.*²⁰ study on 12-year-old Chinese children, and Holla *et al.*¹⁸ study on 13-15-year-old Czech children. On the contrary, two other studies on Chinese adults¹⁶ and Turkish children¹⁹ have demonstrated that the polymorphic allele in the TaqI variation may constitute a risk for developing dental caries. These differences across the studies may be explained with the use of the PCR-RFLP method to determine the VDR gene polymorphisms in the latter two studies in contrast to the use of the TaqMan technique in the

present study, allowing to obtain more precise results. Secondly, another explanation could be that Turkish adults were evaluated in this study.

Current study has found out no differences in the polymorphic TT genotype and T allele frequencies in the TFIP11 rs5997096 variation between the low and high caries experience groups. Several studies are available in the literature, reporting the same results as ours. Of them; Abbasoglu *et al.*³⁸ study examined 23 gene markers in Turkish children, including the TFIP11 rs5997096 polymorphism and found out no relationships between the TFIP11 gene and dental caries. In another study on Turkish children, Patir *et al.*¹² did not find out any relationship between TFIP11 rs134136 and tooth decay. Other studies that did not find any associations between this polymorphism and dental caries were performed on children from Poland³⁹, on young adults from Guatemala²⁴, and on children from Western Norway.⁴⁰ In another study²¹ on 1831 children and young adults from Philippines, Turkey, Argentina, and Brazil; rs134136 and rs5997096 polymorphisms of the TFIP11 gene was not associated with past caries experiences, however, it was found out that the TFIP11 rs134136 polymorphism affected the enamel microhardness of 48 extracted teeth with artificially induced caries lesions. In another *in vitro* study²⁵, the variations of the TFIP11 (rs2097470, rs134143) gene have been shown to affect enamel demineralization in a *Streptococcus mutans* biofilm model. The *in vitro* design of those studies may have led to differences in the results compared to those of the present study.

There are also studies in the literature showing that a single nucleotide polymorphism in TFIP11 is associated with dental caries.^{26,27} The differences in the results of that latter study compared to the former studies have been explained by arguing that; firstly, TFIP11 is responsible for early demineralization and fluoride-mediated remineralization of the enamel and secondly, the environmental heterogeneity might be involved, such as fluoride, which affects the risk of caries.

Epigenetic change of the genetic code are caused by environmental stimuli and hence are responsible for our ability to adapt to different environments.⁴¹ No single host gene that directly regulates dental caries progression has been identified. Epigenetics however, may provide the missing link to these unanswered questions.⁴²

In the literature, there are studies available, using regression analyses and risk models investigating the effect of gene polymorphisms on caries development in combination with the effects of environmental factors.^{12,13,22,35} Among these studies, Slayton *et al.*¹³ have reported a 26.8% accuracy of the caries risk model; which included the polymorphism of the TUFT1 gene and the *S. mutans* count. Another study¹² found out that the best explanation for the past caries experience was provided at an accuracy rate of 40.2% by the model comprising the polymorphisms of the TUFT1 (rs3790506) and AMELX (rs17878486) genes in female children with caries lesions on both anterior and posterior teeth. Similarly; Tennure *et al.*³⁵ reported that in black children with MMP20 (rs1784418) polymorphism and sugar consumption between the meals elevated the caries risk by 74.61%. In this study, the effects of gene polymorphisms and environmental factors on the high caries experience were examined using a risk model and regression analysis. The study findings showed that the presence of dental plaques provided an explanation at a rate of 51.4%. This result is similar to that of a previous study reporting that the dental plaque ranks the first with a rate of 77.6% in the model, which explained the gene-environment interaction on developing risk for caries with an accuracy of 87.8%.²²

Recently, the development of tissue engineering raises regenerative methods in dentistry. Its major component is the mesenchymal stem cells that are seeded on the surface of scaffolds, in order to create a biocomplex.⁴³ Animal studies have reported that mesenchymal stem cells provide alveolar bone regeneration, dentine formation and repair damaged tooth tissues.^{44,45} Gene therapy presents an attractive concept of restoring the oral tissues

lost due to caries by managing the differentiation of stem cells.⁴⁶ Recently, osteogenic genes are presented to promote the bone formation and cellular differentiation using tissue engineering approaches.⁴⁷ However, it has been reported that more genetic research is needed to better understand odontogenesis.⁴³

The limitation of the present study can be the selection and investigation of the genes among the previously reported caries-associated polymorphisms. Instead of selecting these polymorphisms, conducting studies on different populations, as well as selecting new genes that have not been investigated previously and may potentially be associated with enamel formation, can yield different results.

CONCLUSIONS

In conclusion; findings from this study indicated that the VDR TaqI; rs731236 and TFIP11 rs5997096 gene polymorphisms were not associated with high caries experiences in Turkish adults. The presence of dental plaques provided an explanation for the past caries experience at an accuracy rate of 51.4%. Studies with larger sample size in different populations will contribute to better understanding the role of these variants. There is also a need for further studies investigating the effects of variants in different genes to better explain the genetic basis of the dental caries etiology. If future studies can recognize risk or protective genetic factors, researchers will potentially be able to design more effective treatments aimed at preventing dental caries.

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CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

Erişkinlerde Mine Formasyon Gen Varyantları ve Diş Çürüğü Arasındaki İlişki Yokluğu

ÖZ

Amaç: Çalışmalarda mineralizasyonla ilişkili gen polimorfizmlerinin minenin yapısını değiştirerek, diş çürüğüne yatkınlık oluşturabileceği bildirilmektedir.

Bu çalışmada erişkinlerde mine oluşumunda yer alan VDR ve TFIP11 gen polimorfizmlerini ve çürük deneyimi üzerindeki çevresel faktörlerle etkileşimini değerlendirmeyi amaçladık. **Gereç ve Yöntemler:** Çalışmaya 24-40 yaş aralığındaki toplam 160 birey katıldı ve DMFT indeksine göre geçmiş çürük deneyimi düşük ($DMFT \leq 4$) ve yüksek ($DMFT > 13,9$) olan iki gruba ayrıldı. VDR (TaqI; rs731236) ve TFIP11 (rs5997096) polimorfizmlerini değerlendirmek için yanak içi sürüntü örneklerinden DNA izolasyonu yapıldı. Genotipleme işlemi için real-time PCR ile gerçekleştirildi. Çevresel çürük risk faktörleri olarak diş fırçalama sıklığı, karbonhidrat tüketimi, sigara kullanımı ve dental plak miktarı değerlendirildi. **Bulgular:** Yüksek çürük deneyimi ve düşük çürük deneyimi grupları arasında VDR ve TFIP11 polimorfizmlerinin genotip ve alel frekanslarının dağılımları açısından istatistiksel açıdan fark gözlenmedi. İki varyant için homozigot, heterozigot, baskın ve resesif modeller değerlendirildiğinde anlamlı farklılık saptanmadı. Diş fırçalama sıklığı LCE grubundaki bireylerde HCE grubundaki bireylere göre anlamlı derecede daha fazlaydı. Binary lojistik regresyon analizine göre; plak miktarı yüksek çürük deneyimini %51,4 oranında açıkladı. **Sonuçlar:** Bulgularımız VDR ve TFIP11 genlerindeki yaygın görülen varyantların Türk erişkinlerindeki yüksek çürük deneyimleriyle ilişkili olmadığını göstermiştir. **Anahtar Kelimeler:** Diş çürüğü, mine, genler, vitamin D reseptörü.

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DEVELOPING COMPETENCIES FOR THE DENTAL CARE OF PEOPLE WITH SENSORY DISABILITIES: A PILOT INCLUSIVE APPROACH

ABSTRACT

Objectives: Different training programs for healthcare students that have a biopsychosocial approach have been reported to have a significant beneficial impact on their education, attitudes and competencies towards Persons with Disabilities. In Chile, however, there are no explicit legal obligations to provide healthcare professionals with the skills required to offer their services in public or private healthcare facilities to Persons with Disabilities. Given this situation, a pilot one-semester elective course for dental students was carried out focusing on people with visual disabilities and people with hearing disabilities, incorporating Deaf and blind teachers. The aim of this paper is to describe the perceptions and results of this pilot course aimed at identifying and responding to the healthcare needs of people with visual disabilities and people with hearing disabilities, incorporating Deaf and blind teachers. Visual or hearing disability.

Materials and methods: A multi-strategy 17-week elective course was carried out in the first semester of Dentistry School with 14 students enrolled. Educational strategies used were lectures, guided discussion (GD), role-play (RP), standardized patients (SP) and case method teaching (CM), with the participation of Deaf and blind teachers. Once the program had finished, the students answered a survey designed with open-ended questions, and GD, RP, SP, CM, attendance and grades obtained were recorded and analyzed.

Results: Attendance was 82–100%. All students passed the course with the highest score, and thus were able to define the medical approach needed in cases of hearing and visual disability, recognize the cultural and linguistic aspects of people with visual disability and people with hearing disability and learn their means of communication.

Conclusions: This course was successful in helping the students to identify and respond to the healthcare needs of people with visual or hearing disability.

Key Words: Sign language, braille, students, dental.

*Valeria A. Campos^{1,2}
 Ricardo Cartes-Velásquez^{3,4}

ORCID IDs of the authors:

V.A.C. 0000-0003-0697-1345

R.C.V. 0000-0001-5831-7324

¹ Departamento de Prevención y Salud Pública Odontológica, Facultad de Odontología, Universidad de Concepción, Concepción, Chile.

² Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Temuco, Chile.

³ Facultad de Odontología, Universidad Andrés Bello, Concepción, Chile.

⁴ Fundación Kimntrum, Concepción, Chile.

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INTRODUCTION

The current biomedical approach does not encourage the inclusion of People with Disabilities, as it places the *disability* within the person, rather than seeing it as the result of the interactions between the person and the environment.^{1,2} According to the World Health Organization, Persons with Disabilities are two times more likely to find that health-care providers' skills do not meet their needs and three times more likely to report being denied healthcare.³ One of the main reasons for this situation is the lack of training in healthcare professionals regarding subjects such as how to address Persons with Disabilities and the use of a more bio-psychosocial approach, which would satisfy the health-care needs of everyone, including Persons with Disabilities.⁴ In dental health, patients with sensory disabilities have a high prevalence of dental caries and poor oral hygiene.⁵

Visual and hearing disabilities are two of the most prevalent disabilities around the globe,⁶ and according to Bachmann, patients with these disabilities are the most complex population for healthcare providers due to communication barriers.⁴ People with an early onset of moderate to profound hearing loss usually call themselves "Deaf" and define themselves as part of a community with its own culture, values and language, where sign language (SL) is their most important cultural element.^{7,8} People with a visual disability use different devices for reading and writing; people with congenital blindness usually use Braille, screen readers and voice recognition.⁹ People with low vision use optical or electronic magnifiers, prisms, screen readers and voice recognition. It is important to highlight that SL and Braille are recognized worldwide.⁹⁻¹²

Different training programs using a bio-psychosocial approach have been reported to have a significant beneficial impact on healthcare students around the world in terms of their education, attitudes and competencies towards Persons with Disabilities.¹³⁻²⁰ Regarding teaching strategies, it has been established that lectures, guided discussion, role-play, standardized patients

and case method teaching are effective in promoting critical thinking in healthcare students.^{15,19,21-23}

In Chile, even though there are no explicit legal obligations to provide healthcare professionals with the skills required to offer their services in public or private healthcare facilities to Persons with Disabilities,²⁴ to our knowledge, every year more universities incorporate courses to develop such skills among their students. However, most of these courses tend to cover all *disabilities* in one semester or year and do not usually include the active participation of Persons with Disabilities.²²

Given this situation, a pilot one-semester elective course for dental students was carried out focusing on people with visual disabilities and people with hearing disabilities, incorporating Deaf and blind teachers.

The aim of this paper was to describe the perceptions and results of this pilot course aimed at identifying and responding to the healthcare needs of people with visual or hearing disability.

MATERIALS AND METHODS

Design and population

Approval was obtained from the Commission of Research and Bioethics of the School of Dentistry of the Universidad de Concepción, Chile, and the participants gave their informed consent before the study began.

This was a case study of an elective course aimed at identifying and responding to the healthcare needs of people with visual or hearing disability. The participants were undergraduate dental students in their 2nd to 5th year. The elective course, for 14 students, was one of the elective courses offered by the School of Dentistry, via which they earned course credits. There were no other selection criteria.

Elective course description

The expected learning outcomes of the pilot course were:

1. To define the medical approach towards hearing and visual disability.

2. To recognize the cultural and linguistic aspects of people with visual disability and people with hearing disability.
3. To learn the means of communication of people with visual disability and people with hearing disability.

A multi-strategy 17-week elective course was carried out in the first semester of the Dentistry School, with 14 students enrolled. All agreed to take part in the study. The course involved a two-hour lecture plus a two-hour home assignment per week. For evaluations, the Chilean grading system was used, which ranges from 1.0–7.0, where 4.0 is needed to “pass”.

The course was divided into three stages. The first class was introductory, during which the course program, expected learning outcomes, contents, methodology, length of the course and evaluation system were discussed with the students. All PowerPoint material, the syllabus, instructions, rubrics and additional material such as the Convention on the Rights of Persons with Disabilities, and official national laws regarding people with disabilities, among others, were available in a virtual library. Additionally, a WhatsApp group was created between the students and the teacher (VC) in order to facilitate fluent communication.

First stage: Classes 2–5

1. Role-playing: This activity was based on the Deaf Strong Hospital program which mimics actual healthcare scenarios in a Public Family Health Center (*Centro de Salud Familiar, CESFAM*).¹⁹ After each role-playing activity, debriefing sessions were carried out, in which the students reported their personal experience.

a. First, students experienced deaf people’s barriers in healthcare. Each student received individual written and oral instructions for the activity. Different health scenarios were assigned randomly, and medical exam documents and a Fingerspelling alphabet were handed to each student. Each student had to enter the conditioned classroom and wait in the waiting room where he/she had to communicate with a Deaf SL user receptionist, physician and pharmacist. In each

scenario, the receptionist called out the student’s name by fingerspelling and asked in SL the type of health insurance.

b. In the second role-play, students experienced blind people’s barriers in healthcare. Each student received individual written and oral instructions for the activity. Different health scenarios were assigned randomly, and medical exam documents, a blindfold and a cane were handed to each student. Each student had to enter the conditioned classroom, wearing the blindfold and with the help of the cane, and wait in the waiting room where he or she had to communicate with a receptionist, physician and pharmacist. In each scenario the receptionist asked the student his/her name and type of health insurance and handed out a receipt to sign without offering any help or guidance.

2. Guided discussion

a. Movie analysis: The students’ homework was to watch the Indian movie “Black” (<https://www.imdb.com/title/tt0375611/>), which addresses the life of a deafblind girl, inspired by Helen Keller’s life and struggle. After watching the movie, they had to answer written reflective questions at home, and then discuss them in class. The main topics were “What was Michelle’s childhood like before and after the arrival of the teacher?”, “What did you like the most about the movie?”, “What struck you the most about the movie?” and “Did the movie change your way of thinking about deafblind people?”

b. Article discussion: A copy of the brief Comment “Who has special needs?”²⁵ was handed to each student. After individual reading, a group discussion was carried out about the euphemism *Special Needs* and the term “Persons with Disabilities”.

