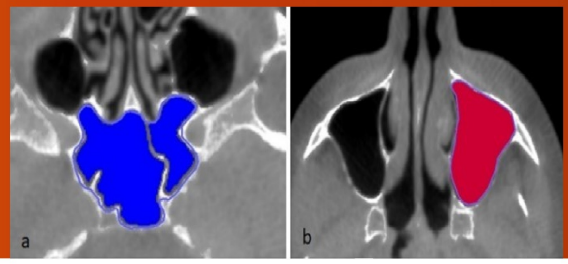
CUMHURIYET DENTAL JOURNAL

The Official Journal of Sivas Cumhuriyet University Faculty of Dentistry



Volume:23 Issue: 4 2020
ISSN : 1302-5805 e-ISSN : 2146-2852

Cumhuriyet Dental Journal

The Official Journal of the Sivas Cumhuriyet University Faculty of Dentistry. The first issue was published in 1998 and journal's name was changed as Cumhuriyet Dental Journal in 2010. Issues are published quarterly since 2018.

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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ISSN 1302-5805

e-ISSN 2146-2852

Volume/23- Issue/4-2020

INDEXING

CUMHURIYET DENAL JOURNAL

AUTHOR GUIDELINE

Cumhuriyet Dental Journal (CDJ) is the Official Publication of the Cumhuriyet University, Faculty of Dentistry. CDJ accepts original experimental investigations and review articles concerning topics of clinical relevance to the general dental practitioner. Case reports and technique articles will be very critically reviewed in terms of interest to the general dental practitioner and the supporting data provided.

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Review Articles: Although a Review article (particularly following a systematic review) may adhere to the format of the Original Research Article, both Review and Focus Articles need not contain Materials and Methods, Results or Discussion sections, and may instead employ other headings as relevant for the topic addressed.

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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 morefree 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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e-ISSN 2146-2852

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.701264

Original research

FREEZE-DRIED PLATELET-RICH PLASMA PROMOTES TRIGEMINAL NEUROPATHIC PAIN RELIEF IN A RAT MODEL

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ABSTRACT

Objective: Neuropathic pain and treatment side effects decreasing quality of life, reducing productivity, and high costs due to long duration of treatment. Regenerative medicine is a new and effective alternative treatment for neuropathic pain, one of which is Platelet-Rich Plasma (PRP) therapy. The objective if this study is to prove that there are differences in neuroregeneration post-crush injury after FD-PRP administration.

Materials and Methods: Neuropathic pain model was made with crushing method, by compressing the infraorbital nerve using the artery clamp for 15 seconds. Rats divided into six groups. Each group was observed on day 14 (A, B, C) and 21 (A', B', C') to observe the macrophages, lymphocytes, and Schwann cells with Hematoxylin Eosin staining seen on horizontal plane of the infraorbital nerve with 400x magnification. Face grooming observations were performed on day 0 (A0, B0, C0), day 7 (A7, B7, C7), day 14 (A14, B14, C14), day 21 (A21, B21, C21).

Results: There were significant differences in face grooming frequencies between groups on day 0 (p=0.002). ANOVA Same Subject test on A`, B`, and C` revealed significant differences in macrophages and lymphocytes with score 0.02 (p-<0.05) and 0.013 (p0.05), respectively. There were significant differences between group A' and B' and between B' and C' (p>0.05).

Conclusions: FD-PRP promotes nerve regeneration in axonotmesis, which was characterized by a decrease in face grooming frequency on day 7 and an increase in the number of lymphocytes, macrophages and Schwann cells on day 21.

Keywords: Platelet-rich plasma, macrophages, lymphocytes, schwann cells, peripheral nerve injuries.

Received : 21.04.2020
Accepted : 26.08.2020

How to Cite: Winias S, Radithia D, Asmaradiva AP, Salsabila NK, Puspitasari M, Sarasati A, Wicaksono S, Mahdani FY, Soebadi B. Freeze-Dried Platelet-Rich Plasma Promotes Trigeminal Neuropathic Pain Relief in A Rat Model. Cumhuriyet Dent J 2020;23:4: 254-261.
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INTRODUCTION

Lesions or somatosensory nervous system diseases cause neuropathic pain. Neuropathic pain that innervates the orofacial area can cause orofacial pain. Neuropathic pain can be caused by mechanical trauma or nerve compression due to surgery or neurological diseases such as post-herpetic neuralgia, which causes functional and structural nerve disorders. The symptoms of neuropathic correlate to the failure of transmission of nerve impulses such as dysesthesia, hyperalgesia, allodynia, paresthesias, and others. Neuropathic pain is generally chronic, and it can decrease the patient's quality of life if not treated adequately.[1]

Pain sufferers have been treated with analgesics such as NSAIDs, anticonvulsants, antidepressants, and opioids. Surgical therapy is viable for neuropathic pain due to neurovascular compression, such as trigeminal neuralgia. Antiviral therapy is viable to cure pain-causing diseases such as post-herpetic neuralgia. Drugs and surgical therapy can relieve symptoms of neuropathic pain, but do not promote and accelerate the regeneration of damaged nerves.[2] Various studies confirmed that nerve regeneration is the most effective and efficient therapy for neuropathic pain management; therefore, effective and efficient therapeutic methods for nerve regeneration is needed.[3]

Platelet-Rich Plasma (PRP) is a bioactive substance that is being developed for a variety of medical therapies due to high growth factor content in α platelet granules. A high concentration of growth factors such as Transforming Growth Factor-β (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and insulin growth factor-1 (IGF-1) can accelerate inflammation and neuroregeneration processes. PRP has been commonly used in the therapy of musculoskeletal disease or injuries.[4,5] Also, various studies have confirmed the PRP regenerative effect on injured nerves. However, there has been no study of the effect of Freeze-Dried PRP (FD-PRP) on injured nerves, particularly in axonotmesis. This study aimed to analyze the effects of FD-PRP on axonotmesis for neuropathic pain relief through cellular observation on lymphocytes, macrophages, Schwann cells, and facial grooming behavioral observation.

MATERIALS AND METHODS

This was a true experimental study with a post-test only control group design. This study received ethical approval by the Health Research Ethical Clearance Commission (HRECC) of the Faculty of Dental Medicine, Universitas Airlangga Number 140/HRECC.FODM/VII/2018. This study used a sample of Wistar rats (Rattus norvegicus), in which all samples were divided into 6 groups, namely the control group (A), crush injury treatment group (B), and crush injury treatment group + FD-PRP (C). Each group was observed on day 14 (A, B, C) and 21 (A', B', C') to observe the macrophages, lymphocytes, and Schwann cells. A crush injury can cause clinically relevant axonotmesis lesions for certain larger-scale molecular and cellular nerve studies, and it is one of the pain indicators for face grooming observation in experimental animals.[6,7] Face grooming observations were performed on day 0 (A0, B0, C0), day 7 (A7, B7, C7), day 14 (A14, B14, C14), day 21 (A21, B21, C21).

Allogeneic FD-PRP was obtained from the blood of 20 rats collected using a syringe containing dextrose citrate acid and centrifuged (Corelab BLC – 2012) for 10 minutes at 4000 rpm. The centrifugation separates the blood and plasma. The plasma was then transferred to another tube using a syringe. The plasma was centrifuged again for 10 minutes at 4000 rpm to accumulate the PRP at the bottom of the tube. The obtained PRP was then sterilized using UV light before freeze-dried. The PRP was then frozen at -83°C for 12 hours and lyophilized (Virtis Benchtop 4K 4BT4K2L-105) for 8 to 12 hours.

Rats in the treatment groups were anesthetized using ketamine per intraperitoneal and asepsized on the cheek region using 10% povidone-iodine. Then surgery was performed to access the infraorbital nerve fibers, which were then crushed for 15 seconds using artery clamp. The wound was then sutured and asepsized using povidone-iodine 10%.

The FD-PRP was dissolved in carboxymethylcellulose (CMC-Na 1%) with a 1:1 ratio and applied using an 18G syringe on the treatment groups 24 hours after the crushing.

Face grooming observation was performed by transferring the experimental animal to a smaller cage without base with a mirror facing the camera for video recording. The recordings were 10-minute videos and recorded between 07.00 – 18.00. Three independent observers examined the video recordings, and the mean was obtained. The face grooming frequency was counted if the front legs of the experimental animal reach the treated region without body grooming after (isolated face grooming).

The macrophages, lymphocytes, and Schwann cells were calculated once the experimental animal anesthetized. The HPA of the infraorbital nerve was obtained from Hematoxylin Eosin (HE) staining. The calculation was performed using a light microscope (Nikon Eclipse e200) with 400x magnification.

RESULTS

In general, we found that macrophages, lymphocytes and schwann cell increased in PRP administration group. The highest face grooming frequency was observed in B0, and the face grooming frequencies of all groups tended to decrease. However, an increase in face grooming frequency was observed in group A21 and group B14. (Figure 1)

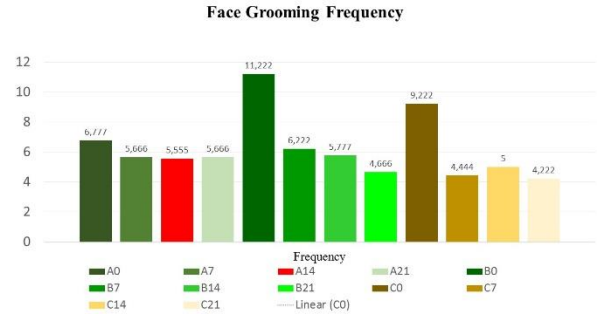

Figure 1 : Face Grooming Frequency Chart

There was a significant difference in face grooming frequency between groups B and C, and there was no significant frequency observed in group A. (Table.1)

Table 1. The Repeated-Measures ANOVA in Face Grooming between Groups

Group	Sig.
A	0.190
B	0.000*
C	0.000*

*significantly difference

There were significant differences in face grooming frequencies between groups on day 0 ($p = 0.002$). Whereas on day 7, 14, and 21, there were no significant differences in face grooming frequencies between groups. (Table. 2)

Table 2. The One-Way ANOVA in Face Grooming between Days

Time of Inspection	Sig.
0	0.002*
7	0.212
14	0.751
21	0.356

*significantly difference

There was an increase observed in lymphocytes, macrophages, and Schwann cells from day 14 to day 21 in each group. The number of lymphocytes and macrophages significantly increased in the treatment groups, while the number of Schwann cells significantly increased in control positive groups. (Figure 2)

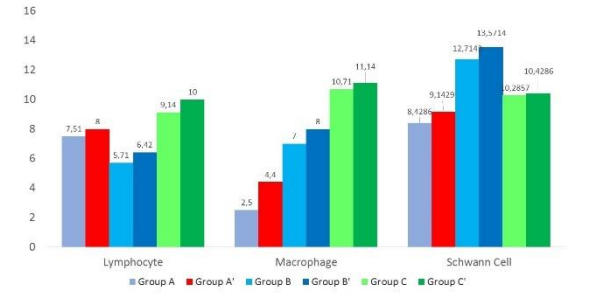

Figure 2 : Mean of Lymphocyte, Macrophage and Schwann Cells

Lymphocytes appeared round shape and almost filled with dark nuclei, macrophages can be found in several forms, commonly foun in oval shape with light-colored cytoplasm and dark nucleus, and Schwann cells are cells around axons, have a single nucleus, and form myelin sheaths. They were observed using a light microscope at 400x magnification. The cells distribution in day 14 and day 21 were presented in Figure 3.

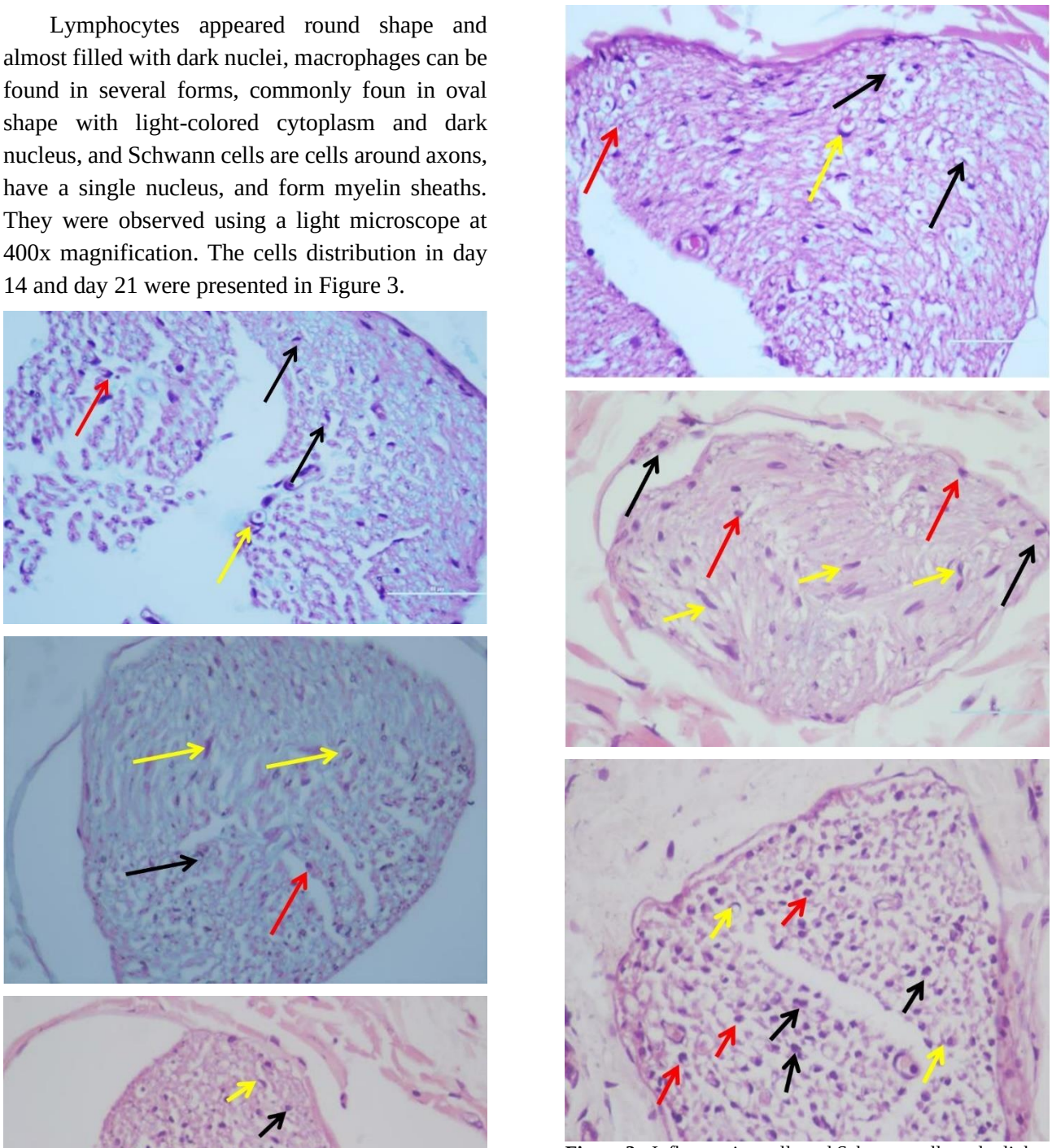

Figure 3 : Inflammation cells and Schwann cells under light microscope with 400x magnification on groups A, A', B, B', C, and C'. Red arrow points lymphocytes cells, black arrow points macrophages cells and yellow arrow points Schwann cells.

ANOVA test was carried out on day 14 and 21 to observe the number of Schwann cells, macrophage cells, and lymphocyte cells. ANOVA test results on day 14 are presented in Table 3, on day 21 are presented in Table 4 and Independent t-test of each cells presented on Table 5.

Table 3. Statistical analysis data relating to the quantity of Macrophages, lymphocytes and schwanncells in each treatment group on day 14.

	Group	Mean±SD	One-way ANOVA test	LSD test		
				A	B	C
Macrophages	A	7.00 ± 0.816	0.000	-	0.007*	0.000*
	B	5.43 ± 0.976		-	-	0.000*
	C	10.71 ± 1.113		-	-	-
Lymphocytes	A	5.71 ± 0.488	0.000	-	0.001*	0.000*
	B	7.57 ± 0.976		-	-	0.004*
	C	9.14 ± 1.069		-	-	-
Schwann cells	A	8.42 ± 1.272	0.000	-	0.000*	0.006*
	B	12.71 ± 1.113		-	-	0.001*
	C	10.28 ± 0.951		-	-	-

*= there is a significant difference ($p<0.05$)

Table 4. Statistical analysis data relating to the quantity of Macrophages, lymphocytes and schwann cells in each treatment group on day 21.

	Group	Mean±SD	One-way ANOVA test	LSD test		
				A'	B'	C'
Lymphocytes	A'	6.43±0.976	0.000	-	0.012*	0.000*
	B'	8.00±1.00		-	-	0.002*
	C'	10.00±1.155		-	-	-
Macrophages	A'	8.00±0.816	0.000	-	0.048*	0.000*
	B'	6.57±0.976		-	-	0.000*
	C'	11.14±1.773		-	-	-
Schwann cells	A'	9.14±1.09	0.000	-	0.000*	0.098
	B'	13.57±1.272		-	-	0.000*
	C'	10.43±1.718		-	-	-

Table 5. Independent T-Test on day 14 and 21

Parameter	Sig.
Lymphocyte	0.205
Macrophages	0.049*
Schwann	0.433

DISCUSSION

The PRP effect on neuroregeneration was observed through face grooming frequency observation and histopathological examination by calculating the number of macrophages, lymphocytes, and Schwann cells. Face grooming frequency was expected to illustrate the effect of FD-PRP on pain arising from nerve injury, and the number of macrophages, lymphocytes, and Schwann cells was expected to demonstrate the effect of FD-PRP on neuroregeneration, increasing the inflammatory response efficiency and promoting Schwann cells proliferation for myelin sheath formation.

The face grooming frequencies of A0, A7, A14, and A21 were close, indicating that face grooming was not influenced by external factors such as stress and de-arousal conditions. Therefore, group A was the comparison for group B and C in face grooming observation. There were significant differences between group A0, B0, and C0. It indicated that crush injury influenced face grooming frequency, which is the response of experimental animals to pain arising from crush-injury treatment. Wound healing and neuroregeneration, as observed in group B and C, influenced the face grooming frequency.[9]

Two hours after FD-PRP injection, the face grooming frequency of group C0 was lower than B0. This suggested that FD-PRP affected face grooming frequency by accelerating the neuroregeneration process, as observed in group C0.[10]

Comparison test results of day 0 revealed significant differences in face grooming frequency, while on day 7, 14, and 21, there was no significant

difference, indicating the wound healing process and relief of pain, therefore decreasing the face grooming frequency.[11] The insignificant result was related to the animals' responses to the given treatment.[12,13]

This study also examined the effect of FD-PRP on neuroregeneration acceleration by calculating the cells involved, namely lymphocyte, macrophages and Schwann cells. Lymphocytes, macrophages and Schwann cells play an important role in inflammation response due to injury. Inflammation promotes an adequate microenvironment in supporting the neuroregeneration process. These cells release various kinds of cytokines, chemokines, and neurotrophic factors to synergistically accelerate neuroregeneration.

Lymphocytes cells revealed significant difference each group ($p< 0.05$). This study in line with Yun et al., (2011) found that one of growth factor which contained in PRP called TGF-β. This growth factor play an important role for lymphocytes proliferation. When PRP was given to injured nerve, the number of inflammatory cells including lymphocytes increased to accelerate neuroregeneration.[14]

The macrophages cells calculation showed a significant result ($p=0.000$, $p < 0.05$). Macrophages in group A made up 9% of cells in normal peripheral nerves, while group B showed a greater number of macrophages compared to group A. This was caused by macrophage migration activated by Schwann cells and local macrophages for clearing out myelin debris.[15] There was a significant improvement in group C due to growth factors contained in FD-PRP, one of which was PDGF in increasing macrophage migration. An increase in the number of macrophages indicates faster healing. Macrophage dysfunction or a decrease in the number of macrophages inhibits the angiogenesis process, which eventually slows the healing process.[16]

There were significant differences between group ($p<0.05$). The significant differences were due to the increased number of Schwann cells in Wallerian degeneration. The number of Schwann cells post-injury increased because of increasing of macrophages cells for clearing out cellular debris and myelin. In successful nerve regeneration, clearing out of cellular and myelin debris by Schwann cells and macrophages completes in 21 to 42 days.[16]

The significant difference on day 21 between group B' and C' indicated that FD-PRP treatment on group C' had accelerated the resolution of inflammation as the Wallerian degeneration was quickly initiated by the contained growth factors.[17]

The non-significant difference on day 21 between group A' and C' indicated that the neuroregeneration in group C' was initiated early by the growth factors; therefore, the number of Schwann cells was not significant to the group A'. These growth factors, including transforming growth factor (TGF-β1), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF- I) contained in FD-PRP, improve the inflammatory mediators' efficiency and accelerate the neuroregeneration processes. TGF-β2 and TGF-β3 also play an important role in Schwann cell proliferation and differentiation.[18]

Lymphocytes revealed no significant differences between day 14 and day 21. During inflammation, T lymphocytes are activated and release cytokines, which activate macrophages and thereby release more cytokines. Lymphocytes and macrophages interact in a bidirectional pathway, and their interactions play an important role in chronic inflammation; therefore, the number of lymphocytes was not significantly different.[19]

Independent T-Test compared between variables of day 14 and 21, and it showed that there was a significant difference in macrophage ($p=0.049$). This was because the healing process of chronically inflamed injured tissue consumes time; therefore, the number of macrophages for clearing out debris remained high.[20]

Independent T-test compared on Schwann cells revealed no significant differences between groups. This was because (1) sprouting axons grow on day 3 to 42 post-injury, and the Schwann cells proliferation and axons and myelin debris clearance by macrophages completes on day 21 to 42 after injury for successful regeneration[16,21,22]

and (2) tissue vascularization, denervation of chronic Schwann cells, endoneurial and perineural fibrosis, axons inability to reach the correct target neuron, the distance for axons to reach the target neuron, cell atrophy, the rate of nerve regeneration, and other factors influencing the neuroregeneration.[23-26]

CONCLUSIONS

FD-PRP increase lymphocytes, macrophages a\nd scwhann cells proliferation, that accelerate peripheral nerve regeneration. These cells initiate several neurotrophic factors to achieve fast recovery. Thus, this study concluded that FD-PRP promotes nerve regeneration in axonotmesis injury, which was characterized by a decrease in face grooming frequency on day 7 and an increase in the number of lymphocytes, macrophages and Schwann cells on day 21.

ACKNOWLEDGEMENT

The authors would like to thank the Stem Cell Center and Tissue Bank of Dr. Soetomo Hospital, Surabaya for its support. This research is financially supported by Faculty of Dental Medicine Universitas Airlangga [grant numbers 1408/UN3/2019].

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.772706

Original research

BETTER WITH OZONE, OR NOT? AN IN-VIVO STUDY OF OZONE THERAPY AS A PRE-TREATMENT BEFORE FISSURE SEALANT APPLICATION

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*Merve Aksoy[2]
Firdevs Tulga Öz[3]

ABSTRACT

Objectives: The aim of the study was to evaluate clinical achievements of Clinpro™ sealant and Teeth mate F-1 fissure sealants with or without ozone treatment as well as retention, marginal discoloration, marginal adaptation and caries formation under in-vivo conditions.

Materials and Methods: The study was carried out on 166 patients whose ages differs between 9-12 and 3 different groups were formed above them according to split-mouth technique. Group 1: Clinpro™ sealant and Teethmate F-1 were applied on a total of 104 first lower permanent molar teeth. Group 2: Clinpro™ sealant and Clinpro™ sealant following ozone treatment were applied on 112 first lower permanent molar teeth. Group 3: Teethmate F-1 and Teethmate F-1 following the ozone treatment were applied on 116 first lower permanent molar teeth. Based on the modified USPHS criteria, the impacts on general achievement, retention, marginal fit, marginal discoloration and caries formation were evaluated in 3rd,6th,12th,18th months. Chi-Square Test and Fisher's Exact Test were used for analyses of the data.

Results: In Group 1; Clinpro™ sealant showed higher clinical success rates in all observation periods. Considering marginal adaptation and retention, Clinpro™ sealant group was statistically significantly different in 18th month observations. In group 2 and group 3, there was not statistically significantly difference ($p>0.05$) between control and experimental groups but clinical success rates of Clinpro ™ sealant and Teethmate F-1 with Ozone Treatment were higher than the same groups without ozone pretreatment for all periods.

Conclusions: It was determined that, Clinpro™ sealant yielded more successful results than the Teethmate F-1 under in-vivo conditions. Although, statistically significantly difference was not detected in Group 2 and Group 3, the Ozone pretreatment method's success rates were clinically higher as Bravo, Alpha and Charlie Scores. It seems that, Ozone pretreatment is a successful method for preventing pit and fissure sealants, clinically.

Key words: Ozone, fissure sealant, anti-bacterial agents, preventive dentistry.

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Received : 27.07.2020
Accepted : 22.09.2020

How to Cite: Çetinkaya M, Aksoy M, Tulga Öz F. Better with Ozone, Or Not? An In-Vivo Study of Ozone Therapy as A Pre-Treatment Before Fissure Sealant Application. Cumhuriyet Dent J 2020;23:4: 262-272.
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INTRODUCTION

Today, preventive dentistry is considered an appropriate approach to halting cariogenic dental infections in both adults and children. Prophylactic methods including fluoride treatment, oral-hygiene education and diet may inhibit smooth-surface caries, but they are less effective against pit-and-fissure caries. Fissure sealants are known to be the most effective agents in the prevention of occlusal caries. In general, under clinical conditions, application of a restorative material is the preferred method of treatment for preventing food retention and bacterial adhesion in deep pits and fissures of the teeth.[1-3] However, the use of resin-based fissure sealants for managing pit-and-fissure caries has also been reported to have a number of drawbacks that can eventually lead to treatment failure, including marginal discoloration, adhesive failure, restoration loss, and caries formation over the long term. In order to improve the success of treatment with resin-based materials, a number of methods have been tried, including pre-treatment with ozone.[4-6]

Ozone gas is one of the most effective antibacterial agents and antioxidants found in nature, and it has long been used in medicine and dentistry for its healing properties with no reported negative effects on general health.[7-9] Numerous in-vitro studies have examined ozone pre-treatment in terms of its antimicrobial activity and its effects on marginal leakage and microleakage of pit-and-fissure sealants.[10-12] A number of in-vivo studies have also been conducted to investigate the antimicrobial effects of ozone therapy and the effects of pre-treatment with ozone gas on the success of resin-based materials.[13-17]

Clinpro™ is a resin-based fluoride-releasing sealant that includes inorganic filler components that is frequently used in clinical practice and that has been featured in many recent studies.[18] Teethmate F-1 is an unfilled, low-viscosity, resin-based material capable of diffusing through deep pits and fissures.[19] To date, there has been no in-vivo study conducted to provide a comparative assessment of the sealants Clinpro™ and Teethmate F-1 used with and without ozone pre-treatment. Therefore, the present study was conducted in order to evaluate and compare the 3-, 6-, 12-, and 18- month clinical success rates of Clinpro™ and Teethmate F-1 fissure sealants applied with and without ozone pre-treatment in terms of marginal discoloration, marginal adaptation, retention, and caries formation. The hypothesis tested was that ozone pre-treatment enhances the clinical success of fissure sealants.

MATERIAL AND METHODS

The study protocol was conducted in line with the principles of the Helsinki Declaration, including all amendments and revisions, and with the approval of the Ankara University Faculty of Dentistry's Board of Ethics (Number:137/1). Access to data was restricted to the researchers, and informed consent was obtained from all participants or their legal representatives prior to any treatment.