Second stage: Classes 6–10

1. Lecture: A traditional lecture was given by the teacher (VC) about the current approaches towards people with hearing or visual disability and their healthcare barriers.

2. Interactive class 1: Led by a blind person. The topics were “How to approach a blind person” and

“How to help a blind person to move around”, addressed through a lecture and practical exercises. Students were challenged to guide the blind teacher and/or had to use a blindfold and receive instructions from a peer.

3. Interactive class 2: Led by a Deaf person. The topics were “How to approach a Deaf person” and “Deaf Culture”, addressed through a lecture and practical exercises. Students were challenged to approach the deaf teacher in a Deaf-culturally accepted way.

4. Case Method Teaching Stage 1: Students were grouped in random pairs and had to visit a CESFAM to identify possible barriers for people with visual or hearing disability. They had to navigate through the CESFAM and interview the health staff, and had to take pictures of the possible barriers. For this activity students received constant feedback from the teacher (VC). Then they had to prepare an oral presentation of their visit, in which they had to define each of the barriers.

Third stage: Classes 11–17.

1. Braille lessons: Three practical classes were conducted by a blind teacher on this writing system. Students learnt the basics of Stage 1 of Braille. Each student was given a slate and stylus to practice in class and at home.

2. Braille practical exam: The fourth class was a practical exam, in which students had to write a medical prescription for a patient in Braille (Figure 1).



Atención en salud de personas con discapacidad auditiva y visual
Universidad de Concepción
Facultad de Odontología

Nombre: Cristina Abasolo

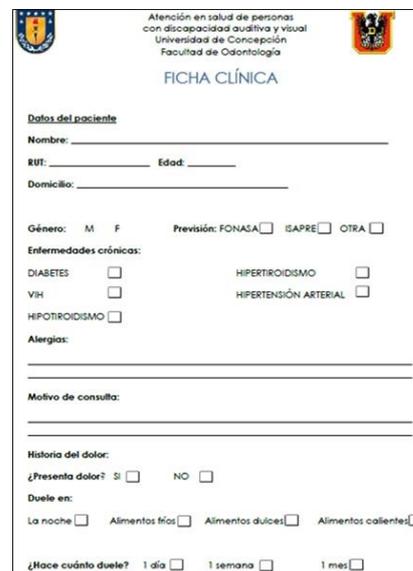
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Amoxicilina 500 miligramos, 1 comprimido cada 8 horas por 7 días.

Figure 1. Medical prescription for Braille

3. Chilean Sign Language lessons: Three practical classes conducted by a Deaf teacher. Basic signs relating to a general clinical record and specific signs for dental examination were taught. Students had permission to record the teacher.

4. Standardized patients: For the Chilean Sign Language practical exam, the Deaf teacher took the role of a Deaf patient, based on a previous script written by the coordinating teacher. Students were given a clinical record (Figure 2), which they had to complete by asking questions in SL to their Deaf patient. Then, they had to communicate the diagnosis, treatment and the date for a follow-up appointment to the Deaf patient.



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FICHA CLÍNICA

Datos del paciente
Nombre: _____
RUT: _____ Edad: _____
Domicilio: _____

Género: M F Previsión: FONASA ISAPRE OTRA

Enfermedades crónicas:
DIABETES HIPERTIROIDISMO
VIH HIPERTENSIÓN ARTERIAL
HIPOTIROIDISMO

Alergias:

Motivo de consulta:

Historia del dolor:
¿Presenta dolor? SI NO
Duele en:
La noche Alimentos fríos Alimentos dulces Alimentos calientes
¿Hace cuánto duele? 1 día 1 semana 1 mes

Figure 2. Clinical record for SL exam

5. Case Method Teaching Stage 2: The same pairs of students had to present reasonable adjustments to the previous barriers identified by them in the CESFAM. Before the oral presentation, students received constant feedback from the teacher (VC). Directors of the different CESFAMs were invited to attend this activity.

Data collection

Once the course had finished, the students answered a survey designed with open-ended questions, according to Castro *et al.*²⁶, to determine if the new teaching intervention had led to any changes in their understanding of the handling approach Persons with Disabilities. Students completed the anonymous questionnaire using the following open-ended questions: 1) “Do you think this course provided you with knowledge or experience that will

help you as a professional? Please explain your answer.”; 2) “In your opinion, what do you think was the most important aspect of the course that influenced your professional training? Please explain your answer.” 3) “Suggestions and comments”. Also, based on the Deaf Strong Hospital survey¹⁵, three questions on a Likert scale 1–5 were added to the survey, 1) “I learned valuable information through my participation in role-play”; 2) “My experience is likely to positively impact my attitudes and behaviors in future interactions with deaf or blind patients.”; 3) “The role-playing time was used effectively”.

In addition, guided discussions, case method teaching, standardized patients, attendance and grades obtained were recorded and analyzed.

Table 1. Quotes from Guided Discussion from movie "Black"

Questions	Quotes
How do you find Michelle’s childhood was before and after the arrival of the teacher?	<i>Before arrival of teacher:</i> “Very dark”; “Lonely”; “Isolated”; “She was treated as an animal”; “Michelle just acted by instinct” <i>After arrival of teacher:</i> “Stable life”; “She learnt how to communicate”; “Happy life”; “Was able to live life”
What did you like the most about the movie?	“The concept of learning and overcoming of Michelle”; “A critic and real vision on how life is for a Deafblind person”; “That the teacher believed in her”; “Michelle was able to be an independent person”
What struck you the most about the movie?	“Michelle’s’ childhood...her parents had forgotten she was a human being”; “To learn how a Deafblind person lives” “No, but I did learn that Deafblind people crave for physical contact”; “Frankly, this subject never came to my mind before watching this movie”;
Did the movie change your way of thinking about deafblind people?	“Yes, I had never dimensioned what it is to live in absolute darkness”; “We usually think they are very dependable and feel pity, but they are just like us”; “Yes, we should respect everyone, as everyone has the same Rights”; “Yes, I realized that Deafblind people also want to achieve what we all want, a profession, a family, etc.”

All students agreed that the correct term is Persons with Disabilities, and not the current euphemism *Special Care patients*. They also internalized the concept that Persons with Disabilities are persons with Rights, and no longer of pity.

All students passed the Braille practical exam and the standardized patient practical exam.

Regarding Case Methods stages 1 & 2, the students correctly identified most of the barriers a person with visual or hearing impairment may encounter when visiting a CESFAM. They identified barriers based on scientific literature, their own experience in the role-play and the

Analysis

Descriptive statistics were used to analyze the students’ scores, including average and range scores. Qualitative results were obtained through the identification of relevant quotes, which were grouped by topic.

RESULTS

Eight students had an attendance of 100%, followed by three students with an attendance of 94%, two with 88% and one with 82%.

Quotes from the guided discussion are shown Table 1.

interactive classes. Also, they were able to identify reasonable adjustments to address the barriers identified. They identified the adjustments based on scientific and non-scientific literature.

Six students obtained a final grade of 7.0, and the remaining eight obtained a grade of 6.9.

Students’ perceptions about the role-play and the course in general are shown in Tables 2 and 3.

Table 2. Distribution of Dental students' evaluation of the Role-Play activity

	Strongly Agree	Agree	Not sure	Disagree	Strongly Disagree
1. I learned valuable information through my participation in Role-play.	13	1			
2. My experience is likely to positively impact my attitudes and behaviours in future interactions with Deaf or Blind patients.	14				
3. The role-playing time was used effectively	12	2			

Table 3. Students quotes from open-end survey

"Totally, every day inclusion is stronger, so I feel the responsibility to know about it, and this course was the first encounter with inclusion in dentistry"

"I think it's one of the courses where I've felt the happiest. Every class I learnt something new about Deaf culture and people with visual impairments. This course has encouraged me to fight for a world without communication barriers"

"I think this course gave me knowledge and experience that will help me in the future because we faced situations for which most of us were not prepared and we learn how to solve them"

"Yes, it gave me so much knowledge that will help me in my future career, because I will be able to help people with sensory disabilities, and these people will feel more included by the health system. Besides, now I can understand how these people feel, as I experienced it by myself"

"The knowledge of Braille and SL focused in dental practice"

"To analyse in detail which are the mistakes and offenses we make every day to people with sensory disabilities, and how to fix these mistakes and avoid these offenses"

"I think the most important part was the Role-Playing, because it gave me the chance to put myself in their shoes and understand their challenges. This encouraged me to learn Braille and SL beyond the evaluation, but it is something really necessary to learn, not just as professionals, but as persons"

"The fact that I can effectively communicate with the patients really had an impact on me, because I learnt the basics of Braille and SL"

Comments and suggestions from the students are shown in Table 4.

Table 4. Comments and suggestions from the students.

Suggestions	<i>"This should be a mandatory subject in our academic education" (7); "More Braille and SL lessons" (2); "More interactions with PwD" (2) "A second part of the course" (2); "More spots available"</i>
Comments	<i>"I liked communicating with Blind and Deaf persons, and understanding what they feel, as I put myself in their place and learnt SL and Braille"; "I found the course a pleasant experience"; "I really enjoyed all the activities"; "It was very didactical, the topics weren't boring or anything like that"; "We learnt the contents in a very didactical way"; "It increased greatly my interest, it opened my eyes"; "The course was developed in a very good way, it is well implemented and coordinated"; "Above all, great human support"</i>

DISCUSSION

A 17-week elective course was carried out in the first semester of Dentistry School with 14 students enrolled. All students passed the course with the highest score, and thus were able to define the medical approach to hearing and visual disability, recognize the cultural and linguistic aspects of people with visual disability and people with hearing disability and learn their means of communication. All these learning outcomes were assessed through practical activities and the participation of Deaf and blind teachers.

What students valued the most was the active presence of Deaf and blind people, because for most of them this was their first encounter with a Deaf or a blind person, and because they felt that they were the appropriate persons to teach about their own experience, difficulties and ways of communication. This elective course fulfils the Convention on the Rights of Persons with Disabilities statement, specifically on *"Recognizing the valued existing and potential contributions made by persons with disabilities to the overall well-being and diversity of their communities..."* and *"Considering that persons with disabilities should have the opportunity to be*

actively involved in decision-making processes about policies and programmes, including those directly concerning them".¹² The importance of the latter is that, even though there is an increasing number of training programs for healthcare students around the world for the care of Persons with Disabilities, none of them involves the active participation of Persons with Disabilities as teachers, which, in our case, promoted a real sense of inclusion. In this regard, role-play was a fulfilling experience, as most students felt that they learnt valuable information that impacted positively on their future interactions with Deaf or blind patients. This type of experience, which has been well documented by Rochester University, has been shown to have a similar effect on pharmacy and medical students.^{15,19,27}

Based on the comments and suggestions, it can be inferred that the students enrolled on this course thinking it would be a rather traditional course, with lectures and written tests and exams. In contrast, they were pleased that the program had a more practical approach, with standardized patients, where their skills were tested through simulated situations with Deaf and blind persons, promoting reflective thinking. The visits to CESFAMs in their own community to identify the barriers a Deaf or a blind person may encounter were also a positive way to promote reflective thinking through the Case Method approach, as they later, on their own and with the teachers' guidance, were able to find a way to address them.^{22,23,28-30} It has been established that the Case Method approach promotes reflective thinking, resulting in confident and consciously competent students, and that standardized patients promote the skills required to offer a better service to patients who have disabilities.^{21-23, 28-33}

Moreover, the Case Method approach was a highly effective strategy, as students (guided by their tutor) were able to identify the barriers a person with visual or hearing impairment may encounter; this situation went beyond the planned scenario, as students identified barriers in the streets near the CESFAMs, and also investigated their access through means of transport, as in some CESFAMs the nearest bus stop was 6 blocks away. In the second stage, the students proposed a number of reasonable adjustments to the problems

they had identified earlier. Reasonable adjustments, i.e. solutions that do not involve significant alterations to the structure or procedure aimed at removing barriers towards full participation, are of great importance since resources in Public Health Centers are very limited, and expensive or time-consuming solutions would be too impractical.^{12,24,34} The reasonable adjustments proposed by the students were shared with directors from the different CESFAMs, in order to give them ideas on how to address the barriers observed.

Regarding the guided discussion (movie analysis), students watched a movie about a deafblind girl, who with her determination and her teacher's assistance learnt to read and to sign, went to university and finally, got her degree. As this story is based on the famous biography of Hellen Keller, it was a great way to change the students' views of Persons with Disabilities, because during the discussion panel the students revealed that they were amazed to discover what Persons with Disabilities with assistance and determination can achieve. This situation helped the students to understand that Persons with Disabilities need to be empowered, that they are subjects of legal rights and not subjects of charity.^{12,35} This topic was also addressed in the article discussion, in which it was reinforced that the correct term is Persons with Disabilities, not deaf-mute, deaf-dumb, the blind or retarded, and that the current euphemism *Special Care patients* should not be used.^{12,25,35}

Among the educational strategies used, WhatsApp was an excellent tool for fluent coordination. The teacher and students were able to share news and events related to People with Disabilities, and send videos regarding specific SL signs and pictures of Braille, and exchange information related to Deaf or blind people in a very informal environment. Also, as the nature of the course was elective, sometimes the established schedule had to be modified or suspended since tests or exams from core courses in the dental undergraduate program were prioritized. This was mitigated through the instant communication that

WhatsApp allows, making it easy to arrange a solution between the students and the teachers.³⁶

The main limitation is that this study was only conducted in one dental school and had a small sample size. Even though it was a small sample, having more than 14 students would affect the interaction between students and teachers, hindering both interaction and learning.³⁷⁻³⁹

Next year, it is expected to incorporate students from multiple undergraduate healthcare programs, so that a greater variety of healthcare disciplines can offer better opportunities to promote collaborative and interdisciplinary work. Also, it is planned to develop a second level of this course, in order to enhance the skills acquired by the students.

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CONFLICTS OF INTEREST STATEMENT

The authors report no conflicts of interest.

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FREQUENCY AND LOCALIZATION OF OVERHANGING RESTORATIONS

ABSTRACT

Objectives: The aim of this retrospective study was to determine the frequency and localization of the overhanging restorations by observing patients' routinely taken panoramic radiographs.

Materials and Methods: The panoramic radiographs of 4,960 patients who applied to a dental clinic in University between 2015 and 2016 and had one or more previous restorations were retrospectively examined. The study group included 243 patients (133 females and 110 males) with a mean age of 39.7 ± 12.5 years. Superposed interdental areas were not evaluated. Frequency distributions and percentages were calculated for the categorical data as to the surface of the maxillary-mandibular premolar and molar teeth, also the presence or absence of root canal treatment. Chi-square tests were used to compare data relating to the localization and frequency of overhanging restorations.

Results: In the radiological evaluation of 243 patients, a total of 280 overhanging restorations were detected. Root canal treatment was present in 45.4% of the teeth with an overhanging restoration. The frequency of overhanging restorations in the maxilla was significantly higher (60.4%) than that of the mandible (39.6%), ($p < 0.05$). The frequency of overhanging restorations in molar teeth (82.9%) was significantly higher than that of premolar teeth ($p < 0.05$). Of all the overhanging restorations, 90.4% were in Class II cavities and 9.6% were in mesio-occluso-distal (MOD) cavities. More than half (57.3%) of the overhanging margins in the Class II restorations were distal; 42.7% were mesial surfaces ($p < 0.05$). The most frequent restorations with overhanging were found in the maxillary molars (49.6%) and the least frequent were in the premolar teeth of the mandible (6.4%).

Conclusions: The restorations with overhanging margins determined most often at the disto-occlusal margins of the maxillary molars. The frequency of overhanging restorations was higher in areas that are difficult to reach during treatment.

Keywords: Dental Marginal Adaptation, Dental Restoration Failure, Permanent Dental Restoration, Molar, Panoramic Radiography.

 *Meltem Tekbaş Atay¹
 Mediha Büyükgöze Dindar²
 Esra Özyurt¹
 Aylin Çilingir¹
 Uğur Erdemir³

ORCID IDs of the authors:

M.T.A. 0000-0002-1762-830X

M.B.D. 0000-0003-3794-4366

E.Ö. 0000-0003-4118-0450

A.Ç. 0000-0002-9848-9136

U.E. 0000-0002-4673-8284

¹ Department of Restorative Dentistry, Faculty of Dentistry, Trakya University, Edirne, Turkey.

² Health Science Vocational College, Trakya University, Edirne, Turkey.

³ Department of Restorative Dentistry, Faculty of Dentistry, İstanbul University, İstanbul, Turkey.

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INTRODUCTION

An overhanging dental restoration is defined as an extension of the restorative material beyond the cavity preparation borders.¹ Overhanging and inappropriate dental restorations and prostheses are the most common etiologic factors of gingival inflammation and periodontal destruction.²⁻⁵ Working in a very limited area in the mouth and having difficulties accessing certain teeth often causes restorations to overhang in the interproximal areas. In these regions, the polishing procedures are difficult to perform because of the anatomical restrictions causing inadequate polishing.⁶ Inadequately polished and overhanging dental restorations prevent patients from practicing oral hygiene in interproximal areas, leading to increased accumulations of plaque and a change in healthy flora.⁷ Over time, inflammation in the region due to increased plaque accumulation and periodontal pathogens causes bone destruction.⁸ Bleeding, gingival inflammation, and bone loss increase in tissues adjacent to overhanging restorations compared to healthy gingiva. Other causes of bone destruction include the infiltration of overhanging restoration due to biological widening, the intrusion of restoration in interproximal gingiva, and chemical damages of the material due to its contents.^{6,9}

The biological width is defined as the size of the soft tissue that connects the tooth's coronal part to the top of the alveolar bone. In studies conducted by Gargiulo *et al.*¹⁰ in 1961, it was reported that in humans, an average of 1.07 mm of connective tissue attachment presents on the alveolar cortex, with an epithelial attachment of just 0.97 mm below the gingival base. The sum of these two distances is defined as the biological width. Inflammation occurs primarily in the gingiva as a consequence of the violation of the biological width.¹¹ If overhanging restoration is not recognized, clinical attachment loss is followed by bone loss, and clinically this results in a deep periodontal pocket or gingival recession.¹² To avoid these unwanted situations, it is important to diagnose and treat overhanging restorations on time.

The most common cause of overhanging dental restorations is iatrogenic due to inadequate physician skill. Creep may play a role in gingival overgrowth of large amalgam restorations.¹³ In some cases, marginal adjustment of the restoration may not be achieved although careful restoration is established. Differences and irregularities in root anatomy can make marginal adjustments difficult.¹⁴ Every restoration change causes some dental tissue loss and the preparation enlarges.¹⁵ For this reason, while the overhanging restorations are being renewed, the new restoration should be done with care, considering the reason for the overhanging.

It is difficult to detect overhanging restorations of posterior teeth with conventional clinical examination methods. Clinical examination alone is inadequate for detecting overhanging fillings when compared to clinical examination with bite-wing radiographs.¹⁶ Bite-wing radiographs alone have been reported to detect more overhanging restorations compared to clinical examination alone.¹⁷ The most reliable method of diagnosing overhanging restorations is to combine both clinical and radiological examinations.¹⁸ Optimal evaluation can be done with bite-wing radiographs, but panoramic and periapical radiographs can also be used to diagnose overhanging restorations. Studies with panoramic radiographs are available in the literature.^{19,20}

The purpose of this study was to determine the frequency and localization of the overhanging restorations by investigating the routinely taken radiographs and to specify the teeth, cavity shapes, localization, and root canal treatment presence were frequently encountered with overhanging restorations and whether there were significant relationships among these variables.

MATERIALS AND METHODS

The permissions necessary for this study were obtained from the Scientific Research Ethics Committee of Trakya University (ID: TÜTF-BAEK 2016/235). In this retrospective study, panoramic radiographs and demographic features of the 4,960 patients who applied to dental clinic

in university between 2015 and 2016 were examined by the same observer (M.B.D.).

The study group includes a total of 243 patients between 18-70 years of age who had at least one obvious overhanging restoration which could be evaluated properly at the panoramic radiographs. The patients whose the demographic features could not be accessed, deciduous teeth, non-contacted fillings and superimposed interdental spaces were excluded from the study.

The digital panoramic radiographs that were utilized were taken with a panoramic x-ray device (Pax-Flex 3D Vatech, Hwaseong, South Korea) at the department of radiology of Trakya University, Faculty of Dentistry. None of the panoramic radiographs were obtained specifically for this study. After panoramic radiographs of overhanging restorations were examined; type, location, cavity design, and root canal treatment presence of teeth were recorded.

Statistical analysis

Table 1. Locations of overhanging restorations.

Location	Premolar	Molar	p-value
	Number (frequency %)	Number (frequency%)	
Maxilla	30 (10.7%)	139 (49.6%)	0.87
Mandible	18 (6.4%)	93 (33.2%)	

Chi-square tests were used to compare the data.

* $p < 0.05$ is statistically significant.

Root canal treatment was observed in 45.4% of the teeth with an overhanging restoration. A total of 90.4% of the overhanging restorations were in Class II and 9.6% were in mesio-occluso-

Table 2. Cavity designs of overhanging restorations.

Cavity Design	Premolar	Molar	p-value
	Number (frequency%)	Number (frequency%)	
MO	6 (2.1%)	102 (36.4%)	0.00*
DO	40 (14.3%)	105 (37.5%)	
MOD	2 (0.7%)	25 (8.9%)	

MO: Mesio-occlusal, DO: Disto-occlusal, MOD: Mesio-occlusal-distal

Chi-square tests were used to compare the data.

* $p < 0.05$ is statistically significant.

The frequency of overhanging restorations was found to be significantly higher on the maxilla (60.4%) than the mandible (39.6%) ($p < 0.05$). The frequency of overhanging

Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 22.0 (SPSS Inc., Chicago, IL, USA). Frequency distributions and percentages were calculated for the categorical data as to the surface of overhanging restorations present on the maxillary and mandibular premolar and molar teeth, also the presence or absence of root canal treatments. Chi-square tests were used to compare the data on the localization and frequency of overhanging restorations. All results were considered significant at $p < 0.05$.

RESULTS

Overhanging restorations were observed in 5% of patients with previously restored teeth. In the radiological evaluation of 243 patients in the study group, a total of 280 overhanging restorations were detected. Of those, 82.9% were in molars and 17.1% were in premolars. Of all the overhanging restorations, 60.4% were observed in the maxilla. Most overhanging restorations were observed in the maxillary molar teeth (49.6%) while the least was observed in the mandibular premolar teeth (6.4%) (Table 1).

distal (MOD) cavities. More than half (57.3%) of the overhanging restoration margins in the Class II cavities were on distal interfaces while 42.7% were on mesial interfaces (Table 2).

restorations in molar teeth (82.9%) was significantly higher than that of premolar teeth ($p < 0.05$). The frequency of overhanging restoration in disto-occlusal cavities was

significantly higher than that of other cavities ($p < 0.05$) (Table 3) (Figure 1).

Table 3. Statistical analyses of location, tooth type and cavity design of overhanging restorations.

Parameters		Number (frequency%)	p-value
Location	Maxilla	169 (60.4%)	0.001*
	Mandible	111 (39.6%)	
Tooth Type	Premolar	48 (17.1%)	0.000*
	Molar	232 (82.9%)	
	MO	108 (38.6%)	
Cavity Design	DO	145 (51.8%)	0.000*
	MOD	27 (9.6%)	

MO: Mesio-occlusal, DO: Disto-occlusal, MOD: Mesio-occlusal-distal
Chi-square tests were used to compare the data.

* $p < 0.05$ is statistically significant.



Figure 1. Panoramic images of overhanging Class II restorations. A, mandibular left second premolar; B, mandibular left first molar.

DISCUSSION

Overhanging restorations may increase plaque accumulation and cause gingival inflammation, periodontal tissue damage, and also particularly decrease in alveolar bone height.^{17,21,22} Also, overhanging restorations are one of the reasons for secondary caries that can cause infection in the pulp.²³ Increased gingival index, hemorrhage index, periodontal pocket depth, and bone loss were observed in the gingiva adjacent to the contact of the overhanging restoration margins.^{6,24-26} Overhanging restorations have been reported to cause flora changes similar to chronic periodontitis in the gingival sulcus adjacent to the overhanging restoration margin.²⁷ An increase in gram-anaerobic bacteria, especially black-pigmented *Bacteroides* in the flora of the gingival sulcus was observed.⁷ Roman-Torres *et al.*²⁸

reported that renewing of the overhanging restorations is associated with a reduction in the amount of *Actinobacillus actinomycetemcomitans* in flora. For these reasons, the timely diagnosis and treatment of overhanging restorations are very important.

The radiographic evaluation has an important role in the diagnosis of overhanging restorations.¹⁸ Normal anatomic contact areas of the posterior teeth make difficult the conventional clinical diagnosis of overhanging restorations. Although bite-wing radiographs are more detailed than panoramic radiographs, the panoramic radiographs routinely taken for oral diagnosis have been used in the literature for various purposes.^{29,30} The fact that bite-wing or periapical radiographs are more successful in the interface evaluation and diagnosing overhanging restorations, the present study has a limitation because of the evaluation of existing panoramic radiographs in order not to expose patients to extra radiation. Besides only the panoramic radiographs were evaluated and oral examinations were not done in this study to detect the overhanging restorations. Therefore, the soft tissue responses to overhanging restorations could not be observed intra-orally. On the other hand, all radiographs were evaluated by one observer as well as in other studies^{30,31} to prevent the differences due to the observer.

In previous studies, the frequency of the overhanging restorations has been reported to be

in the range of 16.5% to 76%. When only evaluating overhanging amalgam restorations, 74% was reported by Gorzo *et al.*³²; 58% was reported by Quadir *et al.*¹⁶; 51% was reported by İbraheem *et al.*³⁵, and 22.2% was reported by Baharlooie *et al.*²⁰ When evaluating all overhanging restorations, Muryani *et al.*²¹ reported 75.4%; Matvijenko *et al.*⁶ reported 50.8%; Tavangar *et al.*³³ reported 36.6%; Gilmore and Sheiham³ reported 32%, and Kuonen *et al.*³⁴ reported 14.1%. According to a recent study conducted by Najm *et al.*²⁴, the frequency of overhanging restorations was reported as 3.2%. Similar to the results of the present study, the frequency of overhanging restorations was 5%. The reason for the restoration frequency being higher in other studies may be due to the difference in methods. The frequency of studies with the radiographic evaluations in conjunction with clinical examinations was found to be higher because a small clinical overhanging can be detected by probing; that might not necessarily be the case with the radiographic examination. In more recent studies, the reported frequency has decreased due to the progress of knowledge, education, and technology.

Similar to other studies^{16,18,33}, this study shows that overhanging restorations are significantly higher in the maxilla. Quadir *et al.*¹⁶ found that the frequency of overhanging restorations was 65% in the maxilla. A similar result was observed in the present study, which was 60.4%. It is thought that the reason for the more overhanging restoration presence in the maxilla is related to the difficulty of indirect sight and limited access to this area during treatment.

This study reported that the frequency of overhanging restorations is higher in molar teeth. This finding is similar to those of previous studies.^{16,33} Overhanging restorations were observed most frequently in Class II cavities. It is expected that this is because Class II is the most common type of cavity.³²

In the present study, most of the overhanging restorations were observed in the maxillary molars (49.6%), supporting the results of other studies.^{16,24,33,35} It was observed that the frequency

of overhanging restoration was the lowest in the premolar region of the mandibula (6.4%). The results of this study and those from Najm *et al.*²⁴ are similar. Najm *et al.*²⁴ also stated that the highest overhanging restoration was in the maxillary molar teeth (31.2%), while the least amount of overhanging restorations was in the mandibular premolar teeth (5%).

The authors of various studies^{16,33,36,37} have shown that overhanging restorations on distal surfaces are higher than overhanging restorations on mesial surfaces. Similarly, in the present study, overhanging restorations were most often encountered on distal surfaces (53.7%).

Although overhanging restorations are usually iatrogenic, it has been understood that dental anatomy and materials used may also be responsible for it. According to the literature, there is a relationship between overhanging restorations and the matrix type used. It has been pointed out that the possibility of overhanging restorations made with environmental matrix systems is higher than that of sectional matrix systems.^{38, 39} Besides, in the restorations made with transparent matrix and reflective wedges, more overhanging restorations were detected than with metal matrix and wooden wedges.⁴⁰ The adaptation of transparent matrices is difficult compared to that of metal matrices and it is not possible to adapt as tightly as with metal matrices. Reflective wedges are very stiff and cannot be adapted well to natural anatomical contours when compared to wooden wedges.

With the increase of physician consciousness and the development of materials used, the frequency of overhanging restorations is decreasing, but they cannot be completely avoided despite clinicians' best efforts. There is still a high frequency of overhanging restorations in teeth with anatomical differences, especially in the areas where the physician has restricted time and access to appropriate materials and the patient load is high. As a result, more emphasis should be placed on the prevention, identification, and rapid removal or correction of overhanging margins of restorations to minimize the risk of periodontal disease.

CONCLUSIONS

Management of restorations with excessive dental hard tissue loss is challenging. For the prevention of overhanging restorations, treatment steps should be followed precisely and effective usage of dental matrices systems and wedges must be taken into consideration. In the future, clinicians would be better able to handle overhanging margins due to the progress of knowledge, education, and technology.

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CONFLICTS OF INTEREST STATEMENT

The authors report no conflicts of interest.