Sample size was calculated based on previous studies using the Power Analysis and Sample Size (PASS) 2008 Statistical Software (Utah, USA). Accordingly, 166 patients (89 males, 77 female) ranging in age from 9-12 years were included in the study. Following clinical examinations, a total of 332 mandibular permanent first molars were selected and randomly divided into three study groups according to CONSORT guidelines (Figure 1).

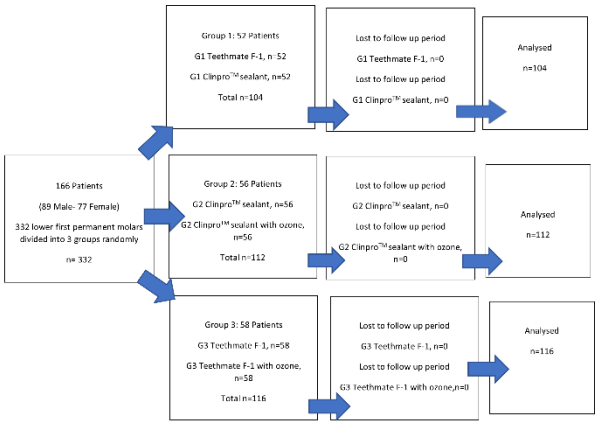

Figure 1. Consort Diagram

Patients were included in the study if their pre-treatment medical history showed them to be "completely healthy" according to ASA criteria and if both their left and right mandibular permanent first molars were fully erupted and intact, with no hypo mineralization defects. All other required dental treatment was performed, and patients were given oral-care instruction before initiating the study.

Prior to sealant application, all tooth surfaces were cleaned with a tooth brush and water[20], and treated teeth were isolated using a rubber dam in order to avoid bacterial contamination and enhance success rates.[21] The area surrounding the teeth to be treated were cleaned with alcohol applied on a cotton pellet, and articulation paper was used to eliminate occlusal interferences.

Participants were randomly divided into one of three study groups, and a split-mouth technique was employed in order to evaluate the effects of two different sealant protocols, which were randomly assigned to either the left or right mandibular first molar. Clinpro™ and Teethmate F-1 sealants with and without ozone pre-treatment were applied as follows:

Group 1: Clinpro™ sealant vs Teethmate F-1

Clinpro™: In line with the manufacturer's recommendation, 37% phosphoric acid (ETCH-37, Bisco Inc., Schaumburg, USA) was applied for 20 seconds on all pits and fissures. Surfaces were washed with water spray for 15 seconds and dried with air spray, until they were white and opaque. Clinpro™ sealant (3M ESPE, St. Paul, MN, USA. 20021121) was applied and homogenized using a dental probe and then polymerized using an LED light-curing unit (Elipar Freelight 2, 3M ESPE, Germany) for 20 seconds (Figure 2).

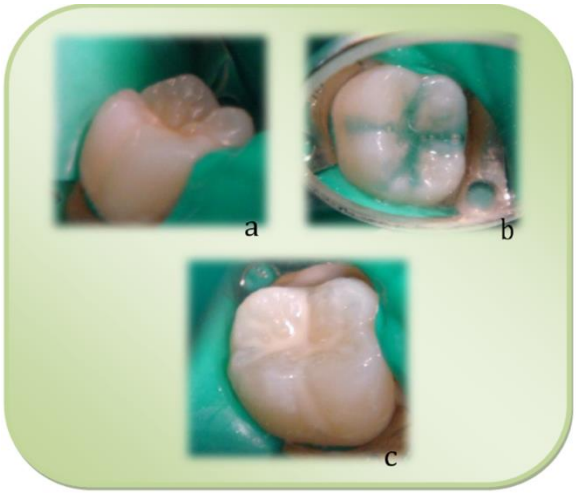

Figure 2. a) G1 Clinpro™ **b)** 37% Phosphoric acid applying, **c)** Polymerisation of Clinpro™ sealant.

Teethmate F-1: In line with the manufacturer's recommendation, 40% phosphoric acid (K-etch, Kuraray Medical, 1621Sakazu, Kurashiki, Okayama 710-8622, Japan) was applied for 20 seconds on all pits and fissures, and surfaces were washed and dried as described above. Teethmate F-1 sealant (Kuraray Medical, 1621 Sakazu, Kurashiki, Okayama 710-8622, Japan) was applied and polymerized as described above for Clinpro™ (Figure 3).

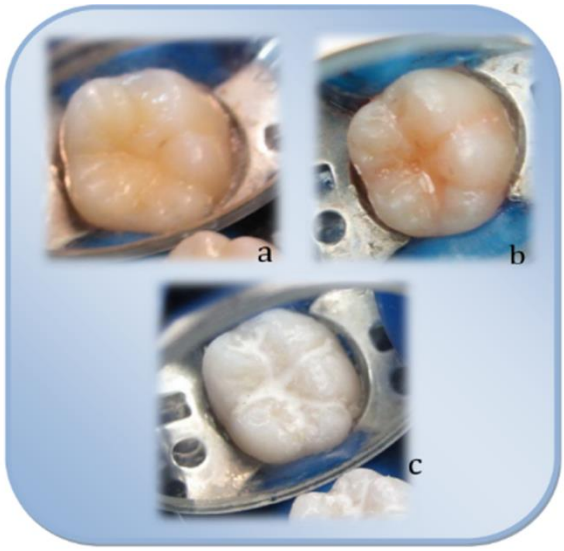

Figure 3. a) G1 Teethmate F-1 First Lower Molar, **b)** 40% Phosphoric acid applying, **c)** Polymerisation of Teethmate F-1.

Group 2: Clinpro™ vs Clinpro™ with ozone pretreatment

Clinpro™: Acid etching and sealant treatment was performed as described above for Group 1, Clinpro™.

Clinpro™ with ozone pretreatment: Ozone pre-treatment was performed using Ozonytron X (Mymed, Germany, 2005), with the CA probe set at 100,000 rpm to apply ozone gas directly on pits and fissures for 30 seconds. Acid etching and sealant treatment was then performed as described above for Group 1, Clinpro™ (Figure 4).

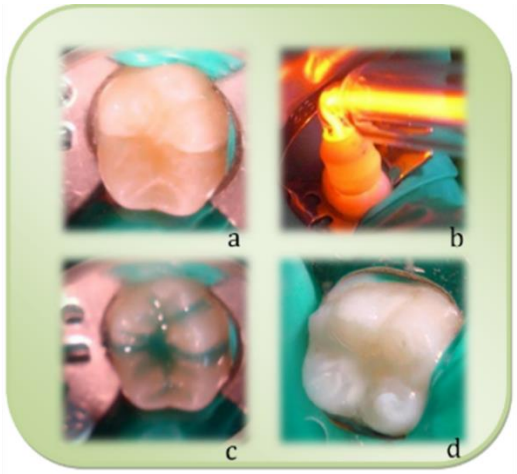

Figure 4. a) G2 Ozone-Clinpro™ First Lower Molar, **b)** Ozone applying at occlusal surfaces, **c)** 37% Phosphoric acid applying, **d)** Polymerisation of Clinpro™ sealant.

Group 3: Teethmate F-1 vs Teethmate F-1 with ozone pretreatment)

Teethmate F-1: Acid etching and sealant treatment was performed as described above for Group 1, Teethmate F-1.

Teethmate F-1 with ozone pretreatment: Ozone pre-treatment was performed as described above for Group 2, Clinpro™. Acid etching and sealant treatment was then performed as described above for Group 1, Teethmate F-1 (Figure 5).

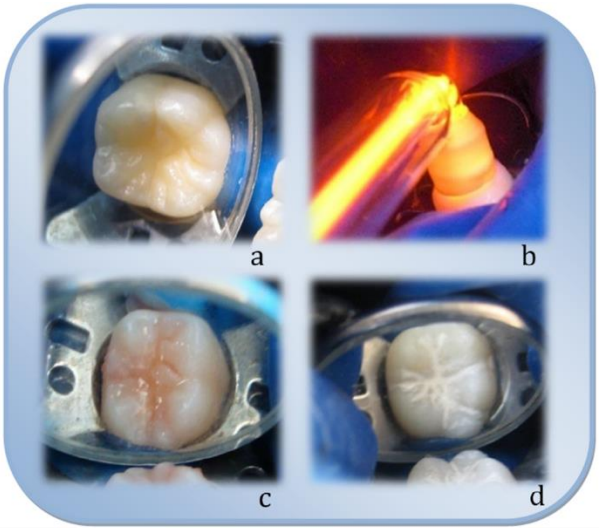

Figure 5. a) G3 Ozone-Teethmate F-1 First Lower Molar, **b)** Ozone applying at occlusal surfaces, **c)** 40% Phosphoric acid applying, **d)** Polymerisation of Teethmate F-1 sealant.

Clinical examinations were conducted at 3, 6, 12, and 18 months following treatment. Success was based on United States Public Health Service (USPHS) criteria[22], i.e., retention of fissure sealants, marginal adaptation, marginal discoloration and caries formation. Criteria were evaluated and scored as follows:

Retention. Retention was evaluated by visual inspection. Teeth were scored as either Alpha (Restoration present), Bravo (Partial loss of restoration, but clinically acceptable), or Charlie (Clinically unacceptable partial loss or total loss of restoration.)

Marginal adaptation. Marginal adaptation was evaluated by probing and inspection. Teeth were scored as either Alpha (Continuity at the margin), Bravo (Slight discontinuity at the margin), or Charlie (Marginal ledge or crevice requiring replacement).

Marginal discoloration. Marginal discoloration was evaluated by visual inspection. Teeth were scored as either Alpha (No discoloration at the margin), Bravo (Superficial discoloration at the margin), or Charlie (Deep discoloration at the margin, penetrated in a pulpal direction).

Caries. Caries were evaluated by visual inspection and probing. Teeth were scored as either Alpha (Caries absent) or Charlie (Caries present).

In each examination period, the teeth scored as Charlie for retention were left out assessment for marginal discoloration and caries formation examinations.

Statistical Analysis

Statistical analysis was performed using the NCSS (Number Cruncher Statistical System) 2007 and PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA). Analysis included descriptive statistics (frequency-ratio) as well as Chi-Square and Fisher's Exact Tests. The Chi-Square Test relies on an approximation to make a global assessment as to whether the relationship between two variables is independent or associated, but it requires a minimum number of samples, whereas Fisher's exact test can be used with small sample sizes.[23] Differences of $p<0.05$ were considered statistically significant.

RESULTS

Clinpro™ vs Teethmate F-1

Table 2 presents the overall clinical success and failure at 3, 6, 12, and 18 months for Group 1.

Table 2. Statistical Analyses of Clinical Success Rates- Group 1

Material	General Success	3rd month		6th month		12th month		18th month	
		n (52)	% 100	n (52)	% 100	n (52)	% 100	n (52)	% 100
Clinpro™ Sealant	**Successful**	50	96.2	47	90.4	44	84.6	43	82.7
	Failure	2	3.8	5	9.6	8	15.4	9	17.3
Teethmate F-1	**Successful**	49	94.2	44	84.6	40	76.9	33	63.4
	Failure	3	5.8	8	15.4	12	23.1	19	36.6
p values		0.647		0.374		0.320		0.016*	

*p<0.05

As the table shows, overall clinical success was significantly higher for Clinpro™ as compared to Teethmate F-1 at 18 months post-treatment (p<0.05). According to Alpha, Bravo, and Charlie scores, no statistically significant differences were observed in any of the individual parameters of retention, marginal adaptation, marginal discoloration, and caries formation at 3, 6, or 12 months. However, at 18 months, statistically significant differences were observed between the two sealants. Retention rates were significantly (p<0.05) higher for Clinpro™ (78.8%) as compared to Teethmate F-1 (65.4%). Marginal adaptation scores were also significantly (p<0.05) higher for Clinpro™ (73.1%) as compared to Teethmate F-1 (53.8%) (Table 3).

Table 3. Findings obtained through the observation periods for the retention, marginal integrity, marginal discoloration and caries formation based on modified USPHS criteria - Group 1.

Clinical Criteria	Observation Periods	Score	Clinpro™ Sealant		Teethmate F-1		p
			n	%	n	%	
Retention	3rd month	Alpha	49	94.2	47	90.4	0.462
		Bravo	1	1.9	2	3.8	0.558
		Charlie	2	3.8	3	5.8	0.647
	6th month	Alpha	45	86.5	45	86.5	1.00
		Bravo	3	5.8	2	3.8	0.647
		Charlie	4	7.7	5	9.6	0.727
	12th month	Alpha	42	80.8	37	71.5	0.251
		Bravo	4	7.7	7	13.5	0.339
		Charlie	6	11.5	8	15.4	0.556
	18th month	Alpha	41	78.8	34	65.4	0.036*
		Bravo	5	9.6	8	15.4	0.250
		Charlie	6	11.5	10	19.2	0.277
Marginal Adaptation	3rd month	Alpha	49	94.3	47	90.4	0.715
		Bravo	1	1.9	2	3.8	1.000
		Charlie	2	5.8	3	5.8	1.000
	6th month	Alpha	44	84.6	43	82.7	0.791
		Bravo	4	7.7	3	5.8	1.000
		Charlie	4	7.7	6	11.5	0.506
	12th month	Alpha	40	76.9	36	69.2	0.377
		Bravo	5	9.6	8	15.4	0.374
		Charlie	7	13.5	8	15.4	0.780
	18th month	Alpha	38	73.1	28	53.8	0.042*
		Bravo	6	11.5	12	23.1	0.120
		Charlie	8	15.4	12	23.1	0.320
Marginal Discolaration	3rd month	Alpha	50	100	49	100	1.000
		Bravo	-	-	-	-	-
		Charlie	-	-	-	-	-

	6th month	Alpha	47	97.9	45	97.8	1.000
		Bravo	1	2.1	1	2.2	1.000
		Charlie	-	-	-	-	-
	12th month	Alpha	43	93.5	40	90.9	0.429
		Bravo	2	4.3	4	9.1	0.429
		Charlie	-	-	-	-	-
	18th month	Alpha	38	86.4	34	82.9	0.660
		Bravo	5	11.4	5	14.6	1.000
		Charlie	1	2.3	1	2.4	1.000
Caries Formation	3rd month	Alpha	52	100	52	100	1.000
		Charlie	-	-	-	-	-
	6th month	Alpha	50	100	49	100	1.000
		Charlie	-	-	-	-	-
	12th month	Alpha	48	100	46	100	1.000
		Charlie	-	-	-	-	-
	18th month	Alpha	44	97.8	42	95.5	1.000
		Charlie	1	2.2	2	4.5	1.000

*p<0.05

Marginal discoloration did not vary significantly between Clinpro™ and Teethmate F-1 at 18 months (p<0.05) and although caries formation was detected in 1 tooth treated with Clinpro™ and 2 teeth treated with Teethmate F-1, the difference was not statistically significant.