Taşkın Restorasyonların Görülme Sıklığı ve Lokalizasyonları

ÖZ

Amaçlar: Bu retrospektif çalışmanın amacı, hastaların rutin olarak çekilen panoramik radyografilerini inceleyerek taşkın restorasyonların görülme sıklığını ve lokalizasyonunu belirlemektir. **Gereç ve Yöntemler:** 2015-2016 yılları arasında Üniversitede bir diş kliniğine başvuran ve önceden bir veya daha fazla restorasyonu olan 4.960 hastanın panoramik radyografileri retrospektif olarak incelendi. Çalışma grubuna yaş ortalaması $39,7 \pm 12,5$ olan 243 hasta (133 kadın ve 110 erkek) dahil edildi. Süperpoze olmuş interdental alanlar değerlendirmeye alınmadı. Dolgulardaki taşkınlıkların alt-üst çene premolar ve molar dişlerin hangi yüzeylerinde olduğu ve dişlerde kanal tedavisi olup olmadığına ilişkin kategorik veriler için frekans dağılım ve yüzde değerleri verilmiştir. Taşkın kenarlı dolguların lokalizasyonu ve görülme sıklığına ilişkin verilerin karşılaştırılmasında Ki kare test kullanıldı. **Bulgular:** 243 hastanın radyografik değerlendirmesinde toplam 280 tane taşkın dolgu varlığı tespit edildi. Taşkın dolgusu olan dişlerin %45,4'ünde kanal tedavisi mevcuttu. Taşkın dolguların üst çenede görülme sıklığı (%60,4), alt çenede (%39,6) olanlara göre istatistiksel olarak anlamlı derecede yüksek bulundu ($p < 0,05$). Molar dişlerde taşkın dolguların görülme sıklığı (%82,9) premolar dişlere oranla istatistiksel olarak anlamlı şekilde yüksektir ($p < 0,05$). Taşkın ara yüz restorasyonlarının %90,4'ü iki yüzlü kavitelere, %9,6'sı mesio-okluzo-distal (MOD) kavitelere aittir. Klas II restorasyonların taşkın kenarlarının yarısından fazlası (%51,8) distal yüzeyde,

%38,6'sı mesial ara yüzeydedir ($p < 0,05$). Taşkın kenarlı restorasyonların en fazla üst çene molar (%49,6), en az alt çene premolar dişlerinde (%6,4) olduğu görülmüştür. **Sonuçlar:** Taşkın kenarlı restorasyonların en sık üst çene molar dişlerinin disto-okluzal yüzeylerinde olduğu saptanmıştır. Taşkın restorasyon görülme sıklığı tedavi sırasında ulaşılması zor olan bölgelerde daha fazladır. **Anahtar kelimeler:** Dental marjinal adaptasyon, dental restorasyon başarısızlığı, kalıcı dental restorasyon, azı dişi, panoramik radyografi.

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MANDIBULAR SEXUAL DIMORPHISM ANALYSIS IN CBCT SCANS IN A SYRIAN SAMPLE

ABSTRACT

Objectives: This study aimed to develop metrical and angular indices to predict the sex of unknown skeletonized human remains in Syria using the mandibular bone.

Materials and Methods: The sample consisted of 99 CBCT scans of the Syrian population (43 males, 56 females) aged between 18-25 years. The collected CBCT images were analyzed on two occasions and by two examiners to test the reliability of measurements. Four measurements were analyzed to be used for sexual prediction analysis as following: Coronoid-Gonion length (the distance between Gonion and the highest lateral point on the Coronoid process), minimum ramus breadth, Gonial angle, and Bigonial width.

Results: All the aforementioned measurements showed significant statistical sex-related differences. The Bigonial width showed the highest difference with ($P < 0.01$ _ mean: 95.17 ± 6.45 mm for males, 86.84 ± 4.81 mm for females), followed by the gonial angle ($P < 0.01$ _ mean: $127.11^\circ \pm 7.87^\circ$ for males, $131.52^\circ \pm 6.08^\circ$ for females). Coronoid-gonion length ($P < 0.01$ _ mean: 58.61 ± 6.78 mm for males, 53.97 ± 5.26 mm for females) and minimum ramus breadth ($P < 0.01$ _ mean: 29.63 ± 2.90 mm for males, 27.89 ± 2.73 mm for females). specificity and sensitivity for the four indices derived function were 79 % and 80 % respectively and the diagnostic accuracy was 79.6 %.

Conclusions: The present study suggested that the sex of mandible in Syrian population could be assessed using metrical and angular measurements which considered as an additional tool for sex identification.

Key Words: Forensic anthropology, forensic dentistry, mandible, sex characteristics.

-  Meced Gazi Aljaber Abofakher¹
-  Amer Owayda²
-  *Maher Suresi³
-  Mahmud Abdülhak¹
-  Nofal Houssein⁴
-  Ömer Hamadah¹

ORCID IDs of the authors:

- M.G.A.A. 0000-0002-3412-8825
- A.O. 0000-0002-6381-6133
- M.A. 0000-0001-6691-6992
- O.H. 0000-0002-2308-0547
- M.A. 0000-0003-1263-5399
- H.N. 0000-0002-4237-6837

¹ Department of Oral Medicine, Faculty of Dentistry, Damascus University, Damascus, Syria.

² Department of Orthodontic and Maxillofacial Orthopedic, Faculty of Dentistry, Damascus University, Damascus, Syria.

³ Department of Oral Histology and Pathology, Faculty of Dentistry, Damascus University, Damascus, Syria.

⁴ Department of Forensic Medicine, Faculty of medicine, Damascus University, Damascus, Syria.

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***Corresponding Author:**

Department of Oral Histology and Pathology, Faculty of Dentistry, Damascus University, Damascus, Syria.

Phone: +96 399 188 03 64

Email: dr.maher.assaf1990@gmail.com

INTRODUCTION

Sex identification using human remains is considered to be an important concern in forensic anthropology practice. Sex identification considered to be very difficult especially when the remains are decomposed or just bones.^{1,2}

Human pelvis bone is considered to be the most important bony standards that involve remarkable sexual dimorphism, with accuracy up to 95 % followed by cranial and long bones with approximate 92% accuracy. All previous bones will be decomposed or lost due to the big size and fragility as postmortem changes.³ The mandibular bone is very important as one of the hardest, most durable of cranial bones and has more form variations according to sex.⁴

3D radiography, such as CBCT and CT scans, is used in bone measurements for many medical purposes.^{5,6} Many anatomical landmarks exhibit the different features between males and females. In general, the mandible size in males is bigger than in females, whereas mandibular gonial angle is more rounded in females compared with males.⁷ The maximum height and width of ramus increased in males more than in females due to differences in masticatory muscles efficacy between males and females. A systematic review⁷ has found that 87.5% of mandibular radiographic studies on adults, also 75% of studies on pre-identified dried mandibular bones of human remain showed significant differences between both sexes and referred to the lower jaw importance as an index in different races for sex determination.⁷

The forensic studies on Middle East populations are very few, although repeated armed conflicts in this area and the increased number of victims daily, there was a high need to find additional sex identifying indices for Syrian population-based on mandibular measurements. This study aimed to assess four mandibular indices for sex identification in the Syrian population including minimum breadth of the right ramus, the distance between coronoid process peak to gonion at a mandibular angle on the right side, right mandibular gonial angle, bigonial width.

MATERIALS AND METHODS

An approval was gained from the Ethics Committee at Damascus university to conduct the study (Approval ref: 283/2017).

This study is a retrospective cross-sectional study.

107 CBCT scans were randomly collected from the department of orthodontics and maxillofacial orthopedic at Damascus university. The inclusion criteria were patients Aged between 18-25 years of Syrian origin. CBCTs with the flowing cases were excluded: acquired or developmental deformation, severe asymmetry, old or new mandibular fracture, loss of two or more teeth and periodontitis. Finally, just 99 CBCT radiographs were included in the current study (56 females – 43 males).

The CBCT images were taken using the same CBCT scanner (Planmeca ProMax 3D Mid; Planmeca Oy; Helsinki, Finland) with a voxel size of 0.3–0.4 mm.

OnDemand3D program™ (Cypermed Inc., Seoul, Korea) was used to study CBCT images. As shown in (Figure 1) and the measurements were made on the 3D reconstructions, on the lateral view the following points were determined Gonion (Go: the most inferior posterior point on the mandibular angle), Gnathion (Gn: the most lower anterior point on the chin). Condylon (Co: the most upper posterior point on the lateral face of condyle), Coronoin (Cor: the most upper posterior point on the coronoid process). After that these measures were taken: The right side Gonial angle (Co-Go-Gn), Mr-Br (the minimum breadth of the right side ramus between anterior and posterior of ramus edges), Cor-Go (the distance between Coronoin and gonion points). On the axial view, the bigonial width (the distance between right and left gonion points) was measured as shown in (Figure 1).

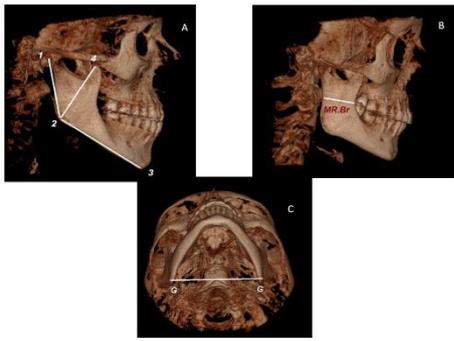


Figure 1: (a) measurement of the gonial angle 1-2-3 and the distance between Co-Go 2-4, (b) measurement of minimum ramus breadth and (c) measurement of the distance between right and left gonion

All measurements were carried out by two observers and on two occasions (the 2nd after two weeks of the first occasion).

Statistical Analysis

Data analyzed using SPSS software (version 20; IBM, Armonk, New York). Data distribution was tested with the Kolmogorov-Smirnov test. The independent sample t-test was employed to explore the differences in Mr-Br, Cor-Go,

bigonial width and the gonial angle between males and females. Discriminant functional analysis was used to determine the accuracy of the aforementioned measures for sex identification purposes. The intraclass correlation coefficient ICC values for all variables were ranged from 94% to 99% and showed a high level of agreement and reliability for both interobserver and intraobserver measurements.

RESULTS

This study was carried out on 99 CBCT scans for individuals aged between 18 and 25 years (56 females, 43 males). Kolmogorov-Smirnov test showed that data was normally distributed. The independent sample T-test showed that all the indices were statistically significant as shown in (Table 2).

Table 1: Descriptive statistics of the Syrian sample including males and females.

Variable	N	Minimum	Maximum	Range	Mean	SD
MinRamBreadth	99	21.60	35.50	13.90	28.65	2.92
Gonial Angle	99	103.30	149.50	46.20	129.60	7.22
Co – Go	95	41.20	70.10	28.90	56.07	6.40
BiGonial width	99	76.65	108.10	31.45	90.46	6.93

MinRamBreadth: minimum ramus breadth.
Co-Go; cordonion-gonion distance

Table 2: T-test for comparison between males and females for all indices, * significant difference.

Variabel	Sex	N	Mean	SD	P-value	Mean difference	95% confidence interval of difference
MinRamBreadth	Male	43	29.63	2.90	0.003*	1.73	0.60
	Female	56	27.89	2.73			
Gonial Angle	Male	43	127.11	7.87	0.002*	-4.40	-7.19
	Female	56	131.52	6.08			
Co – Go	Male	43	58.61	6.78	<0.001*	4.63	2.18
	Female	52	53.97	5.26			
BiGonial width	Male	43	95.17	6.45	<0.001*	8.32	6.08
	Female	56	86.84	4.81			

MinRamBreadth: minimum ramus breadth.
Co-Go: cordonion-gonion distance.
* significant difference

The most significant index between males and females was the bigonial distance (mean; 95.17 mm for males, 86.84 mm for females), followed by Gonial angle and Co-Go distance. Since all variables showed significant differences between males and females, all data subjected to

discriminant functional analysis. the following function was derived:

$$D = -8.535 + 0.042 \text{ Mr-Br} - 0.053 \text{ gonial Angle} - 0.01 \text{ Co-Go} + 0.162 \text{ bigonial width}$$

If D score was less than zero, then this indicates female, and if it was above zero, this indicates male, with males and females centroids,

are 0.864 and -0.715 respectively. On the other hand, specificity and sensitivity of the last function were 79% and 80% respectively and the diagnostic accuracy was 79.6%.

DISCUSSION

Sex identification methodology of skeletal remains involves many ways which can differ between males and females. Many methods are based on the form description of the studied bones, which often influenced by subjective opinions and this decreases its accuracy, also reliability that varies among examiners.⁸ Therefore, the metric measurements of the skeletal features are considered to be more reliable for identification processes.

Several factors must be taken into consideration before determining the significance of a feature to be accepted as an index for sex identification. The morphological structure of this characteristic should reflect a sex-related form, and also must be able to resist decomposition for a long period.⁹

Al-Shamout *et al.*¹⁰ reported that many mandibular features change over time. The gonial angle of mandibular bone was one of the most affected features because of changes in the masticatory forces due to lack of effectiveness of mastoid muscles with aging, which directly affect the muscular activity causing remodeling of the lower jawbone. In contrast, De Oliveira *et al.*¹¹ found that mandibular radiographic cephalometric indices were unable to predict sex under the age of 16 years. Therefore mandible (Lower jaw bone) was adopted in our study, and young ages were chosen for the sample, because this is useful in the case of unidentified corpses resulting from the armed conflict, especially in Syria, and because the bones of elderly people may change its morphology due to Muscular function or osteoporosis.¹⁰

The results of this study were similar to De Oliveira Gamba *et al.*¹² results, Which was conducted on 160 CBCT images of Brazilian society ages between 18 to 60 years. Our results were also agreed with those of Kharoshah *et al.*¹³ study which performed on Egyptian society, and

their results showed that the possibility of determining the sex accurately reached 83.9 % compared to 79.6 % in the current study. The accuracy of mandible index in Syrians resulted in this study is about 79.6% which is close to 81.5% that resulted in Steyn & Iscan study¹⁴ which considered a standard norm in South African Whites.⁸ Despite the results of this study are similar to many studies in the medical literature,¹⁵⁻¹⁸ it disagrees with Ayoub *et al.*¹⁹ that conducted on the Lebanese society, which is ethnically similar to Syrian society. Ayoub *et al.* showed that Gonial angle did not have significant statistical differences between males and females. This difference might be due to using lateral cephalometric radiographs in the Lebanese study, that do not show an obvious difference between left and right sides and may cause a confusing when determining anterior and posterior borders of the ramus and the body of mandible compared with CBCT images which used in this study. The bigonial distance showed the highest difference between males and females, with an average distance in males was 95.2 mm and in females was 86.8 mm. In contrast, this index showed higher accuracy in Indian society with an average distance of 103.5 mm for males and 78 mm for females,¹⁸ and it reached 101.17 mm in males and 93.97 mm in females in Greek society.²⁰ These differences might be a result of ethnic differences between communities.

The small sample size in our study is considered to be as a limitation, therefore, an extended study must be carried out in future with different age groups and greater numbers of individuals.

CONCLUSIONS

The present study suggested that the sex of mandible in the Syrian population might be assessed by using metrical parameters as an additional tool to establish the identity of a person. However, the results of this study should be confirmed in a different sample of Syrian society.

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We want to declare that Damascus University has founded the entire project.

CONFLICTS OF INTEREST STATEMENT

None.

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SHEAR BOND STRENGTH OF ORTHODONTIC BRACKETS TO ZIRCONIUM OXIDE INFRASTRUCTURE TREATED WITH ER:YAG, ND:YAG, AND KTP LASERS: AN EXPERIMENTAL STUDY

ABSTRACT

Objective: The aim of the present study was to investigate the shear bond strength of orthodontic brackets to zirconium oxide infrastructures treated with erbium-doped yttrium aluminum garnet (Er:YAG), neodymium-doped yttrium aluminum garnet (Nd:YAG) and potassium titanyl phosphate (KTP) laser modalities in in vitro settings.

Materials and Methods: A total of 40 zirconium oxide infrastructures were prepared with CAD/CAM technology in accordance with ISO 11405 standard. The specimens were divided into 4 groups as following: Er:YAG, Nd:YAG, KTP, and control groups (n=10). Prior to the application of cementation of orthodontic brackets, the surfaces of the zirconium oxide infrastructures were irradiated using selected laser modalities. Shear bond strength tests were performed on each specimen by using a universal testing machine.

Results: The shear bond strength value of Er:YAG laser group was significantly higher than those of all other groups ($p < 0.05$); although the bonding strength of Nd:YAG laser was higher than that of the KTP laser, this difference was not reached statistical significance ($p > 0.05$). The bonding strength values of Nd:YAG laser group were significantly higher than that of the control group ($p < 0.05$); and the bonding strength values of KTP laser group were significantly higher than that of control group ($p < 0.05$).

Conclusions: The bond strength between the orthodontic brackets and zirconium oxide infrastructures was improved upon using all the laser modalities in the present study, among which, application of the Er:YAG laser was the most successful.

Keywords: Er:YAG lasers, Nd:YAG lasers, KTP lasers, zirconium oxide, orthodontic brackets, shear strength.

 *Melih Ulgey¹
 Zeynep Coban Buyukbayraktar²
 Oguzhan Gorler¹
 Ihsan Hubbezoglu³

ORCID IDs of the authors

M.U. 0000-0001-5859-7071

Z.C.B. 0000-0002-4511-5480

O.G. 0000-0001-6545-8811

I.H. 0000-0001-8984-9286

¹ Departments of Prosthodontics, Faculty of Dentistry, Sivas Cumhuriyet University, Sivas, Turkey.

² Departments of Orthodontics, Faculty of Dentistry, Sivas Cumhuriyet University, Sivas, Turkey.

³ Departments of Restorative Dentistry, Faculty of Dentistry, Sivas Cumhuriyet University, Sivas, Turkey.

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INTRODUCTION

Zirconium oxide ceramics have many benefits like biocompatibility, aesthetics, low cost, good fracture resistance, and accurate fabrication with CAD/CAM systems¹; therefore, they are widely employed in posterior-localized teeth of adult patients. Orthodontic treatments have significantly increased in adult patients recently², which indicates how important bonding strength between orthodontic brackets and zirconium oxide ceramic crowns is.

Better adhesion properties are obtained by using surface treatment methods including airborne-particle abrasion³, acid etching⁴, tribochemical treatment⁵, silanization^{3,5} and laser treatment.^{6,7} In the recent times, the number of studies investigating surface modifications with laser processing has significantly increased since highly automated workstations have been developed and lasers have affordable prices.⁸ Crucial advantages of this process include the highly localized, clean nature of the process, low-distortion and high quality of finish.⁹

Attachment of brackets to zirconium oxide surface is required to be performed against orthodontic forces and exhibit a sufficient strength to avoid bonding failures.¹⁰ Specialist orthodontic practitioners enhance bonding strength to both zirconium oxide and enamel surface through hydrofluoric acid prior to cementation of brackets.¹¹ But, the most common problem encountered in this process is the formation of micro-retention on smooth zirconium oxide surface using hydrofluoric acid.¹² However, the use of hydrofluoric acid prior to cementation may harm oral soft tissues and zirconium oxide restorations.¹³ Therefore, application of laser surface treatment on ceramic surface can be a safe alternative method to increase the attachment of brackets to the ceramic surfaces.¹¹

Although many studies were done to investigate the effect of surface treatment processes on ceramic surfaces on bonding strength, there are no studies examining the effects of Er:YAG, Nd:YAG and KTP lasers applied to zirconium oxide surface on bonding strength. The aim of the present study was to investigate the shear bond strength (SBS) of

orthodontic brackets to zirconium oxide infrastructures treated with erbium-doped yttrium aluminum garnet (Er:YAG), neodymium-doped yttrium aluminum garnet (Nd:YAG) and potassium titanyl phosphate (KTP) laser modalities in in vitro settings. The null hypothesis was that the laser treatments would not alter the shear bond strength of the orthodontic bracket attached to the zirconium oxide infrastructures after surface modifications with Er:YAG, Nd:YAG, and KTP lasers.

MATERIALS AND METHODS

Specimen preparation

The study was approved by the Human Research Ethics Committee of Sivas Cumhuriyet University (No: 2020-02/32). The infrastructures were designed with the help of CAD Software (DWOS, Dental Wings, Canada) in accordance with ISO 11405 standards with a 7 mm-diameter and a 3-mm height. The designed samples were produced by milling pre-sintered zirconium oxide blocks (ST, Uccera, China) by using CAD/CAM milling device (Yenadent, Ankara, Turkey). Therefore, the disc-shaped specimens were removed from zirconium oxide blocks and sprue connections were eliminated. The sintering process of zirconium-oxide specimens were carried out in a high-temperature furnace (Protherm; B&D Dental Origin Milling, USA) in accordance to the manufacturer's instructions.

The specimens were randomly divided into 4 groups according to the surface treatment process to be applied (n=10). The specifications used during laser surface treatment procedures were as follows:

- Control group: No treatment.
- Er:YAG Laser Group: The Er:YAG laser ($\lambda=2.940$ nm) (Smart 2940D Plus; Deka Laser, Florence, Italy) was applied to zirconium oxide infrastructure with non-contact mode for 30 s using very short pulse mode. The laser settings were 3 W, 100 mJ, and 30 Hz (pulse/s).
- Nd:YAG Laser Group: The Nd:YAG laser ($\lambda=1.064$ nm) (Smarty A10, DEKA M.E.L.A. SRL, Italy) was applied to zirconium oxide infrastructure with contact mode for 30 s using short pulse mode. The laser settings were 3 W, 100 mJ, and 30 Hz (pulse/s).

- **KTP Laser Group:** The KTP laser ($\lambda=532$ nm) (Smartlite D, DEKA M.E.L.A. SRL, Italy) was applied to zirconium oxide infrastructure with contact mode for 30 s using pulsed mode. The laser settings were 3 W, 100 mJ, and 30 Hz (pulse/s).

After surface treatment processes, self-etch adhesive system (Transbond XT, 3M Unitek, USA) was applied to the base of orthodontic metal brackets (22-inch slot MBT prescription; American Orthodontics, USA) having the dimensions of 3x4 mm and brackets were bonded at the center of each treated zirconium oxide specimen. Overflowing cements were cleaned and polymerized with a light-curing device (Smartlite, Dentsply, USA) in each direction for a total of 40 s including 10 s for each direction. Before shear bond strength test, the specimens were kept at distilled water at 37 ± 1 °C for 24 h.

After taken the specimens out of the distilled water, all specimens were fixed in the perpendicular position with the help of acrylic resin (Meliodent, Heraeus Kulzer, Germany) to cylindrical metal molds with a length of 2.5 cm and a diameter of 1.5 cm. The specimens were placed to a universal test machine (Lloyd LF Plus, Ametek Inc, UK) for shear bond strength test. The load was vertically applied through a 1-mm thick straight knife-edged blade for blunt cutting process in accordance with the ISO TR 11405 specification. The test was performed at 0.5 mm/min crosshead speed under laboratory conditions (Figure 2). The amount of load per unit area was calculated by recording loads at failure in newton (N) and converting them into Megapascal (MPa) values.



Figure 2. A representative image of control group.

Statistical Analysis

The data presented as the mean – standard deviation (SD) were assessed by using the analysis of variance followed by the post hoc Tukey test for pairwise comparisons after Kolmogorov–Smirnov normality test was performed. The data are shown with whisker plots including mean and SD lines and scatter dots presenting raw data. The value of 0.05 was accepted as statistical significance.

RESULTS

Figure 1 shows the shear bond strength values of zirconium oxide infrastructures and orthodontic brackets subjected to surface treatment procedures (Er:YAG, Nd:YAG, and KTP laser applications).

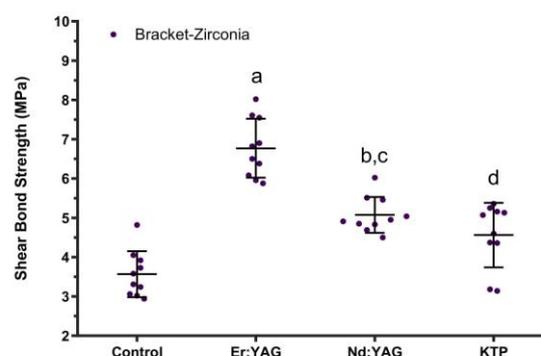


Figure 1. Values of shear bond strength between orthodontic brackets and zirconium oxide infrastructures treated with Er:YAG, Nd:YAG, and KTP lasers. Data were expressed as mean (midline) and SD (whiskers). ^aP<0.05, Er:YAG group vs. Nd:YAG, KTP, and control groups. ^bP>0.05, Nd:YAG group vs. KTP group. ^cP<0.05, Nd:YAG group vs. control group. ^dP<0.05, KTP group vs. control group.

ANOVA and t tests indicated that overall, all laser applications increased the bond strength of orthodontic brackets to zirconium oxide infrastructures. The SBS value was significantly higher in Er:YAG laser group compared to all other groups ($p<0.05$). The bonding strength was higher in Nd:YAG laser compared to the KTP laser; however, this difference was not statistically significant ($p>0.05$). The SBS values were significantly higher in Nd:YAG laser group compared to control group ($p<0.05$); whereas, KTP laser group had significantly higher SBS values than control group ($p<0.05$) (Table 1).

Table 1. Shear bond strength values (MPa) among all groups

Groups	Mean	Standard deviation	Minimum	Maximum
Control ^a	3.63	0.55	2.90	4.82
Er:YAG Laser ^b	6.77	0.74	5.88	8.02
Nd:YAG Laser ^c	5.07	0.45	4.50	6.02
KTP Laser ^c	4.56	0.82	3.14	5.36

*Different lower case letter represents statistical significant among groups, verified by one-way ANOVA and Tukey's test ($p < 0.05$).

DISCUSSION

In this study, the null hypothesis, stating the laser treatments would not alter the shear bond strength of the orthodontic bracket attached to the zirconium oxide infrastructures after surface modifications with Er:YAG, Nd:YAG, and KTP lasers was rejected. Overall, these results indicated that while Er:YAG laser had the highest effectiveness in the zirconium oxide infrastructures than the other modalities, KTP laser had the least effectiveness in all the infrastructures.

Although the minimum bonding strength value is 6-8 MPa for orthodontic brackets to stand against orthodontic forces¹⁴, some authors have stated that a bond strength value of 2.86 MPa is clinically acceptable.^{15,16} Since ceramics have inert surfaces, surface treatment applications can be useful to ensure attachment of brackets on ceramic surfaces.¹³ Laser surface treatment applications can be an alternative method to hydrofluoric acid etching application used routinely in clinics due to their advantages such as not causing any pain or sensitivity, being applied in a short time and eliminating problems related to acid applications.¹⁷

Er:YAG laser is a laser at 2940 nm wavelength forming craters in anfractuous form leading microexplosions on the surface of dentin.¹⁸ The use of Er:YAG laser to increase adhesion in dental materials may be an useful application. Hou *et al.*, examined different Er:YAG laser power settings in terms of the shear bond strength of different CAD/CAM ceramics. They determined that Er:YAG laser application provided a statistically significant increase in all groups when compared to the control group. The bond strength of IPS Empress CAD and IPS e.max CAD could be increased using certain power settings.¹⁹ Sabuncuoglu *et al.*, made a

comparison concerning the effects of different porcelain surface treatment methods on the shear bond strength and fracture mode of orthodontic brackets. They stated that laser etching with Nd:YAG or Er:YAG laser application was more effective and less time-consuming than both hydrofluoric acid and sandblasting for the treatment of deglazed feldspathic porcelain.²⁰ Yassaei *et al.*, assessed the shear bond strength of orthodontic brackets bonded to ceramic after etching with Er:YAG laser than 9.6% hydrofluoric acid (HF). They determined no significant difference between Er:YAG laser and acid etching applications. The authors also suggest that Er:YAG laser is a proper method for bonding of orthodontic brackets to porcelain surfaces.¹³ Xu *et al.*, analyzed how Er:YAG laser conditioning bond strength of orthodontic brackets affected porcelain surfaces. They stated that porcelain surfaces etched by 250 mJ, 20 Hz of Er:YAG laser through hydrofluoric acid could have a sufficient bond strength and lower porcelain fracture rate for orthodontic bracket bonding.²¹ Similarly, in the present study, Er:YAG laser application increased the bonding strength values.

Poosti *et al.*, examined shear bond strength of orthodontic brackets to porcelain surface after conditioning by Er:YAG and Nd:YAG laser by comparing with traditional methods. They determined that both Er:YAG at 2 W and 3 W and surface roughening alone showed significantly lower bond strengths than the Nd:YAG laser or 9.6% hydrofluoric acid-etching treatment ($p < 0.05$).²² These results are different from results of the present study. This difference may be due to the difference in the infrastructure where the brackets are adhered.

The use of Nd:YAG laser in dental field, treatment of tooth hypersensitivity, cavity cleaning, tooth whitening and disinfection of dental tissues is preferred.²³ Nd:YAG laser is used in many studies for the purpose of increasing bonding strength.^{24,25} Cevik *et al.*, assessed the shear bond strength of orthodontic brackets bonded to different kinds of ceramic surfaces after different surface conditioning methods. They determined that Nd:YAG laser treatment increased bonding strength values between porcelain systems to orthodontic brackets.²⁶ In their another study, Cevik *et al.*, assessed the effect of six different surface conditioning methods on the shear bond strength of ceramic brackets bonded to feldspathic porcelain. They determined that the Nd:YAG laser application on feldspathic porcelain surface statistically significantly increased compared to control group.²⁷ Akyil *et al.*, investigated the shear bond strength of a resin cement to zirconium oxide surfaces subjected to air abrasion, silica coating, CO₂, Er:YAG, or Nd:YAG laser irradiation, or irradiated by each laser after air abrasion. They determined that Nd:YAG laser irradiation after air abrasion is an alternative treatment method used to improve the bond strength between resin cement and Y-TZP material.²⁸ The results of the present study are compatible with the results of these studies.

For photochemical bleaching, absorption of chelate compounds is important and the best absorption is provided by argon laser (515 nm) and KTP laser (532 nm) due to their ideal wavelengths.²⁹ In addition, KTP laser is used in dental field; desensitization of cervical dentine, laser-enhanced fluoride uptake, periodontal pocket disinfection, root canal disinfection and minor soft tissue surgery processes.³⁰ In the dental literature, data about the investigating the effect of KTP laser on bond strength is limited. Kustarci *et al.* assessed the impacts of antimicrobial pretreatments [chlorhexidine gluconate (CHX), Clearfil Protect Bond (CPB), and potassium-titanyl-phosphate (KTP) laser] on microleakage under metal orthodontic brackets. They observed the lowest microleakage scores in the control group. CPB, KTP, and CHX groups did not show

significant differences with the control group ($p > 0.05$).³¹ In a study conducted in our laboratory, the shear bond strength (SBS) of ceromer and nanohybrid composite to direct laser sintered (DLS) Cr-Co and Ni-Cr-based metal infrastructures subjected to Er:YAG, Nd:YAG, and KTP laser applications was investigated. The results of that study indicated that Er:YAG, Nd:YAG, and KTP laser methods were effective in increasing bonding of these structures based on order of success, thus supported the bonding of ceromer and nanohybrid composite superstructures to the DLS and Ni-Cr based infrastructures.³² The experimental work presented here provides one of the first investigations evaluating the effect of KTP laser on bonding strength of orthodontic brackets to ceramic infrastructures.