Clinpro™ vs Clinpro™ with Ozone Therapy

Clinpro™ with Ozone Therapy showed higher overall clinical success rates than Clinpro™ without Ozone Therapy at 3, 6, 12 and 18 months, but the differences were not statistically significantly (p>0.05). No statistically significantly differences (p>0.05) were observed in any of the individual criteria during any of the observation periods (Table 4).

Table 4. Findings obtained through the observation periods for the retention, marginal integrity, marginal discoloration, and caries formation based on modified USPHS criteria - Group 2.

Clinical	Observation	Score	Clinpro™ Sealant		Ozone- Clinpro™ Sealant		p
			n	%	n	%	
Retention	3rd month	Alpha	53	94.6	55	98.2	0.618
		Bravo	-		-		-
		Charlie	3	5.4	1	1.8	0.618
	6th month	Alpha	47	83.9	49	87.5	0.589
		Bravo	4	7.1	4	7.1	1.000
		Charlie	5	8.9	3	5.4	0.716
	12th month	Alpha	44	78.6	47	83.9	0.468
		Bravo	5	8.9	4	7.1	1.000
		Charlie	7	12.5	5	8.9	0.541
	18th month	Alpha	43	76.8	45	80.4	0.645
		Bravo	5	8.9	5	8.9	1.000
		Charlie	8	14.3	6	10.7	0.568
Marginal Adaptation	3rd month	Alpha	53	94.6	55	98.2	0.618
		Bravo	0	0	0	0	-
		Charlie	3	5.4	1	1.8	0.618
	6th month	Alpha	46	82.2	47	83.9	0.801
		Bravo	5	8.9	5	8.9	1.000
		Charlie	5	8.9	4	7.2	1.000
	12th month	Alpha	42	75.0	44	78.6	0.654
		Bravo	6	10.7	5	8.9	0.751
		Charlie	8	14.3	7	12.5	0.781

	18th month	Alpha	41	73.2	42	75.0	0.829
		Bravo	6	10.7	6	10.7	1.000
		Charlie	9	16.1	8	14.3	0.792
	3rd month	Alpha	53	100	55	100	1.000
		Bravo	-	-	-	-	-
		Charlie	-	-	-	-	-
	6th month	Alpha	50	98	53	100	0.490
		Bravo	1	2	0	0	-
Marginal Discolaration		Charlie	-	-	-	-	-
	12th month	Alpha	46	93.9	49	96.1	0.675
		Bravo	3	6.1	2	3.9	-
		Charlie	-	-	-	-	-
	18th month	Alpha	43	89.6	46	92	0.738
		Bravo	5	10.4	4	8	-
		Charlie	-	-	-	-	-
	3rd month	Alpha	56	100	56	100	1.000
		Charlie	-	-	-	-	-
	6th month	Alpha	53	100	55	100	1.000
		Charlie	-	-	-	-	-
Caries Formation	12th month	Alpha	51	100	53	100	1.000
		Charlie	-	-	-	-	-
	18th month	Alpha	48	97.96	51	100	1.000
		Charlie	1	2.04	-	-	1.000

With the exception of 1 caries that was observed at 18 months in a tooth treated using Clinpro™ Sealant without Ozone Therapy, no caries was detected in Group 2.

Teethmate F-1 vs Teethmate F-1 with Ozone Therapy

Teethmate F-1 with ozone therapy showed a higher overall clinical success rate than Teethmate F-1 without Ozone Therapy at 3, 6, 12 and 18 months, but the differences were not statistically significantly ($p>0.05$). No statistically significant differences were observed in retention, marginal adaptation, or marginal discoloration during any of the observation periods (Table 5). With the exception of 2 caries observed at 18 months in a tooth treated using Teethmate F-1 without Ozone Therapy, no caries was detected in Group 3.

Table 5. Findings obtained through the observation periods for the retention, marginal integrity, marginal discoloration, and caries formation based on modified USPHS criteria - Group 3

Clinical	Observation	Score	Teethmate F-1		Ozone- Teethmate F-1		p
			n	%	n	%	
	3rd month	Alpha	53	91.4	54	93.1	1.000
		Bravo	2	3.4	2	3.4	1.000
		Charlie	3	5.2	2	3.4	1.000
	6th month	Alpha	51	87.9	51	87.9	1.000
		Bravo	3	5.2	3	5.2	1.000
Retention		Charlie	4	9.6	4	6.9	1.000
	12th month	Alpha	42	72.4	44	75.9	0.672
		Bravo	8	13.8	7	12.1	0.782
		Charlie	8	13.8	7	12.1	0.782
	18th month	Alpha	38	65.5	39	67.2	0.844
		Bravo	9	15.5	9	15.5	1.000
		Charlie	11	19	10	17.2	0.809
Marginal Adaptation	3rd month	Alpha	53	91.4	54	93.2	1.000
		Bravo	2	3.4	2	3.4	1.000

		Charlie	3	5.2	2	3.4	1.000
		Alpha	50	86.2	49	84.5	0.793
	6th month	Bravo	3	5.2	5	8.9	0.486
		Charlie	5	8.6	4	6.9	1.000
		Alpha	39	67.3	42	72.4	0.544
	12th month	Bravo	9	15.5	7	12.1	0.590
		Charlie	10	17.2	9	15.5	0.802
		Alpha	35	60.3	35	60.3	1.000
	18th month	Bravo	10	17.2	10	17.2	1.000
		Charlie	13	22.4	13	22.4	1.000
		Alpha	55	100	56	100	1.000
	3rd month	Bravo	-	-	-	-	-
		Charlie	-	-	-	-	-
		Alpha	52	96.3	53	98.1	1.000
	6th month	Bravo	2	3.7	1	1.9	-
Marginal Discolaration		Charlie	-		-	-	-
		Alpha	46	92	48	94.1	0.715
	12th month	Bravo	4	8	3	5.9	-
		Charlie	-	-	-	-	-
		Alpha	40	85.1	42	87.5	0.734
	18th month	Bravo	7	14.9	6	12.5	-
		Charlie	-	-	-	-	-
	3rd month	Alpha	58	100	58	100	1.000
		Charlie	-	-	-	-	-
	6th month	Alpha	55	100	56	100	1.000
		Charlie	-	-	-	-	-
Caries Formation	12th month	Alpha	54	100	54	100	1.000
		Charlie	-	-	-	-	-
	18th month	Alpha	48	96	51	100	0.567
		Charlie	2	4	-	-	-

DISCUSSION

Fissure sealants are considered an important form of treatment for the prevention of occlusal caries.[1,3] In order to enhance the clinical success of sealant application, a variety of different techniques and materials have been investigated, including ozone therapy.[18]

Ozone has long been known for its antibacterial activity and is used in dentistry as a prophylactic to halt the progression of early-stage caries. Ozone's oxidation capacity reduces bacterial plaque formation. The healing activity associated with ozone is attributed to its ability to maintain an antibacterial environment. Once this environment is secured, recover is achieved through the self-healing capacity of the affected tissue.[8]

In-vivo studies offer the most reliable method for assessing clinical success rates of treatment techniques and materials used in the mouth because intraoral conditions like occlusal forces and temperature and humidity changes cannot be accurately reproduced in-vitro.[24] Moreover, due to the uniqueness of the oral environment, which varies among individuals[25], this study was conducted as an in-vivo study with a split-mouth design that compared treatment of left and right mandibular permanent first molars in the same subject.

The mandibular first molars are the first permanent teeth to erupt in the oral environment, placing them at higher risk than other teeth in terms of bacterial accommodation and caries. Clinically, fissure sealants are most often used to treat the occlusal surfaces of first mandibular molars. According to an earlier clinical study, mandibular first molars have higher treatment success rates than maxillary first molars.[26] For this reason, the

present study was conducted with mandibular permanent first molars.

In line with other previous studies, the present study used a rubber dam to isolate teeth and avoid bacterial contamination.[21] Prior to treatment, occlusal surfaces were cleaned with water and burs only; pumice was not used, since Scanning Electron Microscopy (SEM) studies have shown small particles to remain trapped in pits and fissures.[20] Etching with phosphoric acid was performed according to the manufacturers' instructions (20 seconds with 35% phosphoric acid for Clinpro™ and 20 seconds with 40% phosphoric acid etching for Teethmate F-1). Light-curing was performed with an LED light source because LED lights have been shown to provide better polymerization activity than halogen lights.[27]

Ozone therapy may be performed either before or after the application of a fissure sealant. Previous studies have examined the effects of ozone therapy as a prophylactic before application of a sealant; therefore, this method was also used in the present study.[10]

Previous studies have shown pre-treatment with ozone to have a favourable effect on the penetration and bond strength of resin-based materials to enamel.[4,14,27] Ozone has also been shown to reduce marginal leakage of fissure sealants in vitro.[5,12] For example, in an in-vitro study by Cehreli *et al.* [5], fissure sealant (Fissurit FX) was applied to extracted molars either with or without ozone pre-treatment and with or without application of a bonding agent (Clearfil Protect Bond); SEM evaluation conducted following thermocycling and basic fuchsine dyeing found ozone treatment to significantly improve marginal adaptation and decrease marginal leakage ($p<0.001$).

In a previous in-vivo study by Baysan and Lynch[28] comparing the remineralization of root surfaces when sealant was used with and without ozone treatment, ECM (Electronic Caries Monitor) and DIAGNOdent values showed a significantly higher level of remineralization occurred with ozone treatment ($p<0.05$). Another previous in-vivo study examined both remineralization and retention of teeth treated with three different fissure sealants (Fuji Triage, Aegis FS, Helioseal) with and without ozone pre-treatment. Retention rates were measured at 1, 3, 6, 9, 12 months, and at the end of 12 months, sealant restorations were removed using an air-abrasion device, and DIAGNOdent scores were measured to compare remineralization values before and after treatment. According to the study report, ozone application improved remineralization for all groups; however, based on DIAGNOdent scores, the increases in remineralization observed with Fuji Triage and Aegis FS following ozone treatment were significantly higher ($p<0.05$) than those observed with Helioseal following ozone treatment.[15] These findings were similar with the recent study, though statistically significant results were not detected in.

CONCLUSIONS

The clinical performance of Clinpro™ sealant was found to be significantly better than Teethmate F-1 in terms of retention and marginal adaptation ($p<0.05$). While ozone pre-treatment improved the success rates of both these fissure sealants, the differences were not statistically significantly ($p>0.05$). However, given the antimicrobial effects of ozone treatment and the importance of securing an antibacterial environment before performing any restorations, ozone therapy may be recommended before the application of a fissure sealant.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflicts of interest.

Ozon Tedavisi Uygulanan ve Uygulanmayan İki Fissür Örtücünün In-Vivo Koşullarda Değerlendirilmesi

ÖZ

Amaç: *Bu çalışmanın amacı, ozon tedavisi uygulanan ve uygulanmayan Clinpro™ sealant ve Teethmate F-1 fissür örtücülerin genel klinik başarılarının yanı sıra tutuculuk, kenar renklenmesi, kenar bütünlüğü ve çürük oluşumlarının in-vivo koşullarda değerlendirilmesidir.*

Gereç ve Yöntemler: *Yaşları 9-12 arasında değişen 166 hasta çalışmaya dahil edilmiştir ve 3 farklı grup oluşturulmuştur. Grup-1'de 104 adet rastgele seçilen alt daimî birinci büyük azı dişine Clinpro™ sealant ve Teethmate F-1 uygulanırken; Grup-2'de 112 adet rastgele seçilen alt daimî birinci büyük azı dişine Clinpro™ sealant ve ozon tedavisinden sonra Clinpro™ sealant; Grup-3'te 116 adet rastgele seçilen alt daimî*

birinci büyük azı dişine Teethmate F-1 ve ozon tedavisi sonrasında Teethmate F-1 uygulanmıştır. Tedaviden sonra 3., 6., 12. ve 18. aylarda, genel başarı, tutuculuk, kenar bütünlüğü, kenar renklenmesi ile oklüzal yüzeylerdeki çürük oluşumları USPHS kriterlerine göre değerlendirilmiştir. ***Bulgular:*** *Grup1'de, Clinpro™ sealant'ın Teethmate F-1'e göre tüm gözlem periyotlarında genel başarısının daha yüksek olduğu ve aradaki farkın 18. ayda istatistiksel olarak anlam kazandığı (p<0,05), Grup-2 ve Grup-3'te istatistiksel olarak anlamlı bir fark olmamakla birlikte ozon tedavisi uygulanan gruplarda genel başarının daha yüksek olduğu tespit edilmiştir (p>0,05). Örtücülerin tutuculuk ve kenar bütünlüğü değerlendirildiğinde Grup-1'de, Clinpro™ sealant'ın Teethmate F-1'e göre 18.ayda daha başarılı olduğu (p<0,05), kenar renklenmesi açısından istatistiksel olarak anlamlı bir fark olmadığı, Grup-2 ve Grup-3'te ozon tedavisi uygulanan ve uygulanmayan fissür örtücüler arasında tutuculuk, kenar renklenmesi, kenar bütünlüğü açısından istatistiksel olarak anlamlı bir fark bulunmadığı tespit edilmiştir (p>0,05).* ***Sonuç:*** *Clinpro™ sealant, Teethmate F-1'e göre istatistiksel olarak daha başarılı bulunmuş fakat fissür örtücü öncesi yapılan ozon tedavisinin genel başarı ve tutuculuk, kenar bütünlüğü, kenar renklenmesi, çürük gelişimi üzerindeki etkilerinin istatistiksel olarak anlamlı bir fark yaratmadığı tespit edilmiştir.* ***Anahtar Kelimeler:*** *Ozon, fissür örtücüler, çocuk diş hekimliği.*

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.777128

Original research

RELATIONSHIP OF FOREHEAD AESTHETICS WITH FRONTAL SINUS SIZE AND SKELETAL MALOCCLUSIONS

Hasan Camcı[3]

ABSTRACT

Objectives: Forehead inclination and aesthetics have received very limited attention, although forehead is critically important in obtaining total facial aesthetics. The aim of this study was to evaluate the relationship between frontal sinus fields, forehead morphology and skeletal malocclusion.