This study has some limitations in terms of the laser settings used. In this study, during the application of surface treatment to infrastructures, all laser parameters were set as 3W. We believe that this will be useful in the comparison of the effectiveness of lasers. In further studies, using different laser powers and measuring the bonding strengths of different ceramic systems will be useful in terms of comparing the attachments of orthodontic brackets to different infrastructures.

The main idea of the current study was to include different laser applications including Nd:YAG, Er:YAG, and KTP lasers, and investigation if the bond strength between zirconium oxide infrastructures and orthodontic brackets would be affected by different specifications of these laser modalities. These experiments suggested that laser treatments applied on zirconium oxide surface, especially the Er:YAG laser, can modify and enhance the bonding between zirconium oxide surface and orthodontic brackets.

CONCLUSIONS

Er:YAG, Nd:YAG, and KTP lasers may be considered as effective methods to increase bonding strength of orthodontic brackets to zirconium oxide infrastructures. Er:YAG laser has the highest effectiveness in the bonding strength of zirconium oxide infrastructures to orthodontic

brackets. In present experimental setting; Er:YAG, Nd:YAG, and KTP lasers were more effective for improving the retention of orthodontic brackets to zirconium oxide infrastructures based on order of effectiveness.

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CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no competing interests.

Ortodontik Braketlerin Er:YAG, Nd:YAG ve KTP Lazer ile Pürüzlendirilen Zirkonyum Oksit Alt Yapılara Olan Makaslama Bağlantı Dayanımı: Bir Deneysel Çalışma

ÖZ

Amaç: Bu çalışmanın amacı, Er:YAG, Nd:YAG ve KTP lazer ile pürüzlendirilmiş zirkonya altyapıların ortodontik braketlere olan bağlantı dayanımını *in vitro* olarak araştırmaktır. **Gereç ve Yöntemler:** Bu çalışmada, CAD/CAM teknolojisi kullanılarak ISO 11405 standartlarına uygun olarak üretilmiş 40 adet zirkonya örnek kullanıldı. Örnekler 4 gruba ayrıldı: Er:YAG, Nd:YAG, KTP ve kontrol grup. Ortodontik braketlerin simantasyonu öncesi, zirkonya alt yapıların yüzeyleri seçilen düzenleme yöntemlerine göre pürüzlendirildi. Makaslama bağlantı dayanımı testi, her örnek için universal test cihazında uygulandı.

Bulgular: Er:YAG lazer grubunun makaslama bağlantı dayanımı, diğer tüm gruplara göre istatistiksel olarak anlamlı derecede yüksekti ($p < 0,05$); Nd:YAG lazer grubunun bağlantı dayanımı değerleri KTP lazere göre yüksek olmasına rağmen, bu farklılık istatistiksel anlamlı değildi ($p > 0,05$). Nd:YAG lazer grubunun bağlantı dayanımı değerleri, kontrol grubunun bağlantı dayanımına göre anlamlı derecede yüksekti ($p < 0,05$). KTP lazer grubunun bağlantı dayanımı değerleri, kontrol grubuna göre anlamlı derecede yüksekti ($p < 0,05$). **Sonuçlar:** Bu çalışmada ortodontik braketler ile zirkonya alt yapılar arasındaki bağlantı dayanımını tüm lazer uygulamaları arttırmıştır. Bunlar arasında Er:YAG lazer en başarılı uygulamadır. **Anahtar Kelimeler:** Er:YAG lazerleri, Nd:YAG lazerleri, KTP lazeri, zirkonyum oksit, ortodontik braketler, kayma mukavemeti.

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MULTIPLE DENTIGEROUS CYSTS IN A CHILD: A CASE REPORT AND RADIOGRAPHIC FOLLOW-UP

ABSTRACT

The aim of this study is to describe a case of dentigerous cysts of a young patient successfully treated as well as clinical and radiographic follow-up. A 10-year-old female patient was referred to the Buccomaxillofacial Surgery and Traumatology Service of the Regional University Hospital of Campos Gerais. The patient's panoramic radiograph revealed a well-defined radiolucent unilocular area associated to the crown of the second permanent right lower molar, which was impacted. In addition, the permanent upper canines were enclosed, suggesting a dentigerous cyst. A conservative treatment was determined, through marsupialization of the lesions. The histopathology examination confirmed the clinical diagnosis of dentigerous cysts. Longitudinal clinical and radiographic monitoring was essential to certify the regression of the lesion, as well as bone neoformation, thus promoting the eruption and maintenance of the affected permanent teeth.

Keywords: Dentigerous cyst, panoramic radiography, diagnosis.

-  Bruna Carolyne Siefert de Oliveira¹
-  Amanda Regina Fischborn²
-  *Caique Mariano Pedroso¹
-  Jéssica Daniela Andreis²
-  Camila Maggi Maia Silveira³
-  Luiz Felipe Manosso Guzzoni¹
-  Marcelo Carlos Boroluzzi⁴
-  Gilson Cesar Nobre Franco⁴

ORCID IDs of the authors:

B.C.S.O. 0000-0001-6247-2941
 A.R.F. 0000-0002-7470-484X
 C.M.P. 0000-0002-7504-7597
 J.D.A. 0000-0002-4435-4850
 C.M.M.S. 0000-0001-6434-5532
 L.F.M.G. 0000-0002-7032-4516
 M.C.B. 0000-0003-2756-5047
 G.C.N.F. 0000-0001-7082-7837

¹ State University of Ponta Grossa, Ponta Grossa - PR, Brazil.

² MSc in Integrated Clinical (Etiology and Diagnosis of Oral Diseases), State University of Ponta Grossa, Ponta Grossa - PR, Brazil.

³ Integrated Clinical, Department of Dentistry, State University of Ponta Grossa, Ponta Grossa -PR, Brazil.

⁴ Diagnosis and surgery, Department of Dentistry, State University of Ponta Grossa, Ponta Grossa -PR, Brazil.

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INTRODUCTION

Dentigerous cysts are responsible for approximately 24% of true cysts and they are the most frequent after root cysts.^{1,2,3} The occurrence of these cysts is higher in men at the posterior region of the mandible, followed by the maxilla front region. The teeth usually involved are the lower third molar, upper canine, third upper molar, lower pre-molar, supernumerary tooth or ectopic tooth, respectively.^{1,4,5} These are usually asymptomatic lesions, randomly investigated, when a permanent tooth eruption is delayed, or as an imagiological finding during radiographic examination. It appears as a radiolucent unilocular area, with a well-defined corticated margin, around the crown of an impacted tooth.^{4,6,7} Histologically, these lesions present a thin stratified epithelium, which is sometimes bilaminar along with keratinizing metaplasia.^{6,8} Many of the odontogenic cysts share clinical and radiographic aspects, thus, the diagnosis must be based on both radiographic and histopathological findings.^{4,6,7} Clinical management consists of enucleation or marsupialization, the latter being a more conservative approach. This method consists in joining the cyst cover to the oral mucosa, which allows the spontaneous eruption of the dental element when there is enough space.^{5,9,10,11}

Success in the treatment of dentigerous cyst depends on the correct choice of treatment, as well as on the performance of clinical and radiographic monitoring, thus preventing recurrence. In this context, the aim of this study is to describe a case of a dentigerous cyst in a young patient, taking into account the success of the treatment and the importance of long-term radiographic follow-up.

CASE REPORT

A 10-year-old female patient was referred by her orthodontist to the service of Buccomaxillofacial Surgery and Traumatology Service of the Regional University Hospital of Campos Gerais. The radiographic image revealed a well-defined unilocular radiolucency associated to the crown of the permanent lower right second molar impacted and displaced to the lower border of the mandible, as well as the presence of a radiolucent area associated

with the upper canine teeth, suggesting a dentigerous cyst. (Figure 1).



Figure 1. Panoramic radiograph showing cystic lesions related to the crown of the permanent lower right second molar and upper canines.

The clinical examination did not reveal the presence of local infection, cortical bone expansion or facial asymmetry, and the patient did not report any associated symptoms. After obtaining the free and informed consent form from her guardian, the patient was submitted to complete blood count examination, prothrombin activity time (PAT), activated partial thromboplastin time (aPTT), creatinine, urea and fasting blood glucose tests. In addition, a computerized tomography was requested to assess complementary features of the lesion, like its size and effect on adjacent structures to the lesion. The blood test results were normal, and the Computerized Tomography evidenced imaging characteristics compatible with the provisional diagnosis. (Figure 2. A-D).





Figure 2. A-B. Computerized tomography of the face with axial cut evidencing a well-defined image of the permanent lower right second molar affected.



Figure 2. C-D. Axial cuts demonstrating the presence of a well-defined image of the permanent upper canines and lower right molar affected, respectively.

A conservative treatment was determined, through marsupialization of the lesions. The surgery was carried out at the hospital under general anaesthesia. During the surgery, an elliptical incision posterior to the first lower right molar was performed for the marsupialization of the cystic lesion in the adjacent tooth. The deciduous upper canines were extracted for the marsupialization of the cystic lesions of the permanent upper canines. A small sample of the material was collected and sent for

histopathological analysis. After the surgery, an antibiotic therapy was prescribed consisting of amoxicillin oral suspension (250 mg/ 8 hours) for 7 days and analgesia using dipyrone oral solution for 5 days. The patient was instructed regarding her oral hygiene, and rinsing with a 0.12% chlorhexidine solution.

The histopathological specimen showed features which were suggestive of dentigerous cysts (Figure 3).

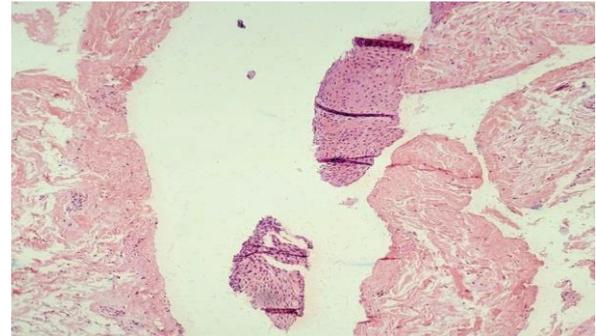


Figure 3. Histological exam confirming the diagnosed hypothesis of a typical dentigerous cyst, covered with a non-keratinized stratified epithelium. Dysplastic alterations were not observed. (Coloration H&E, 40x).

The patient returned periodically for follow-up examinations. Suitable maintenance of the surgical site was observed and no complications were found. In the third week after the surgery, the sutures were removed. After 10 weeks, it was possible to observe clinically the cusp of the lower right second molar in an active eruption process. Within 26 weeks of surgical follow-up, the radiographic examination showed bone neoformation in the regions previously occupied by the cystic lesions and a proper tooth eruption process. In addition, the presence of a radiolucent unilocular area in the region of the right mandibular ramus was observed, however of a smaller diameter, with not well-defined borders, which might have resulted from the cystic decompression. (Figure 4. A-B).





Figure 4. A-B. Radiographic follow-up after 10 weeks (A) and 26 weeks (B) post-operation, bone neo-formation was observed and a suitable eruption process of the teeth involved along with a radiolucent area on the right mandibular ramus.

After a one-year follow-up period, with radiographic evaluation, the lower right second molar was observed to be in eruption and infra-occlusion process. The radiolucent area in the mandibular ramus did not show any evidence of evolution (Figure 4. C).



Figure 4. AC. After a year of radiographic follow-up, the lower right second molar showed infra-occlusion and a persistent radiolucent area on the mandibular ramus, without evolution.

The patient and her guardian were instructed about the importance of the radiographic analysis every six months for a suitable follow-up.

DISCUSSION

This report demonstrates a dentigerous cyst in a young patient with classic radiographic features. This odontogenic cyst is considered a common jaw lesion, and is usually found in routine radiographs at the end of the second and third decades of life.^{5,9,12} In this case report, the lesions showed an unusual presentation since they had an early diagnosis. According to previous studies by Ferreres *et al.*¹³, Gendeviline *et al.*⁸ and Langaroodi *et al.*¹⁴, the main teeth involved are third molars, in patients ranging in age from 15 to 34 years old. Contrasting with findings described in the literature, in the present clinical case, the involvement of lower right second molar and the maxillary canines was observed in a patient in the first decade of life.

The dentigerous cyst radiographic image revealed a well-defined unilocular radiolucy, which must be differentiated from other lesions that might present the same radiographic aspect such as odontogenic keratocyst, ameloblastoma and ameloblastic fibroma.^{12,15,16} Although the radiographic examination provides important information, histopathological examination is fundamental for a definite confirmatory diagnosis.¹⁶ The histopathological diagnosis criteria for dentigerous cysts include the presence of a cystic cavity with non-keratinized epithelium and a fibrous conjunctive tissue wall,¹³ which was observed in this case through the histopathological analysis of the sample obtained in during the surgical procedure.

Dentigerous cyst treatment options include the complete enucleation or marsupialisation, the latter being a more conservative surgical alternative.^{5,9} Cases of treatment with marsupialization emphasize the relevance of choosing a less traumatic therapeutic method and preventing young patients from being exposed to unnecessary surgical procedures. In this context, this type of treatment is based on the fact that marsupialization tends to decrease the intraosseous pressure, in which the impacted tooth is able to perform its own eruption without the need for more aggressive interventions.¹⁷ Some authors use enucleation as the first choice of treatment in cases of small size cysts, as well as marsupialization previously performed followed by enucleation.⁸ However, the literature is not univocal regarding the best approach. In this sense, consideration should be given to the size and location, patient's age, cyst closeness to vital structures, tooth position in relation to the cyst and the degree of inclination and root formation.^{15,17} Thus, the marsupialization was the treatment of choice in this case, since it is more conservative, allowing the maintenance of the permanent teeth involved.¹⁰ Although this surgical method proves to be effective, there is a controversy, in which cell debris left in situ can proliferate and consequently turn into benign or malignant odontogenic tumors.¹⁷ In our case, there

was no recurrence of the lesion and the proposed treatment proved to be effective.

The use of general anaesthesia (GA) was the option chosen for the surgical treatment, since it is an efficient approach enabling the whole procedure to be carried out in a single session.^{18,19} Treatment under GA is indicated in children's treatment for several reasons such as to reduce long surgical procedures, and with extremely non-cooperative patients, who might be anxious or non-cooperative, among others.^{18,19} The treatment can be carried out at the hospital, outpatient surgery center or at the dentist's office and presents low incidence of adverse reactions when carried out safely and efficiently by qualified professionals following the pre-established protocols and guidelines.²⁰ In this case, the use of GA to treat patients with dentigerous cyst proved to be an effective alternative, without complications, as also occurred in the clinical case reported by Bozkurt *et al.*²¹

Ghandour *et al.*¹² reported the use of marsupialization in a 13-year-old female patient, in which the panoramic radiograph revealed the absence of any radiolucent lesion and well-succeeded eruption of the tooth involved. Arjona-Amo *et al.*⁹ carried out marsupialization on a young patient for the treatment of dentigerous cysts that impacted the lower first molars and after three months both teeth erupted. Therefore, marsupialization as a conservative method, can be the first option for the treatment of dentigerous cysts in young patients, thus preserving and favoring the spontaneous eruption of developing permanent teeth.

CONCLUSIONS

Dentigerous cyst management is determined by several factors, such as patient's age, cyst size, location, closeness to vital structures, among others. Marsupialization can be the first option to treat dentigerous cysts in young patients, since it is a more conservative approach that allows the eruption and maintenance of the permanent teeth involved. The importance of choosing the standardized clinical operating protocols for treatment success is highlighted. Likewise, the

longitudinal radiographic follow-up is considered extremely important to the success of the treatment.

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CONFLICTS OF INTEREST STATEMENT

No conflicts of interest to disclose

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APPLICATION OF 3-D IMAGING IN A FAMILIAL CASE OF CLEIDOCRANIAL DYSPLASIA

ABSTRACT

Cleidocranial dysplasia (CCD) is a rare inherited disorder affecting dental and skeletal tissues. CCD usually has an autosomal dominant pattern of inheritance and common clinical features seen are aplastic or hypoplastic clavicles, late closure of fontanelle, open skull sutures, retained deciduous teeth, late eruption of permanent teeth and presence of multiple impacted supernumerary teeth. Here, we present a case of CCD in a female patient with positive family history. The diagnosis was confirmed clinically and radiographically. The newer radiographic advancement, CBCT was used to validate the radiographic findings.

Keywords: Cleidocranial dysplasia, impacted teeth, supernumerary teeth, CBCT, CBFA1 gene.

-  Anwasha Biswas¹
-  *G Subhas Babu²
-  Kumuda Rao¹
-  Soundarya Sakthivel¹
-  Ananya Madiyal¹
-  Renita Castelino¹

ORCID IDs of the authors:

- A.B. 0000-0001-6716-6409
- G.S.B. 0000-0001-9383-7886
- K.R. 0000-0002-6214-1381
- S.S. 0000-0002-3213-9395
- A.M. 0000-0002-0907-3244
- R.C. 0000-0002-8696-549X

¹ Department of Oral Medicine and Radiology, A B Shetty Memorial Institute of Dental Sciences, NITTE deemed to be University, Deralakatte, Mangalore.

² Department of Oral Medicine and Radiology, NITTE deemed to be University, Deralakatte, Mangalore.

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INTRODUCTION

Cleidocranial dysplasia is an autosomal dominant bone disease affecting the skeletal and dental system.¹ Martin in 1765 reported the first case of cleidocranial dysostosis.² CCD also called 'Scheuthauer Marie-Sainton syndrome', 'Marie-Sainton disease' or 'Mutational dysostosis'. CCD affects the bones undergoing intra-membranous ossification, especially skull, clavicles, and flat bones. Hence it is termed as "Cleidocranial dysplasia". It was later found that CCD also affects bones undergoing endochondral ossification and hence, it was said to be a generalized disorder affecting various skeletal structures.³ The exact etiology is unknown, but the prime cause in about 20-40% cases is CBFA1 gene, on chromosome 6p21.⁴ The clinical features of CCD include hypoplasia of mid-face and clavicles giving an appearance of sloping shoulders that can be opposed at the midline; sometimes there may be aplasia of shoulders and abnormalities affecting hands like brachydactyly, short and broad thumbs, tapering fingers.⁵ The CBFA1 gene also plays major role in tooth development and differentiation of ameloblasts and odontoblasts, thus affecting morphogenesis of tooth.⁷ However, in 40% of cases with CCD, there was no genetic factor.⁶

The dental findings noticed with multiple impacted and supernumerary teeth, are retained primary teeth and delayed permanent teeth eruption resulting in malocclusion.⁸ Features of skull include brachycephalic skull due to delay in ossification of sutures of the cranium and fontanelles; and frontal bossing. Parietal and occipital bones are also affected. Face may appear smaller compared to the cranium. Zygomatic bones and Maxilla are hypoplastic. Paranasal sinuses are also underdeveloped.

With the early diagnosis of CCD, an effective treatment planning can be instituted. Cone-Beam Computed Tomography (CBCT) is a recent advancement in the field of maxillofacial imaging which provides a better 3-D outlook of dentition and skull morphology to the clinician, thus assisting in better treatment planning for the patient. The treatment goal is to give functioning masticatory system to patient and for improved

facial appearance. The treatment objective can be achieved with the help of prosthesis, after doing required extractions. The reposition of permanent teeth can be achieved by combination of surgical and orthodontic interventions.

CASE REPORT

A 8-year-old girl reported to our department with the chief complaint of presence of unerupted teeth in upper and lower jaw since 1 year leading to an unaesthetic facial appearance. She reported delayed eruption of permanent teeth after exfoliation of primary teeth, which led to difficulty in chewing and speech. The patient had the habit of mouth breathing since 2 years. It was also her first visit to a dentist. Medical history revealed that the patient was diagnosed with Cleidocranial dysplasia shortly after birth as per the reports submitted by the patient's parent. Patient was also epileptic and under medication (Carbamazepine 5 mg per day) for the past 4 years. On eliciting family history, patient was a single child and her parents had a nonconsanguineous marriage and father was also a known case of Cleidocranial dysplasia who also gave history of similar complaint in his childhood.

General physical examination revealed that the patient had a short stature and skull was brachiocephalic (Figure 1a).



Figure 1a: Physical examination of patient showing a brachiocephalic skull with frontal bossing

Frontal bossing was evident with a depressed bridge of nose and hypoplastic maxilla and zygomatic arches was underdeveloped resulting in a concave facial profile. On digital palpation, depression was felt over the sagittal suture of

skull and aplastic clavicles were noted. The patient could approximate her shoulders towards midline (Figure 1b).

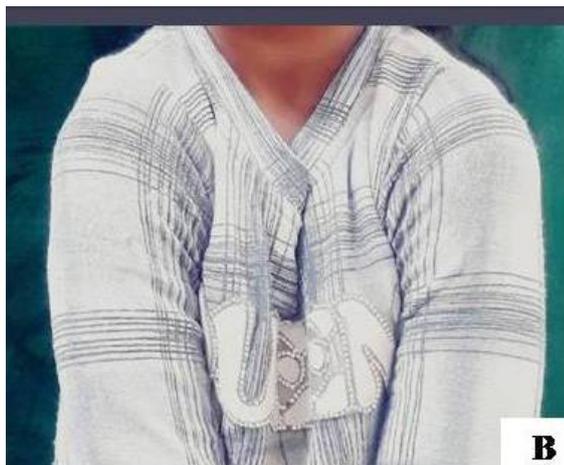


Figure 1b: Approximation of the shoulders to midline to demonstrate absence of clavicles

Extremities showed broad thumbs, brachymetatarsia, overlapping of toes and partial cutaneous fusion of 4th and 5th fingers on right hand of patient (Figure 1c, Figure 1d).



Figure 1c and 1d: Extremities showing broad thumbs and brachymetatarsia.

Intra-orally, hyperpigmentation of dorsum of tongue, high arched palate and generalized gingival inflammation were present. Besides presence of few primary teeth, permanent mandibular lateral incisors were missing. The permanent molars were erupting in both the arches (Figure 2a, Figure 2b). Based on the findings of clinical examination and case history, a provisional diagnosis of Cleidocranial dysplasia was made.

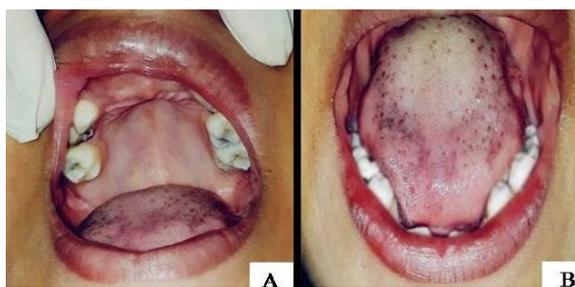


Figure 2a and 2b: Intraoral examination of patient showing hyperpigmentation of tongue, high arched palate and multiple missing teeth

A Panoramic radiograph was made which showed altered bilateral condylar morphology, multiple impacted teeth in the maxillary and mandibular arches, coronal radiolucency with respect to lower deciduous first molar, missing teeth with respect to lower right and left lateral incisor and erupting permanent first molars. Developing permanent tooth buds were seen beneath the primary teeth and supernumerary tooth buds were seen around the permanent tooth buds. (Figure 2c).



Figure 2c: Panoramic radiograph of patient showing altered condylar morphology, multiple impacted and missing teeth.

The patient was subjected to CBCT, that revealed mixed dentition with multiple impacted supernumerary teeth with respect to maxilla and mandible. Figure 3a revealed CBCT panoramic reconstruction of patient revealing multiple impacted supernumerary teeth with respect to maxilla and mandible.

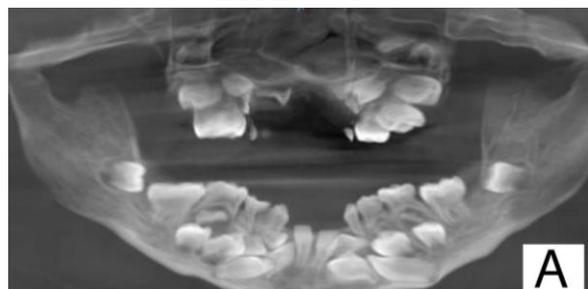


Figure 3a: CBCT panoramic reconstruction of patient revealing multiple impacted supernumerary teeth with respect to maxilla and mandible

Figure 3b and 3c revealed CBCT anterior view with 3D reconstruction.

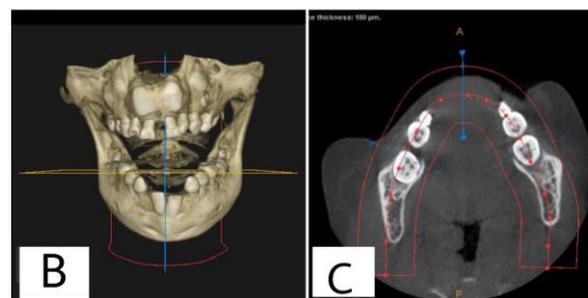


Figure 3b and 3c: CBCT anterior view 3D reconstruction of patient revealing multiple impacted supernumerary teeth with respect to maxilla and mandible.

Figure 3d, 3e, 3f and Figure 3g, 3h, 3i shows CBCT of left and right half of the patient's face with 3D reconstruction and cross sections at every 2.5 mm respectively.

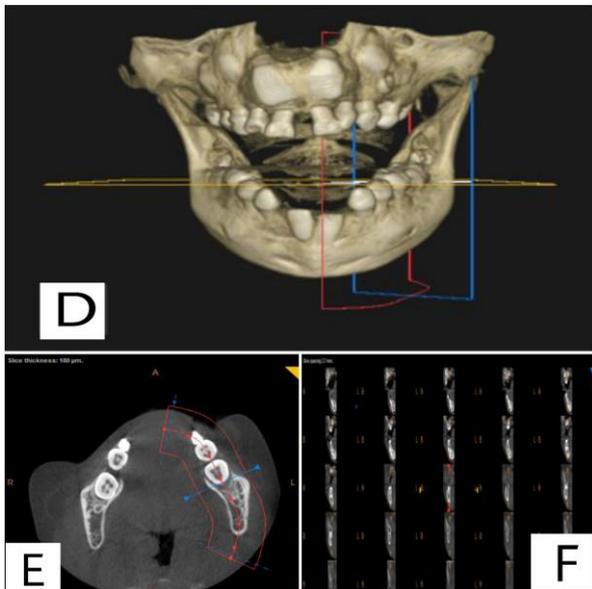


Figure 3d, 3e and 3f: CBCT of left half of the patient's face with 3D reconstruction and cross sections at every 2.5 mm

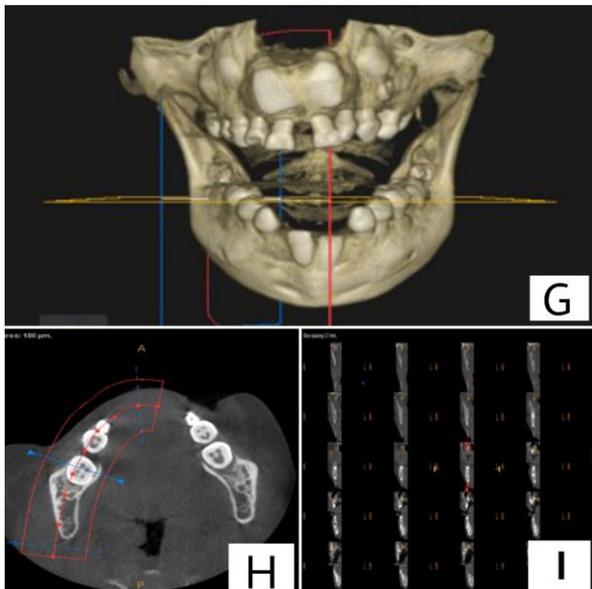


Figure 3g, 3h and 3i: CBCT of right half of the patient's face with 3D reconstruction and cross sections at every 2.5 mm

No abnormal findings were observed in Hematological investigations. Thus, the diagnosis of Cleidocranial dysplasia was arrived at, with the help of CBCT. A multi-disciplinary approach was planned for a comprehensive management of the patient.

General examination of the patient's father was also made which revealed that he could

approximate the shoulders to the midline (Figure 4a).

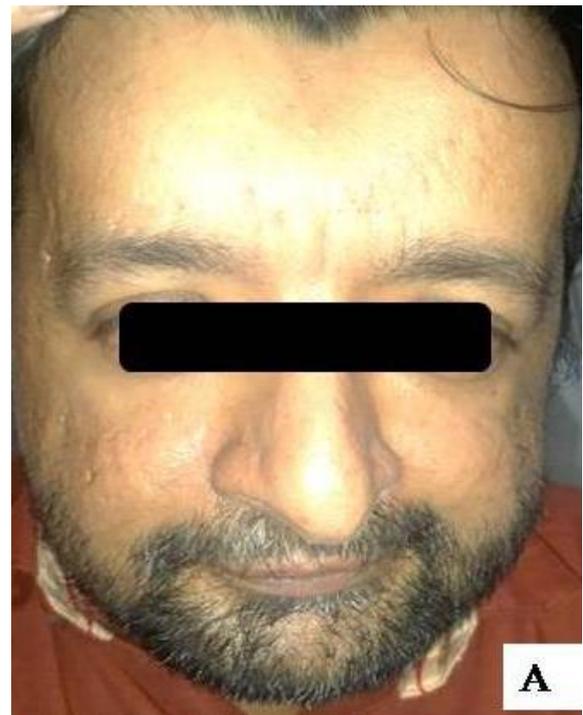


Figure 4a: Physical examination of patient's father showing brachiocephalic skull with frontal bossing

Extra oral examination revealed frontal bossing and brachycephaly (Figure 4b).



Figure 4b: Patient's father demonstrating approximation of shoulders to midline indicative of missing clavicles

On Intra oral examination multiple anterior and posterior teeth were found missing. Panoramic Radiograph of patient's father revealed altered condylar morphology, multiple impacted teeth in the maxillary and mandibular arches, root stump with respect to upper right maxillary first permanent molar, retained teeth with respect to upper right permanent canine, left first premolar,

lower left lateral incisor, left canine, right lateral incisor, canine and second premolar and multiple carious teeth with respect to lower left second premolar, second molar and right first molar (Figure 4c).



Figure 4c: Panoramic radiograph of patient's father showing multiple impacted and missing teeth.