Materials and Methods: A total of 224 extraoral lateral photographs and cephalometric radiographs from skeletal class I, class II and class III patients in post-pubertal period were investigated. Extraoral photographs and cephalometric radiographs were superimposed for each patient before measurements. The photographs were evaluated in three groups: Group A: Skeletal Class I malocclusions (n=85), Group B: Skeletal Class II malocclusions (n=74), Group C: Skeletal Class III malocclusions (n=65). All subjects from the three groups were classified according to the types of the foreheads, and then on the photographs, four different angle measurements (A1, A2, A3, A4) were performed. In addition, the frontal sinus (FS) fields were calculated using the RadiAnt Dicom Viewer (Beta version) on cephalometric and posterior-anterior radiographs. One-way ANOVA, Chi-square, and Student t tests were used for statistical evaluation.

Results: No significant relationships between the skeletal malocclusions and the forehead types were seen. In Group C, the FS fields and A2 angles were higher than in the other groups. No gender-related variations were detected for any measurements.

Conclusions: There was a limited correlation between malocclusions, frontal sinus and forehead morphology. Gender was not an element that creates morphological differences in forehead aesthetics.

Keywords: Forehead aesthetics, frontal sinus, skeletal malocclusions.

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Received : 24.08.2020
Accepted : 25.09.2020

How to Cite: Camcı H. Relationship of Forehead Aesthetics with Frontal Sinus Size and Skeletal Malocclusions. Cumhuriyet Dent J 2020;23:4: 273-282.
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INTRODUCTION

One of the most important element of orthodontic diagnosis and treatment planning is soft tissue analysis.[1] Numerous measurement techniques have been developed on lateral cephalometric radiographs for the comparison of initial and final results.[2,3] In the assessment of facial aesthetics, the focus is generally on the lower two-thirds of the face. The forehead has an important role in facial aesthetics and is located in the first third of the face. However, orthodontists may not focus enough to forehead inclination and morphology when evaluating facial aesthetics. Forehead silhouette is a simple curvature reflecting frontal bone morphology and seen straight, round or angular depending on anatomic structure of the frontal bone. Age-dependent morphological changes occur in facial soft tissue.[4,5] Frontal region is one of these affected areas. Someone with an round forehead subconsciously gives a message that the person is young. Foreheads have an round structure in the first years of life but this curvature becomes straight with age.[6] As an round forehead creates a younger appearance, this is frequently demanded especially by women.[7]

In examination of skeletal class II malocclusions lower third of the face is frequently focal point, whereas in class III cases the focus is the midface region.[8,9] However, facial aesthetics should be considered with all the three parts. It is for the benefit of proper facial reconstruction for those with facial anomaly, syndromic patient, terrible accident, etc.[10–12]

Growth and Development of the Frontal Sinuses

Frontal sinus (FS) is located in the anterior part of the cranial bone and surrounded by two thick cortical bones. Its development begins at the 4th or 5th week of intrauterine life. In the postnatal period, the development continues until puberty or even early adulthood.[13] The first frontal pneumatization of the bone begins gradually at age around one. When the infant is 2 years of age, secondary pneumatization begins. FS continues to grow from 2 years until adolescence and reaches the final capacity in pneumatization.[14] Only, it may start appearing on the computer tomography (CT) after the age of 3. The suture between the two bones becomes ossified during infant growth, and the FS is divided into two as of right and left. Right and left FS could develop asymmetrically and independently, and one side may not develop at all. In one study, the right and left FSs were shown to be between 3% and 5% aplastic.[13] Factors affecting this abnormal development of the FS include factors such as race, geography, and climate.[15,16]

The anatomical structure of the FS is unique to each individual in terms of size and shape. Therefore, It can be used as a biomarker for the identification of individuals, such as fingerprints.[17] Growth and development of the FS are linked to other neighboring cranial structures. For instance, Aslıer *et al.* reported a positive correlation between FS and maximum cranial length and nasal height.[18] Similarly, the FS could affect the frontal slope and surrounding morphology.

The aim of this research was to investigate the relationship between the FS and skeletal malocclusions, and also to analyze its effect on the forehead morphology and types. We therefore hope to help maxillofacial or plastic surgeons achieve better results in facial reconstruction.

MATERIALS AND METHODS

Experimental Groups

This research was approved by Clinical Research Ethics Committee of Afyonkarahisar Health Science University (ID:2019-224). In the power analysis to determine sample size, it revealed that at least 65 patients were required for each group in order to obtain sufficient statistical power ($n>37$, $\alpha=0.05$, and $1-\beta=0.80$). Informed consent forms were obtained from all the patients included in the study. Hand-wrist radiographs were evaluated during the selection of the samples and only patients in the MP3 and Ru phases were included. Two hundred twenty four patients in post pubertal period (43 male with mean age 17±2 and 181 female with mean age 16±2) were divided into three groups based on cepholometric analysis (ANB angle). Group A: Skeletal Class I malocclusions (n = 85), Group B: Skeletal Class II malocclusions (n = 74), Group C: Skeletal Class III malocclusions (n = 65). Patients with the following characteristics were not included in the study: a history of previous orthodontic treatment, mouth

breathing, maxillary transverse deficiency due to mouth breathing, and sinusitis complaints.

Profile Photo And Cephalometric Radiograph Evaluation

Lateral cephalometric radiographs were taken using Planmeca Promax device from all the patients with their true horizontal plane parallel to the ground. Extraoral profile photos were taken by using a Canon 60D-EOS camera with a Sigma 105mm f / 28 EX DG macro lens placed on a tripod 1.5 m away from the patients. Particular attention was paid to the foreheads for being bare when taking the profile photos. According to the photographs, the foreheads were categorized as straight, round or angular (Figure 1).

Figure 1 Types of Forehead. R: Round, S: Straight, A: Angular

The points identified by Andrew (Trichion, glabella, superion, FFA point) were taken as reference for forehead measurements[19] (Figure 2).

Figure 2 Anatomical landmarks on the forehead

The definitions of these landmarks are as follows[20]; Trichion (T): the most superior aspect of the forehead on the hairline in individuals with a relatively straight forehead, Glabella (G): The most inferior aspect of the forehead, Superion (S): Most superior aspect of the forehead when the forehead is either rounded or angular in contour, FFA point: Midpoint between trichion and glabella for straight foreheads or the midpoint between superion and glabella for rounded or angular foreheads. Before measurements, the lateral cephalometric film and the profile photo were superimposed to determine soft tissue landmarks properly by using AudaxCeph Version 5.X orthodontic software for each patient (Figure 3).

Figure 3 Superimposition of lateral photograph and lateral cephalometric radiograph

By taking into consideration Andrew's anatomical landmarks and true horizontal (TH) plane, one angular measurement for straight foreheads and four angular measurements for round or angular foreheads were performed (Figure 4-5).

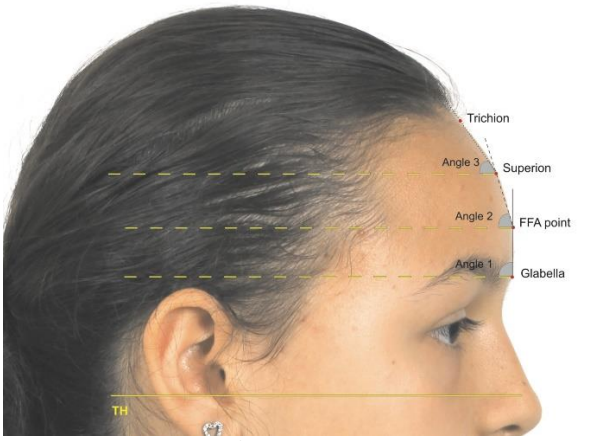

Figure 4 Schematic view of Angle 1 (TH/G-FFA), Angle 2 (TH/FFA-S), Angle 3 (TH/S-T)

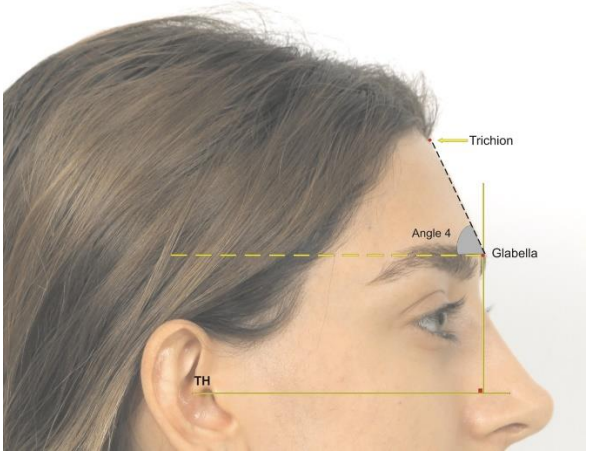

Figure 5 Schematic view of Angle 4 (TH/G-T line)

Angle 1 (A1) : Between TH and G-FFA line, Angle 2 (A2): Between TH and FFA-S line, Angle 3(A3): Between TH and S-T line, Angle 4 (A4): Between TH and G-T line[21]. Cephalometric analysis were performed by the same experienced researcher (H.C.)

Calculation of the Frontal Sinus Fields

The frontal sinus fields of all patients were calculated on both lateral cephalometric (CEP cm^2) and posterior-anterior (PA cm^2) radiographs. For the fields calculation on the radiographs, RadiAnt Dicom Viewer (Beta version) was used. The radiographs were imported into the software in DICOM format. The fields on both CEP and PA radiographs were calculated with the software's 'Closed Polygon' option after the calibration (Figure 6). Patients with aplastic sinus or uncertain sinus borders were excluded from the study.

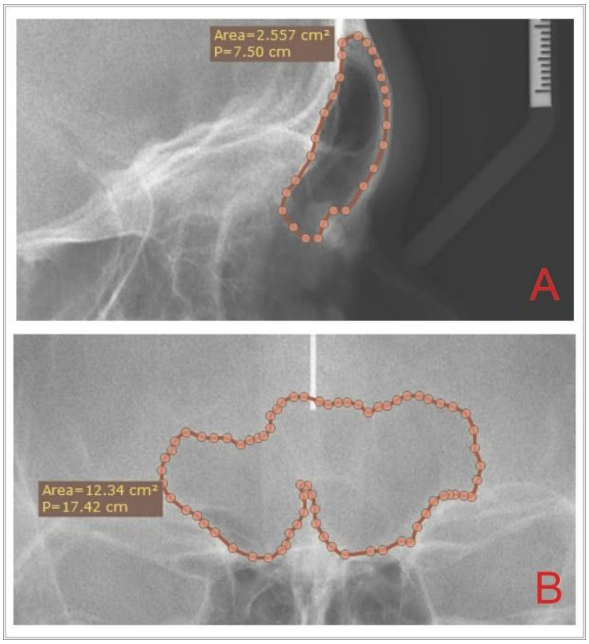

Figure 6: Calculation of FS fields. A: Cephalometric radiograph, B: Posterior-anterior radiograph.

Statistical Analysis

All statistical tests were done by SPSS 22.0 package program (SPSS Inc, Chicago, III). One-way analysis of variance models (ANOVA) and Tukey post hoc test was used to compare the measurements between the three groups. Independent T-test were performed to compare the measurements by gender. The homogeneity of the distribution of forehead types in the malocclusion groups was evaluated by Chi-square test. The level of significance was determined as $P < 0.05$. In ten randomly selected patients, all parameters were re-performed one month later by the same experienced researcher. The intraexaminer variability was tested using these dual measurements via Pearson correlation test (Table 1).

Table 1 Pearson test results,* P<0.01

Measurements	A1	A2	A3	A4	CEPcm^2	PAcm^2
Correlation coefficient	0.998*	0.996*	0.997*	0.950*	0.976*	0.903*

A1: Angle 1, A2: Angle 2, A3: Angle 3, A4: Angle 4, CEP cm2: lateral cephalometric area, PA cm2: posterior-anterior area

RESULTS

In Group C, both the CEP and PA fields of the FS were higher than the other groups. However, a statistically significant difference was only between Group C and Group A. Similarly, the A2 measurement was the highest in Group C, and only a significant difference between Group A and Group C was seen (Table 2).

Table 2 Comparison of measurements by malocclusion groups, *P<0.05

Measurements	A1	A2	A3	A4	CEPcm^2	PAcm^2
	Mean±SDs	Mean±SDs	Mean±SDs	Mean±SDs	Mean±SDs	Mean±SDs
Group A	80.1±4.6^a	76.2±5.0^a	58.4±5.0^a	71.8±5.2^a	1.99±0.8^a	8.46±3.57^a
Group B	80.9±5.7^a	76.4±4.8^{ab}	58.8±5.7^a	73.1±6.0^a	2.11±0.8^{ab}	8.49±3.20^{ab}
Group C	108.0±136.0^a	79.0±4.1^b	60.2±4.8^a	83.7±77.1^a	2.46±0.9^b	9.83±3.24^b

In each column, different superscripts indicate statistically significant difference between groups (p<.05), SDs: Standard deviations, A1: Angle 1, A2: Angle 2, A3: Angle 3, A4: Angle 4, CEP cm^2: lateral cephalometric area, PA cm^2: posterior-anterior area

There was no difference between the groups in the other measurements (P>0.05). Sinus fields of men in both CEP and PA were found to be bigger than females. However, that difference was not statistically significant (Table 3). And also no statistically significant difference between men and women was found in the angular measurements.

Table 3 Comparison of the measurements by gender.

	Gender	N	Mean	Std. Deviation	Significance
CEP cm^2	F	181	2.01	.80	Ns
	M	43	2.82	.98	
PA cm^2	F	181	8.45	3.2	Ns
	M	43	10.61	3.6	
A1	F	109	87.89	70.4	Ns
	M	4	77.77	6.0	
A2	F	109	76.94	4.9	Ns
	M	4	78.60	4.4	
A3	F	109	59.13	5.1	Ns
	M	4	56.10	5.0	
A4	F	181	76.56	46.5	Ns
	M	43	72.18	4.9	

F:Female, M: Male, Ns: No significance, A1: Angle 1, A2: Angle 2, A3: Angle 3, A4: Angle 4, CEP cm2: lateral cephalometric area, PA cm2: posterior-anterior area

The relationship between forehead types and skeletal malocclusions was presented in Table 4. In comparison of the distribution of the forehead types for the each group, no correlation was found between the skeletal malocclusion and the forehead types.

Table 4: Evaluation of the relationship between the types of the forehead and the skeletal malocclusion. Chi-Square test results P>0.05

		Types of Foreheads			
		Angular	Straight	Round	
Group A	Count	19	34	32	85
	Expected Count	18.2	42.1	24.7	85.0
Group B	Count	16	41	17	74
	Expected Count	15.9	36.7	21.5	74.0
Group C	Count	13	36	16	65
	Expected Count	13.9	32.2	18.9	65.0

Individuals with the same forehead types in all groups were compared with each other. In other words; class I, class II, class III patients with straight (Cl1s, Cl2s, Cl3s) foreheads were compared among themselves, the patients with round foreheads (Cl1r, Cl2r, Cl3r) among themselves and individuals with angular foreheads (Cl1a, Cl2a, Cl3a) among themselves.

When the measurements of angular forehead type (Cl1a, Cl2a, and Cl3a) were compared for all values, no difference was found between groups (P>0.05). The comparison of straight foreheads (Cl1s, Cl2s, Cl3s) in terms of CEP, PA fields, and angle measurements did not state any significant difference. In the comparison of round foreheads (Cl1r, Cl2r, Cl3r), Cl3r was observed to be

significantly bigger than Cl1r only in terms of CEP fields (Table 5).

Table 5: Comparison of round foreheads between the groups, *: P<0.05

Measurements	A1	A2	A3	A4	CEPcm^2	PAcm^2
Groups	Mean±SDs	Mean±SDs	Mean±SDs	Mean±SDs	Mean±SDs	Mean±SDs
Cl1r	81.0±4.2^a	76.1±4.9^a	59.8±5.0^a	71.7±4.1^a	1.6±0.7^a	6.9±3.2^a
Cl2r	81.2±5.4^a	75.0±5.0^a	60.4±5.4^a	71.8±4.7^a	1.7±0.5^a	7.6±3.0^a
Cl3r	129.5±182.8^a	78.4±4.1^a	61.6±5.1^a	113.7±155.0^a	2.23±0.8^b	7.5±1.7^a

In each column, different superscripts indicate statistically significant difference between groups (p<.05), SDs: Standard deviations, A1: Angle 1, A2: Angle 2, A3: Angle 3, A4: Angle 4, CEP cm2: lateral cephalometric area, PA cm2: posterior-anterior area

DISCUSSION

The expectations and demands of humans from both dental aesthetic procedures and facial cosmetic surgery are increasing especially in the 21th century.[22,23] This situation emphasizes the importance of evaluating facial aesthetics as a whole during orthodontics diagnosis and treatment. Though the forehead, which constitutes the upper 1/3 of the face, is an important part of this whole, there are limited studies in the literature about forehead aesthetics.[20,24]

Malocclusions or types of anatomical structures might show different distribution between the genders because of sexual dimorphism.[25–27]A female could perceive the same malocclusion or normal anatomical structure differently than a male because of the perception of aesthetics and attractiveness.[28] In our study, no correlation was noted between gender and forehead slope or type. However; in terms of maximum patient satisfaction, extra attention is required to analyze the anatomical differences between the genders and the variability of aesthetic perceptions. Another factor that has impact on the aesthetic perception or anatomical structures change is age. For example; an older person's forehead has more inclination than a younger indiviual.[29] Thus, male and female patients in post-pubetal period were included in the study.

Forehead slope and aesthetics could be evaluated with different measurement techniques. Hwang *et al.* evaluated forehead aesthetics by measuring an angle called FHA (forehead angle) on the cephalometric radiograph.[20] This angle was located between the nasion perpendicular line (NP) and the line passing through the intersection point of the NP and forehead and the soft tissue of the glabella. However, this angle might vary depending on the morphology of the nasal bone. These evaluations in the anterior-posterior direction contain limited information about forehead aesthetics. In addition to this assessment in the sagittal direction, the forehead width (distance between lateral canthus) in the coronal view might be evaluated. Lee *et al.* have also studied forehead aesthetics by measuring forehead surface area or volume on 3D computed tomography (CT) images.[30]

Bad oral habits have negative side effects on dentofacial structures.[31,32] These habits change the growth direction of anatomical structures. For instances; due to mouth breathing the face becomes long and narrow (adenoid face type or long face syndrome).[33,34] Similarly, inadequate ventilation or chronic infection of the frontal sinus as an anatomical structure adjacent to the forehead, might affect the forehead inclination. Further studies are needed to determine whether this relationship exists. For these reasons, the patients with mouth breathing or chronic sinusitis were excluded from the study.

Forehead reflects the curvature of the frontal bone, but the soft tissue thickness that covers the bone surface is also a factor that might affect the aesthetic of the forehead.[35] In the study of Kamak *et al.*, soft tissue thickness differences among skeletal malocclusions were observed.[36] Additionally, Hwang *et al.*[37] reported that gender plays a role in soft tissue thickness. Therefore the thickness of soft tissue could be a factor that influences the findings.

The norm values of cephalometric measurements, soft tissue analysis or aesthetic

criterias show racial differences.[38,39] There might be different aesthetic expectations might be seen in each nation.[40,41] Therefore, with a limited study on a particular race, it is not possible to make a clear statement about the presence or absence of the relationship between the forehead slope, types and skeletal malocclusions.

The types of the skeletal malocclusions is closely related with the parameters located the lower and middle 1/3 of the face.[42,43] Similarly, in our study, a significant relationship was found between the types of malocclusions and the some parameters of forehead slope.

The frontal sinus (sphenoid, ethmoid, maxillary, and frontal) is one of the four paranasal sinuses in the head and is the only paranasal sinus absent at birth. These four sinus development periods are different from each other and vary by gender.[44] Spaeth *et al.* evaluated paranasal sinuses using CT images in the patients aged 0 to 25 years. They reported that the development of FS was completed at the age of 18 in boys, 15-16 in girls, and the final sizes were higher for boys.[16] However, in our study, no relation was found between gender and frontal sinus size. Racial and regional variations in which studies are conducted may be the explanation for this. The sinus assessment technique may be another factor. The size of the FS was assessed in our study by calculating the field. However, the method used in the other study was to measure the maximum width (mediolateral distance) and length (ventrolateral distance) of the FS.

After puberty, FS reaches the final proportions.[45] Its average volume is 10cc, which ranges from 0 to 37cc.[46] The volume of FS varies considerably in individuals of the same age.[47] Moreover, the right and left frontal sinuses of the same individual can develop asymmetrically and independently.[46] FS might be aplastic at varying rates in different populations.[48,49] This rate can reach 40 percent in some societies.[50] The patients with aplastic FS were excluded from the study in our research. However, there was a considerable variation between the minimum and maximum values of the field calculations (Table 6). We believe that this may have had an impact on the findings of the study.

Table 6. Mean values of the CEP and PA measurements. There was a considerable discrepancy between the maximum and minimum values.

		Mean	Std. Deviation	Minimum	Maximum
CEP cm^2	Group A	1.99	.86	.31	4.50
	Group B	2.11	.84	.70	4.70
	Group C	2.46	.94	.79	7.14
	Total	2.17	.89	.31	7.14
PA cm^2	Group A	8.46	3.57	.37	16.96
	Group B	8.49	3.20	1.58	20.12
	Group C	9.83	3.24	3.06	22.43
	Total	8.87	3.40	.37	22.43

CEP cm2: lateral cephalometric area, PA cm2: posterior-anterior area

The relationship between the size of paranasal sinuses and the types of skeletal malocclusion is controversial. Dhiman *et al.* reported that the frontal sinus area was larger in skeletal Class III malocclusion than in skeletal Class I and Class II.[51] This is consistent with the findings of our study. However, Sabharwal et al. suggested that the frontal sinus size was independent of the type of malocclusion.[52]

Frontal bone is one of the strongest structures in the face.[53] Frontal bone fracture is rare because it is more resistant to mechanical forces than other maxillofacial bones. However, motor vehicle accidents, gunshot, assault or falls could cause frontal bone fractures. High-impact traumas and compressive forces deform the convexity of the front bone into concavity that significantly impairs facial aesthetics. As a consequence, the frontal bone needs to be reconstructed functionally and

esthetically. The main goal of treatment is therefore to create a safe frontal sinus and restore the facial contour. The findings of the study could be useful for a plastic or maxillofacial surgeon who needs to reconstruct the face of a severely damaged patient.

Our research was performed without gender considerations and the FS was evaluated by 2-dimensional field measurement. Additionally, due to its difficult determination in the 2D film, the frontal sinus septum was ignored in calculations. The results could have been affected by these limitations. Further studies are needed to evaluate the relation of forehead aesthetics, malocclusions, and FS with more advanced techniques and more specific and larger examples.

CONCLUSIONS

- There was a limited correlation between malocclusions, frontal sinus and forehead morphology.
- Gender was not an element that creates morphological differences in forehead aesthetics.

CONFLICTS OF INTEREST STATEMENT

The author declares no conflict of interest in this study.

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.776746

Original research

PREDICTIVE INDICATORS FOR MANAGING DENTAL CARE IN PATIENTS WITH DIFFERENT ABILITIES

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ABSTRACT

Objectives: To determine predictive indicators for dental health management of patients with different abilities, according to a new screening protocol.

Materials and methods: This was an observational, analytical, retrospective, cross-sectional study. The sample consisted of all patients with different abilities, diagnosed with autism, Down syndrome, cognitive deficit, cerebral palsy and others, who received care between 1999 and 2019 at the Oral Health Service of the *Centro Ann Sullivan del Peru* and met the inclusion criteria. Data were obtained from the clinical histories to determine the predictive indicators for managing dental care in these patients. A multivariate statistical analysis was performed using binary logistic regression test. The research project was evaluated by the Research Project Reviewing Committee and then referred to the Human Ethics Committee from a university, securing the relevant approvals.

Results: Of the 589 cases evaluated, mean age was 14.5 years, 67.9% were male; systemic diagnosis of different abilities was autism in 62%, the most frequent treatment was caries (52.1%); and final management of these patients was conscious in 96.4% of the cases. With regard to type of classification and horizon, 67.1% of the cases corresponded to classification A, with 35.9% of these in horizon I. With regard to diagnosis of different abilities, autism presented 40.2% of the cases in classification A and horizon I. A predictive equation of 97.1% probability of whether or not sedation was used was obtained. Regression analysis showed that this model has high sensitivity (100%) and low specificity (15%).

Conclusions: Findings show that the type of management for dental care was conscious in nearly all the cases dealt with. This was significantly influenced by the Classification and Horizon Screening Protocol indicator, which established the best predictive model for whether or not to use sedation. The model adequately classifies patients who do not require sedation.

Keywords: Dental care for disabled, dental anxiety, disease management, conscious sedation.

Received : 04.08.2020
Accepted : 16.10.2020

How to Cite: Cava-Arangoitia OCJ, Guevara-Canales JO, Morales-Vadillo R, Cava-Vergiú CE, Uribe MR, Ortega LM. Predictive Indicators for Managing Dental Care in Patients with Different Abilities. Cumhuriyet Dent J 2020;23:4: 283-290.
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INTRODUCTION

Patients with different abilities include people who have physical, developmental, mental, sensorial, behavioral, cognitive or emotional impediments. These limiting conditions may require differential medical management, intervention of special medical care and/or use of specialized services or programs.[1,2] People with different abilities may present high prevalence of oral diseases such as dental caries[3]; possibly due to barriers in communication regarding their own health education and poor oral hygiene habits.[4,5] The term "different abilities" was coined to focus attention on what these people can do rather than on what they cannot do within the community.[6] A visit to the dentist's office will always be an unwanted experience, which may be frightening or traumatic for many patients[7-9] The more so for patients with different abilities, who may display a range of reactions, including nervousness, sweating hands, anxiety and even panic.

The different attitudes and behaviors will be more marked at the beginning of dental treatment. This may overwhelm the operator, creating difficulty in making a prognosis for that treatment, which may lead to the operator giving up on providing the patient with an opportunity to be treated while conscious.[10] It would therefore be ideal and timely to find a way to classify patients according to their behavior in order to predict the possibility of providing oral health care while they are conscious. This is why it is presented detailed dental treatment while conscious for patients with different abilities, based on the application of a new protocol for dividing patients, according to behavior, into Classification (A, B or C) and Horizon (I, II, II, IV or V). This classification protocol is based on more than 32 years' experience of the main author of this study in dental care and follow-up of these patients, analysis of the performance of the different personalities, characters and reactions, and the advice and accounts provided by parents about their children. The screening protocol seeks to achieve a planned methodology for providing dental care while the patient conscious, by designing a technique that is simple to apply and which considers the individual characteristics of any patient with different abilities (child, teenager or adult). This would enable the creation of a "Classification and Horizon Screening Protocol according to patient behavior".

The **Classification** is a division according to behavior for rapid recognition of patient type, by making a good clinical history and considering the family living with the patient. It divides patients into: Group A: Patients who could initially be considered the easiest to treat, with clinical operator working calmly and comfortably without need to resort to physical restraint, conscious sedation or full sedation. Patient is cooperative, extroverted, with self-help skills and with prior positive dental experience. Group B: Patients opposite to Group A, who apparently display very uncooperative behavior and from whose clinical history may be deduced multiple understandable causes for their nervous, restless, bored, intolerant and sometimes aggressive or self-aggressive behavior. Group C: Patients who may belong to Group A or B, but who, due to other reasons, require care in the operating room, e.g., due to serious diseases such as cancer, or to family reasons such as unexpected traveling which requires the treatment to be completed sooner than planned.