DISCUSSION

CCD is commonly misdiagnosed clinically for various other conditions such as Noonan syndrome, Turner's syndrome, hypothyroidism and other skeletal Dysplasia. CCD has a prevalence of 1 per million live births. Its etiology is related to mutation in the gene coding for osteoblast transcription factor Runx2/Core binding factor, which is essential for membranous and endochondral ossification.⁹ The clavicles are hypoplastic, or aplastic, because they are the first bones to ossify. In about 10% of cases, complete absence of clavicles is observed with affected acromial end.¹⁰ Maxillary hypoplasia along with mastoid air cells and accessory sinus are also noted. Sometimes, conduction deafness is also noticed in cases of CCD.¹¹ The thoracic cage appears to be "bell-shaped" with short and oblique ribs.

Various syndromes and anomalies have similar features as of CCD. Differential diagnosis of CCD includes various skeletal dysplasia like Mandibuloacral dysplasia (MAD), Yunis-Varon syndrome (YVS), Crane-Heise syndrome, Pycnodystosis, Hypophosphatasia (HPP), CDAGS syndrome and osteogenesis imperfecta (OI). Patient anterior fontanelles is a significant feature of CCD as a result of defective ossification of Wormian bones in the suture. Similar features can be seen in Pycnodysostosis, Osteogenesis Imperfecta and Congenital Hypothyroidism. Yunis-Varon syndrome is a rare genetic disorder having complete absence of clavicles, similar to CCD, but other manifestations like intellectual disability, abnormalities of hands and feet.

Delayed closure of fontanelles, presence of Wormian bones in sutures. These findings may be seen in other conditions like Pycnodysostosis, Mandibuloacral dysplasia and Yunis-Varon syndrome. Pycnodysostosis is differentiated from CCD. Based on its increased bone density and dwarfism.¹³ Crane-Heise syndrome is another syndrome differentially diagnosed from CCD, by the presence of features like large sized head, mineralized deficiencies of skull, cleft lip and palate, dysmorphic low-set ears, hypoplasia of clavicles and scapulae, abnormalities of cervical vertebrae. Mandibuloacral dysplasia is characterized by short stature, undeveloped mandible and clavicles, delayed closure of cranial sutures. The atrophy of skin with hyperpigmentation, rashes and papular lesions in the trunk and extremities can also be seen. As the age progresses some individuals may develop alopecia. Progressive stiffness of joints may also be noted. Radiograph of fingers and toes reveal acro-osteodysplasia and delayed ossification of carpal bones. CDAGS syndrome is characterized by craniosynostosis, delayed closure of the fontanelles, hypoplasia of clavicles, anal and genitourinary abnormalities, and skin eruption. Hypophosphatasia is characterized by a generalized deficiency in mineralization and ossification. Children affected may exhibit features poorly mineralized bones and widened cranial sutures, short ribs, and a narrow thorax. There is decrease in alkaline phosphatase activity in serum and tissues.¹³ Due to maxillofacial hypoplasia and osteogenic deficiencies, there is defect in clavicle, cranial and various vertebral and dental abnormalities, Many patients have chest malformations, some severe cases can lead to early respiratory distress in infants and some suffer from hematological disease. Due to osteogenesis and ossification, osteopenia is present in most patients of CCD causing short stature below the two percentiles between 4 and 8 years of age. Although motor development may be delayed, but in most cases mental development is normal.¹² Intraorally, the presence of supernumerary and impacted teeth in the premolar area is the most important feature. Delayed exfoliation of primary teeth and delayed eruption

of permanent teeth are other common features.¹⁴ Similar findings have been noticed in this case report.

Most supernumerary teeth are generally asymptomatic and can be found during routine radiographic examinations, but sometimes they can lead to several pathologies such as tooth displacement, root resorption of the adjacent tooth, cyst formations.¹⁵ In the present case with impacted supernumerary teeth are common features. Permanent third molars and canines are the most commonly impacted teeth.¹⁶ The etiology behind unerupted teeth is disturbance in resorption of alveolar bone, pre absence of cellular cementum, premature loss of gubernacular canal and disturbance in union between dental follicle and oral mucosa. The abnormalities of skull include multiple Wormian bones, open metopic fontanelles, delayed closure of sutures.¹⁴

Radiologic investigations play a role important in the diagnostic management of CCD. Various radiographic techniques that are available for the investigation of CCD include Orthopantomogram (OPG), Cone beam computed tomography (CBCT), Micro Computerized tomography (Micro-CT), Computed tomography (CT), etc. Micro-CT methods are recent advancement that allows 3-dimensional measurements of images that are being used in pediatric dentistry.¹ CBCT is being used in several medical applications, such as angiography, mammography, radiotherapy guidance and it was accepted by the Food and Drug Administration (FDA) in 2001 for the visualization of maxillofacial structures and is being used in almost all areas of dentistry.¹⁸ Using CBCT, it is possible to obtain sectional images on the axial, sagittal and coronal planes. Various bone abnormalities are best diagnosed on 3D whole-body CT images. After the birth of child, a whole-body radiographic investigation is required for any live-born infant, preterm foetus or stillborn with a suspected constitutional disorder of bone, so that any bony anomaly can be diagnosed at an early stage.¹⁹ Thus, by a thorough clinical examination and CBCT radiological examination,

a confirmed diagnosis of CCD is obtained.

Management of CCD patient is quite challenging. The prognosis is based on early treatment intervention.²⁰ Comprehensive craniofacial management of CCD patient involves the contribution of radiologist, oral maxillofacial surgeon, pedodontist, prosthodontist and orthodontist to provide for better masticatory function and facial aesthetics.

CONCLUSIONS

The diagnosis of this disorder is often missed or diagnosed at a later age. The patient with CCD often suffers with skeletal handicap and dental, hearing problems and psychological distress. Treatment is based on an interdisciplinary holistic approach by a lineup of dental and medical specialists working as a team.

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CONFLICTS OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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AGENESIS OF FRONTAL SINUSES IN ASSOCIATION WITH KINDLER SYNDROME: A RARE CASE REPORT

ABSTRACT

Kindler syndrome, as a rare subtype of Epidermolysis Bullosa, sets in motion a series of genetic conditions causing minor traumas and blisters on skin and making the skin susceptible to sunburn. The present study presented a case report of a 32-year-old female diagnosed with Kindler syndrome. Coronal and axial cone-beam computed tomography (CBCT) images clearly exhibited the agenesis of frontal sinuses. The condition was completely obvious in the images, which is a rare occurrence and has not been previously reported. The underdevelopment or aplasia of the paranasal sinuses is a rare phenomenon, which relates primarily to the frontal sinuses (12%) and secondarily to the maxillary sinuses (5-6%). Similarly, the agenesis of the sphenoid sinuses is an extremely rare condition. Therefore, raising our awareness about the paranasal sinus anomalies associated with the Kindler syndrome can lead to new discoveries about this syndrome. Further, with respect to the other patients suffering from Kindler syndrome, obtaining the basis of such knowledge together with evaluating CBCT or CT images in order to detect abnormalities can facilitate the management of the problems arising from paranasal sinus abnormalities.

Keywords: Frontal sinus, epidermolysis bullosa, syndrome.

 Ali Bagherpour¹

 *Shahin Moeini¹

ORCID IDs of the authors:

A.B. 0000-0002-6281-1867

S.M. 0000-0001-5251-6218

¹ Department of Oral and Maxillofacial Radiology, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran.

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INTRODUCTION

Kindler syndrome is a rare autosomal recessive genetic disorder induced by mutation in the *KIND1* or *FERMT1* genes (fermitin family homolog 1).¹ The first case of this syndrome dates back to 1954 and was described by the pediatrician Theresa Kindler, reporting the case of a 14-year-old female English patient who had developed blisters on her feet, legs, arms, and hands, along with changes in skin pigmentation, after exposure to the sun. Kindler syndrome is considered as a rare subtype of Epidermolysis Bullosa which sets in motion a sequence of genetic conditions responsible for the development of blisters on skin as well as minor traumas, and render the skin prone to sunburn.²

In addition, this syndrome can affect various mucous tissues, such as the mouth and eyes, and can lead to other health problems as well. The oral manifestations of Kindler syndrome include multiple painful oral ulcers in the mucosa, severe periodontitis with spontaneous bleeding, angular cheilitis, and desquamative gingivitis (i.e. red, shedding, and ulcerated appearance of the gums). Furthermore, poor dentition with premature loss of teeth, leukokeratosis of the lips (i.e. severely keratinized or ulcerated leukoplakia), xerostomia, caries, halitosis, gingival bleeding, and fragility of mucosa are considered as the other signs of the syndrome. These manifestations may impair proper nutrition intake and might cause growth and development problems.^{3,4}

CASE REPORT

A 32-year-old female diagnosed with Kindler syndrome was referred to a private oral and maxillofacial radiology center in Mashhad, Iran. Her chief complaint was headache and she had already been examined by an ENT specialist, advising the referral. The patient signed a written informed consent for publication of this rare case report.

She had no previous history of facial trauma, irradiation, or systemic disease affecting the skeletal system such as Paget's disease, osteopetrosis, or fibrous dysplasia. No abnormality or cystic fibrosis was found in her clinical and laboratory examinations. During further oral examination, pathological skin involvement and a

blister were observed, which were believed to be associated with the syndrome (Figure 1).



Figure 1: Skin involvement and blister related to kindler syndrome.

CBCT-based findings revealed the agensis of frontal sinuses in coronal and axial views (a 3mm-thick slice). Nasal turbinates appeared normal and there was no sign of pneumatization in bilateral frontal sinuses (Figures 2-3).

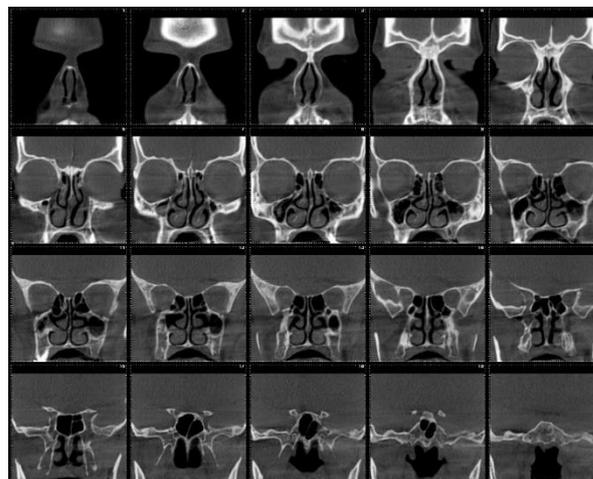


Figure 2: Coronal views in CBCT showed agensis of frontal sinuses.

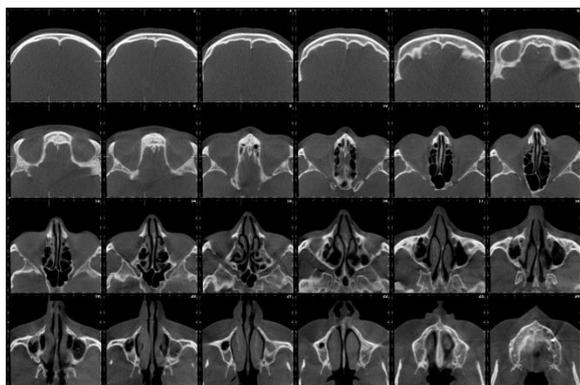


Figure 3: Axial views in CBCT showed agensis of frontal sinuses and maxillary sinus hypoplasia.

Further, the hypoplasia of maxillary sinuses and deviated nasal septum were observed in the axial view (Figure 3).

DISCUSSION

Kindler syndrome, as a rare type of Epidermolysis Bullosa, sets a series of genetic conditions which causes the skin to blister, experience minor traumas and be prone to sunburn. Among the oral manifestations of this syndrome, multiple painful oral ulcers in the mucosa, periodontal attachment loss, gingival bleeding, and fragile mucosa are considered as the most significant signs. They may lead to poor intake of nutrition and improper growth and development.³

Paranasal sinuses develop as an evagination of the mucosa from the nasal cavities during the third and fourth fetal months. The frontal sinus is absent at birth and starts to develop after the age of 2. The growth of the sinus increases at the age of 6 and continues until the late teenage years. The frontal sinus is the last one to develop among the paranasal sinuses. However, this sinus is reported to be bilaterally absent among 3-4 to 10% of the population. All paranasal sinuses undergo major pneumatization after birth, along with the development of the facial cranium and teeth.⁵ As a rare condition, the underdevelopment or aplasia of the paranasal sinuses is mainly associated with the frontal (12%) and then maxillary sinuses (5-6%). In the same vein, another extremely rare condition is the agensis of the sphenoid sinuses. The agensis of the paranasal sinuses occurs more frequently in craniosynostosis, osteodysplasia (Melnick-Needles), and in the case of Down's syndrome (the hypoplasia of the frontal sinus).⁶

Although the above Down's syndrome, Osteodysplasia and Craniosynostosis are considered as the differential diagnosis for the agensis of paranasal sinuses, other characteristics can also help to differentiate them. For instance, in craniosynostosis radiographic features are restriction of skull growth is perpendicular to the affected suture line. dysmorphic head shapes are associated with each type of craniosynostosis.⁷ The radiographic characteristics of osteodysplasia are noticeable in the skull, where the bones of the cranial vault irregularly thicken with the sclerosis of the base, especially in the anterior/mid fossa and mastoids.⁸ The physical characteristics of the Down's syndrome appear at birth, which include an epicanthic fold, brachycephaly, flat nasal bridge, upward angle of the eyes, single palmar crease, and increased nuchal skin.⁹

However, none of these characteristics were observed in the case of the present study. Accordingly, the agensis of frontal sinuses in the patient's CBCT may be related to the Kindler syndrome. Therefore, by evaluating the frontal sinuses in CT or CBCT of the other patients with kindler syndrome, it can be found out whether the absence of the frontal sinus is associated with this syndrome.

The developmental anomalies of paranasal sinuses among patients with cystic fibrosis are significantly prevalent compared to the normal population. Developmental pathologic abnormalities may be misdiagnosed as sinusitis or neoplasm.^{5,10} Further, paranasal air sinuses neoplasms are a rare condition and only 0.2% of which are malignant. Squamous cell carcinomas (SCC) are the most common neoplasm in paranasal sinuses.^{11,12}

In addition, the configuration and development of frontal sinus in each person depends on the constitutional factors such as age, gender, hormones, and craniofacial configuration along with the environmental factors including climatic conditions and local inflammation.^{13,14,15}

Based on previous studies, the frequency of bilateral frontal sinus aplasia is extremely low and this aplasia occurs more commonly in young women compared to men.^{14,15}

The case reported in the present study can be considered unique since the patient is a female diagnosed with kindler syndrome, combined with the aplasia of bilateral frontal sinuses and hypoplasia of maxillary sinuses. These conditions could be related to her syndrome. Previously reported cases have been associated with other syndromes such as primary ciliary dyskinesia.¹⁶

To the best of our knowledge, there is no study on Kindler syndrome which reported paranasal sinus abnormalities. Therefore, it is recommended to check the new findings of the present study across a wide range of patients with Kindler syndrome.

CONCLUSIONS

Increased awareness about the paranasal sinus anomalies associated with the kindler syndrome can provide the opportunity to make new findings related to this syndrome. In addition, with regard to the other patients suffering from the kindler syndrome, gaining the required knowledge, along with checking CBCT or CT images for abnormalities, can be very useful for managing the problems originated from paranasal sinus abnormalities. The significance of this issue can be seen, for example, in a case which the pathological cause of a headache may be associated with this condition.

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None

CONFLICTS OF INTEREST STATEMENT

None

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