The **Horizon** is a subclassification which does not relate to patient behavior, but to the level of complexity the treatment may reach and whether it is possible to complete it while the patient is conscious. The criterion is to begin with the simplest part and move forward according to complexity, in accordance with patient performance and cooperation. Horizon I is the simplest level, and Horizon V is the most complex. Horizon provides information on how far the treatment can advance while the patient it is conscious, to provide quality of life to the patient and family.

This study contributes to the professional theoretical knowledge of dentists by showing what events can influence the management of dental care by means of the new Classification and Horizon screening protocol for patients with different abilities. It makes a social contribution by providing the possibility of these patients not

being exposed to sedation or general anesthesia, which might involve risks and/or complications. The aim of this study was to determine predictive indicators for managing dental care in patients with different abilities according to a new screening protocol using the indicators classification, horizon, need for readiness prior to dental treatment and type of treatment.

MATERIALS AND METHODS

Ethical aspects

The research project was evaluated by the Research Project Reviewing Committee for approval of the *Facultad de Odontología, Universidad de San Martín de Porres* (FO-USMP), Peru, (Certificate No. 010-2020-CRPI/INVE-FO-USMP) and then sent to the Human Research Ethics Committee of FO-USMP (Certificate No. 002-2020-CEI/INVE-FO-USMP), where it secured approval.

The clinical histories of patients who met the inclusion criteria were registered under a code. Researchers undertook to preserve the confidentiality of all data recorded and/or obtained from patients, clinical histories or outcomes, to ensure that they would only be handled by the principal investigator, while the other participating investigators would not have access to them. The investigators declare no conflict of interest.

Methodological design

This was an observational, analytic, retrospective, cross-sectional study. The sample consisted of patients with different abilities diagnosed with autism, Down syndrome, cognitive deficit, cerebral palsy or other conditions, who received care between 1999 and 2019 at the Dental and Oral Health Service of Centro Ann Sullivan of Peru (CASP) and who met the inclusion criteria. Sample size was approximately 627 patients of whom 589 met the inclusion criteria. Inclusion criteria were having been duly medically diagnosed as having some different ability condition, and receiving dental care at CASP accompanied by their parents on the visit. Exclusion criteria were patients with different abilities whose parents or guardians would not cooperate with the order of the protocol for care of their children and patients with any kind of systemic disease requiring immediate hospitalization.

Data collection

Data from the clinical histories of patients who met the selection criteria were evaluated. These data were used to identify predictive indicators for managing dental care of the patient with different abilities. The clinical histories of patients medically diagnosed as having different abilities were reviewed, thus providing data related to Classification and Horizon, need for previous readiness and treatment type.

Statistics

The data recorded on index cards for data collection were transferred to Microsoft Office Excel software (Microsoft, Inc., Redmond, Washington, USA) to create a database.

Categorical variables were described in tables of absolute and relative frequencies, represented in graphs expressed in percentages. The numerical variable Age was described with measures of central tendency and dispersion, and graphed using a histogram. To determine predictive indicators for managing dental care in patients with different abilities, a multivariate statistical analysis was used by means of a binary multiple regression test. Values were considered statistically significant for $p<0.05$.

Analysis was performed on a computer with Windows® Operative System (Microsoft, Inc., Seattle, Washington, USA) using the SPSS software package version 25 in Spanish.

RESULTS

627 cases were found to have received care at the CASP Dental and Oral Healthcare Service between 1999 and 2019, of which 38 did not meet the total data required for statistical analysis. Of the 589 cases evaluated, ages ranged from 1.3 to 63 years, mean age was 14.5 years; 67.9% were male, and the most common systemic diagnosis of different abilities was autism in 62% of the cases. The most frequent treatment was caries (52.1%). Final management of these patients was conscious in 96.4% of the 589 cases evaluated. Table 1 provides the descriptive data.

Table 1: Descriptive data of factors that influence the type of dental care management for patients with different abilities

				Frequency	Percentage	Statistic
Sex	Male			400	67.9	
	Female			187	31.7	
	Lost			2	0.3	
Age (years)	Mean					14.456
	95% of C.I. for the mean	LL				13.528
		UL				15.384
	Median					10.000
	SD					11.410
	Minimum					1.3
	Maximum					63.0
Different abilities diagnosis	Autism			365	62.0	
	Down syndrome			88	14.9	
	Cognitive deficit			51	8.7	
	Cerebral paralysis			14	2.4	
	Asperger			4	1.2	
	PDD			7	1.2	
	West syndrome			6	1.0	
	OD			51	8.7	
Classification and horizon	A		I	142	71.0	
			II	86	63.2	
			III	114	63.7	
			IV	36	63.2	
			V	12	100	
	B		I	43	21.5	
			II	44	32.4	
			III	52	29.1	
			IV	18	31.6	
	C		I	15	7.5	
			II	6	4.4	
			III	13	7.3	
			IV	3	5.3	
Dental treatment	Caries	No		281	47.7	
		Yes		307	52.1	
		Lost		1	0.2	
	Sealants	No		577	98.0	
		Yes		11	1.9	
		Lost		1	0.2	
	Fluorine	No		490	83.2	
		Yes		95	16.1	
		Lost		4	0.7	
Need for preparation		No		47	8.0	
		Yes		541	91.9	
		Lost		1	0.2	
Type of management	Conscious			568	96.4	
	Sedation			21	3.6	

CI = Confidence interval, LL = Lower limit, UL = Upper limit, SD = Standard deviation, PDD = Pervasive developmental disorder, OD = Other diagnoses.

Table 2 shows type of Classification and Horizon according to systemic diagnosis, where 67.1% of the cases corresponded to classification A, with 35.9% of these in horizon I, and Table 3 show how dental care was managed.

Table 2: Type of Classification and Horizon according to diagnosis of different abilities

Classification and Horizon		Autism		Down syndrome		Cognitive deficit		Cerebral palsy		Asperger		PDD		West syndrome		OD	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
A	I	102	40.5	19	31.7	9	34.6	2	22.2	1	14.3	2	40.0	1	33.3	6	21.4
	II	56	22.2	11	18.3	5	19.2	1	11.1	3	42.9	2	40.0	1	33.3	7	25.0
	III	72	28.6	16	26.7	7	26.9	5	55.6	2	28.6	1	20.0	1	33.3	10	35.7
	IV	18	7.1	7	11.7	5	19.2	1	11.1	1	14.3	0	0	0	0	4	14.3
	V	4	1.6	7	11.7	0	0	0	0	0	0	0	0	0	0	1	3.6
B	I	21	22.6	8	36.4	3	17.6	1	25.0	0	0	1	100	1	33.3	8	47.1
	II	31	33.3	5	22.7	3	17.6	2	50.0	0	0	0	0	1	33.3	2	11.8
	III	32	34.4	8	36.4	5	29.4	1	25.0	0	0	0	0	1	33.3	5	29.4
	IV	9	9.7	1	4.5	6	35.3	0	0	0	0	0	0	0	0	2	11.8
	V	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C	I	6	33.3	1	33.3	5	62.5	0	0	0	0	1	100	0	0	2	33.3
	II	3	16.7	1	33.3	0	0	1	100	0	0	0	0	0	0	1	16.7
	III	9	50.0	1	33.3	1	12.5	0	0	0	0	0	0	0	0	2	33.3
	IV	0	0	0	0	2	25.0	0	0	0	0	0	0	0	0	1	16.7
	V	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

PDD = Pervasive developmental disorder, OD = Other diagnoses.

Table 3: Type of dental care management according to diagnosis of different abilities

	Conscious		Sedation		Total	
	N	%	N	%	%	N
Autism	356	62.7	9	42.9	365	62.0
Down syndrome	87	15.3	1	4.8	88	14.9
Cognitive deficit	45	7.9	6	28.6	51	8.7
Cerebral palsy	14	2.4	0	0	14	2.4
Asperger	6	1.1	1	4.8	7	1.2
PDD	7	1.2	0	0	7	1.2
West syndrome	6	1.1	0	0	6	1.0
OD	47	8.3	4	19.0	51	8.7

PDD = Pervasive developmental disorder, OD = Other diagnoses.

The Forward Wald binary logistic regression method was used for statistical analysis to determine the best statistic predictive model for dental care management. Type of dental health care management was considered as dependent variable, while independent variables were sex, diagnosis, classification, horizon, caries treatment, use of sealants and fluorine application. A 5% significance level was considered ($p<0.05$). A significance value of $p<0.001$ was obtained, which indicates that the model as a whole has significant fit in the second step of introduction of influencing variables using the Forward Wald method. The model reports that patients who require dental care management under sedation are those who have the independent variables "Classification" and "Horizon" as triggering factors. These variables were also found to be

significantly associated to the dependent variable through a predictive equation with 97.1% correct classification, described as the probability of using sedation, as follows:

Y = -17.151 + 13.898 (Horizon I) + 14.301 (Horizon II) + 16.487 (Horizon III) + 17.469 (Horizon IV) + 1 (Horizon V) - 4.051 (Classification A) - 1.431 (Classification B) + 1 (Classification C).

Probability of using sedation = $\frac{1}{1+e^{-y}}$

In addition, regression analysis showed that our model has high sensitivity (100%) and low specificity (15%), so it adequately classifies patients who do not require sedation and deficiently classifies patients who do require sedation.

DISCUSSION

In 2018, the World Health Organization estimated that over a billion people (about 15% of the world population) have some form of disability.[11] This is higher than the previous WHO estimate, which was 10% in the 1970s. Thus, disability is part of the human condition, since nearly everyone, at some time in life, will have a temporary or permanent disability.[6]

With regard to oral health, Faulks *et al*.[12] have defined people who require special dental care as those with a disability or restriction to activity which directly or indirectly affects their oral health, within their personal and environmental context, using the International Classification of Functioning, Disability and Health model.

Many studies from different parts of the world have established that people who require special dental care have poor oral health and high levels of unmet needs in terms of prevention and periodontal, restorative and functional treatment.[13-16] Indeed, dental care is one of the most frequent unmet health needs for people with special needs.[17,18]

For many people with intellectual or developmental disabilities, available treatment consists mainly of emergency extractions, often under physical immobilization[19] and general anesthesia, rather than comprehensive planned care.[14,20,21]

Patients included among those with special needs should have access to a *dental home* for primary oral health care. This refers to the continuous relationship between that patient and his/her dentist, including all aspects of oral health, provided in a comprehensive, accessible, coordinated, family-centered manner.[22]

Dentists may lack some knowledge and skills required to meet the oral health needs of these patients, which may explain their lack of confidence and unfavorable attitudes, and may be related to the time spent with these patients or to being limited to merely symptomatic, rather than comprehensive treatment.[23] Thus, dentists who regularly manage patients with special needs could be a source of relevant information for research into obstacles and recommendations on how to overcome those obstacles.[22] In addition, the specialty Dentistry for Special Patients should be recognized, to enable seamless primary and secondary oral healthcare services to be organized and provided to these people.[24] Unfortunately, most undergraduate programs in North America do not include minimum didactic and practical clinical experience in the care of people with disabilities.[25]

When a patient is treated while conscious, he/she is not exposed to any kind of sedation which could involve some level of risk. The key to success is to provide friendly care by learning about patient's motivations, e.g., finding out about a patient's favorite music and playing it during the clinical session, or giving the patient a warm welcome. Thus, from 1982 to 2014 in Peru, dental treatment for people with different abilities performed in operating rooms and under general anesthesia has descended from 95% to 4%.[26]

CONCLUSIONS

The results show that in almost all the cases considered, dental care was managed while the patient with different abilities was conscious, and this is significantly influenced by the indicator provided by the division regarding behavior and the level of complexity in the dental treatment,

called the Classification and Horizon Screening Protocol. This indicator provided the best prediction for whether or not sedation was needed, and the model adequately classifies patients who do not require sedation.

ACKNOWLEDGEMENT

None

CONFLICT OF INTERESTS

The authors declare no conflict of interest with potential applications or effects of the results of this research.

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.746201

Original research

PREDICTORS OF DENTAL ANXIETY IN CHILDREN: SELF-PERCEPTION AND PARENTAL FACTORS

ABSTRACT

Objectives: To identify the factors involved with dental anxiety in children.

Materials and Methods: A cross-sectional study was conducted with children aged 8–12 years and their parents. They were interviewed and examined. Oral health and treatment need perceptions were assessed using a self-reported index. Information about DMFT and dental anxiety scores were collected from children and their parents. Regression analysis was obtained to find association between anxiety in children scores and all variables studied. Spearman's correlation coefficients were calculated to determine possible correlations between variables.

Results: Significant association was observed between the child's oral health self-perception, DMFT and parents' anxiety. It was found a significant correlation between dental anxiety and oral health self perception to children and adults. Moreover, dental anxiety in children is positive correlated to DMFT.

Conclusions: Dental anxiety in children is affected by parental dental anxiety. Moreover, it seems child's dental anxiety is influenced not only by DMFT scores but also by oral health self-perception.

Keywords: Dental anxiety, oral health, self-assessment, child, parents.

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Received : 01.06.2020
Accepted : 06.10.2020

How to Cite: Fernandes EC, Tôrres ACSP, Nóbrega FJO, Santos PB. Predictors of Dental Anxiety in Children: Self-Perception and Parental Factors. Cumhuriyet Dent J 2020;23:4: 291-299.
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INTRODUCTION

Dental anxiety is a common condition, which can affect people of all ages, mostly children and adolescence.[1,2] The prevalence of dental anxiety in children varies across international literature ranging from 3% to 43% depending on populations and age groups. The development of dental anxiety has a multifactorial and not well-known background. It can influence patient management, dental attendance and can persist even in adulthood, leading to dental avoidance.[3,4]

Existing evidence about parental effect and oral health condition on anxiety in children are conflicting. Even though many studies have showed a positive correlation between those and dental anxiety.[5,6,7] It seems there is a vicious circle, in which children with dental anxiety are more prone to delaying their treatment, thereby leading to worsening of their problems and fueling the dental anxiety that was already present.[8]

Patient perception of oral condition has multidimensional nature and it is not only influenced by clinical conditions but also by psychosocial features, as dental anxiety.[9] People with dental anxiety can report a negative perception about their oral health influenced by misconceptions about dental treatment, regardless of their real context.[10]

Identifying potentially modifiable factors that may affect the interaction between the negative self-perception and clinical status is crucial to achieve a patient management success. Therefore, assessing the background of anxiety in children can determine the most appropriate and effective management strategy. So, the dentist plays an important role in decreasing or eliminating dental anxiety in the patient and promoting good general oral hygiene habits.[10,11]

MATERIAL AND METHODS

Study design and subjects

The study participants included 230 people, who were 115 school-children (51 girls, 64 boys) ranging 8-12 years old from schools and 115 respective parents (81 mothers 34 fathers). The average children's age was 9.2 years (SD = 1.2).

From August 2015 to August 2017, data were collected from children enrolled in public schools. Sample was selected including all schools of the city. In each school, all children ranging 8-12 years were invited to participate, and their parents as well. Children and their parents who agreed were included in the data collection at school, which consisted of an interview and clinical oral examination.

A member of the research team explained the questionnaire instructions to the children and their parents, who individually completed each assessment. Moreover, a researcher was available for further clarification or explanation of the survey whether needed.

Informed consent from parents and school staff was obtained for all participants. This study received ethical approval from the local ethics committee of authors institutions (No. 1448908).

Dental anxiety scores

An adaptation of the 5-item Modified Dental Anxiety Scale (MDAS) for the Portuguese language was used to all adults. Participants reported levels of dental anxiety on a 5-point Likert scale, with 1 meaning, 'relaxed/not worried' and 5 meaning, 'very nervous/very worried'.[12]

Faces representing the level of anxiety were included on the Likert scale to facilitate the children's understanding of the questionnaire items (MDAS(f)). A total score was calculated by adding the child's responses, which ranged from 5 to 25 (higher scores reflected greater anxiety levels).[13]

Dental health status: An experienced paediatric dentist conducted a clinical examination and determined the dental status of each participant using a flat-surface mouth mirror and gauze. The index for decayed, missed and filled primary teeth (dmft), and permanent teeth (DMFT) were recorded.[14]

Self-assessed oral health status: Children and their parents were asked, 'How would you describe your dental status?' The response format included three options: Good, Fair or Bad.

Treatment need perception: Those information were obtained from the question: "As for today, in your opinion, how many treatments do you need?"

The response format included three options: A lot, Some or Not at all.

Data analysis

The data obtained on the questionnaires and scales were analyzed with the aid of the Statistical Package for the Social Sciences (SPSS®, Version 15.0, Chicago, USA). Regression analysis was obtained trying to find association between anxiety in children scores and all variables studied. Spearman's correlation coefficients were calculated to determine possible correlations between variables. The level of significance was set to 5% ($P \leq 0.05$).

RESULTS

A hundred fifteen children and their parents were included in this study. Mean of dental anxiety in children and their parents was 20.57 and 11.32, respectively. Table 1 shows the distribution of the sample and descriptive analyses to all variables. Most of all people consider their dental health needs to be 'some', and dental status to be 'fair'.

Table 1. Descriptive analysis of the investigated variables

Variables	Subjects		
	Average		**Sd**
Child's Anxiety	20.57		5.69
Parental Anxiety	11.32		4.56
Dmft	3.83		1.75
Dmft	6.42		2.38
	Percentual Of Subjects		
	Good	**Fair**	**Bad**
Child's Self-Assessed Oral Health Status	32 (28%)	75 (65%)	8 (7%)
Parental Self-Assessed Oral Health Status	44 (38%)	65 (56%)	6 (6%)
	Not At All	**Some**	**A Lot**
Child's Treatment Need Perception	30 (26%)	71 (62%)	14 (12%)
Parental Treatment Need Perception	**34 (30%)**	**66 (57%)**	**15 (13%)**

Association between anxiety in children and all independent variables is shown in Table 2. Significant association was observed in the analysis between the child's oral health self-perception, dtmf and parental anxiety, considering anxiety in children as dependent variable ($P<0.005$).

Table 2 - Results of multiple linear regression analysis, considering child anxiety as the dependent variable.

Variables	Child's Anxiety
	P
Parental Anxiety	0.016*
Dmft	0.001*
Dmft	0.074
Child's Self-Assessed Oral Health Status	0.039*
Parental Self-Assessed Oral Health Status	0.479
Child's Treatment Need Perception	0.151
Parental Treatment Need Perception	0.903
R= 0.468	R2 = 0.219

*Statistically significant at $P < 0.05$.

The data also demonstrate a significant correlation between dental anxiety and oral health self-perception to children and adults. Moreover, children's dental anxiety is positive correlated to DMFT. To all people, oral health self-perception is correlated to treatment need perception (Tables 3 and 4).

Table 3. Correlation coefficients among parental anxiety, DMFT index, Self-assessed oral health status and treatment need perception – Spearman's (r) correlation coefficients.

Variable	Parental Anxiety	Dmft	Parental Self-Assessed Oral Health Status	Parental Treatment Need Perception
Parental Anxiety				
Dmft	0.0626 P=0.510			
Parental Self-Assessed Oral Health Status	-0.2041	-0.1507		
	P=0.030*	P=0.111		
Parental Treatment Need Perception	- 0.0237	- 0.2136	0.3866	
	P= 0.803	P=0.023*	P=0.000*	

*Statistically significant at P < 0.05.

Table 4. Correlation coefficients among children's Anxiety, dmft index, Self-assessed oral health status and Treatment need perception – Spearman's (r) correlation coefficients.

Variable	Child's Anxiety	DMFT	Child's Self-Assessed Oral Health Status	Child's Treatment Need Perception
Child's Anxiety				
DMFT	0.3162 P= 0.001*			
Child's Self-Assessed Oral Health Status	-0.1953	-0.1777		
	P=0.038*	P=0.060		
Child's Treatment Need Perception	0.095	- 0.0191	0.3968	
	P=0.312	P=0.841	P=0.000*	

*Statistically significant at P < 0.05.

DISCUSSION

Dentists should know more about aetiology of anxiety to gain a better understanding of it and therefore to help paediatric patients and their families to cope with dental anxiety. Indeed, the evaluation of anxiety should be part of a paediatric anamnesis.[15,16,17] An understanding of psychologic and behavioural sciences has long been a central part of dental education and essential for a better clinical practice. Patients who have anxiety regarding their dental health and treatment needs, tend to delay appointments and avoidance behaviour towards oral care.18 For this reason, evaluation of anxiety levels and treatment needs can be supportive for a good oral health maintenance and psychological improvement of children. Once child behaviour is changed, it is possible to prevent not only diseases, but also help to streamline appropriate oral hygiene habits and regular dental appointment.[12,19,20]

There are many instruments available for the assessment of dental anxiety, both in children and adults. In this study, the Modified Dental Anxiety Scale[12], a reliable and quick five item scale to administer was adopted for all parents. It has a scale ranging between mild, moderate, and phobic levels of anxiety.[21] Although there are some measures which allow for the particular identification of characteristics of the individual's dental anxiety, the MDAS provides a quick and easy-to-use screening tool. It has been found to be acceptable both to patients and dentists.[22-24]

A version is also available for use with children[25] (Modified Child Dental Anxiety Scale, MCDAS), although there are various scales and techniques used in order to assess anxiety in children. In the current study, anxiety in children rates were recorded by MCDAS – faces[13], as it has been shown to be reliable and valid scale.[1,26,27] Since children are limited by the level of cognitive functioning required to complete the various other numeric rating scales, we understand a self-reported and an easy index for children are necessary.[28] The faces version of the MCDAS has been tested in a series of studies with children. Aminabadi *et al.*[29] and Fakhruddin *et al.*30 have also used this scale for recording anxiety in their studies.[31,32] And a good test-retest reliability was demonstrated in children between 8 and 12 years old.[13]

The aetiology of dental anxiety is believed to be multifactorial with sociodemographic status playing important roles as risk factor.[33] That means homogeneity of children and parents in terms of sociodemographic issues is crucial to avoid confounding factors. In this study, only one type of school was included to obtain a better representation of children and their parents, regarding economic and demographic factors. This aspect reinforces the robustness of our results.

An understanding of the factors underlying the aetiology and maintenance of dental anxiety and how children learn it may assist dentists to reduce this fear in children. It has been suggested that fears are learned by observing the behaviour of other individuals and the outcomes of those behaviour. Parents usually serve as a model for development of anxiety, and parental behaviour towards oral health may be considered a modelling process in which children imitate adult models.[34-37]

In the present study, associations were found between the children and parents' dental anxiety scores, although both showed lower dental anxiety score. That lower anxiety score was unreported in other studies, although no information was shared about dental experience, history of dental phobia, history of dental pain, and diagnosed behaviour disorders.[10,38-41] Those aspects are known to cause dental anxiety.[42] Our findings indicate the parents had an influence on anxiety in children level, in a good or bad way. Indeed, negative or positive dental attitudes and experiences transmitted by parents are related somehow to anxiety in children, even though there is not an agreement in the international data about this finding. Previous studies report no association between parental and MCDAS scores[37,41,43-45], whereas others found that parental anxiety was associated with children anxiety.[3,46,47]

That difference can be explained by the fact that mothers are usually more involved in childcare.[37] Our findings showed the children were most often accompanied by their mothers during dental care (70,44%). Previous studies found that maternal anxiety level was predictive of dental anxiety score in children, and these findings are supported with our results.[15,37,48]

A recent literature review[49] suggested that parental dental anxiety may affect child dental anxiety by transmission of anxiety model and verbal learning that it would cause decreased self-efficacy for pain and affect perceptions of dental stimuli. Moreover, parental anxiety could also impact a child's learning history when it results in avoidance of dental appointments.

Self-reported measures regarding oral health and treatment need are cost- and time-effective.[50,51] It is hypothesized that they can give an opportunity to determine dental health for a single person and populations also. This kind of evaluation can help people who avoid clinical examination, for reason of either cost or fear of embarrassment of their oral condition.[18,52]

Our findings show there is no correlation between DMFT/dmft scores and self-reported dental status for children and their parents. In general, the present study results indicate that most of the children and their parents consider their dental health needs to be 'some', and dental status to be ‘fair’ which it was also reported before.[18] Even though, people tend to underestimate their dental treatment needs[53],

possibly that is why those variables were not correlated.

On the other hand, it was found a positive correlation between self-reported dental health needs and oral health dental status. It was stated that the use of patient self-assessment was a good predictor of patient dental status and it could serve as a tool for public and preventive dentistry, as well as to help the dental practitioner in managing patients according to their complaints and self-assessment.[50] According to the results of this study, simply asking straightforward questions regarding child's dental status may predict the results of their dental anxiety. Tables 3 and 4 show that the dental status self-perception is positive correlated to dental anxiety for children and adults. Once there is a tendency toward a correlation between the dental anxiety and the need for dental treatment shows that, despite minimally invasive dentistry, dentist's appointment remains closely associated with unpleasant experiences.[15,54,55]

Hence, it is reasonable to understand that since children found their oral health status fair, their dental anxiety score was also small.[56]

It is recognized that dental anxiety is associated with irregular dental attendance[48,57,58,] which can head to a poor oral health, with a higher incidence of caries lesions in children with dental anxiety.[57,59-61] It is important that dentists must consider that dental fear may also affect oral health self-perception. Our results show that children dental anxiety is directly affected by their oral health and also by their self-perception about oral health status. That means dental anxiety is likely to be a predictor of dental caries, and may be a risk factor for dental caries and incidence.[61] Tables 4 also shows a positive correlation. In this sample, the more the children caries experience, the more dental anxiety in children was significantly higher. That can be explained since dental anxiety children might perceive that dentists will only provide symptomatic care for their declining oral health, rather than a comprehensive treatment. This behaviour is most likely related to their previous negatively perceived experiences.[62] De Jongh *et al.*[48], confirmed that avoidance of dental care is related to a worse oral health and likelihood of experiencing anxiety. Besides, it was suggested that patients with high dental anxiety would particularly benefit from minimal interventions to break their avoidance pattern.[37,48]

CONCLUSIONS

We suggest family factors may serve to help determine the pattern of learning in which child's dental anxiety is affected by their parental dental anxiety. Moreover, it seems children's dental anxiety is influenced not only by DMFT scores but also by the oral health self-perception.

ACKNOWLEDGMENT

The authors would like to acknowledge National Council for Scientific Development (CNPq) for its Support.

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.756607

Original research

THE ROLE OF TITANIUM MESH IN LIMITING DIMENSIONAL CHANGES OF MAXILLARY SINUS GRAFT: RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Objectives: The dimensional changes that occurs during the healing of the grafting material in external sinus lift procedure is always present and sometimes may lead to difficulties in placing the dental implants in the second stage. This study aims to evaluate the efficacy of adding a titanium mesh as a new fixed floor of the sinus to minimize these changes.

Materials and Methods: this randomized clinical trial included 8 patients who needed bilateral 2-stages sinus lift. On one side sinus lift was carried out using Deproteinized Bovine Bone Matrix (DBBM) mixed with A-PRF+ (Group A) while on the other side a titanium mesh was fixed as a new sinus floor above the same mixture (Group B). CBCT radiographs were taken preoperatively (T0), directly postoperatively (T1), and 9 months after surgery (T2). Linear measurements were recorded in the same points in all three radiographs, graft volume was measured and compared using 3D Slicer software.

Results: the mean bone height (BH) at T1 was 13.08 and 13.56 mm while at T2 was 11.56 and 13.56 mm in groups A and B respectively. The mean reduction in BH was 1.68 and 0.00 mm in groups A and B respectively. The graft volume (GV) at T1 was 1.49 and 1.63 cc and at T2 was 1.19 and 1.53 cc, the mean volume shrinkage was 0.29 and 0.1 cc in groups A and B respectively. The differences between the groups in both linear and volumetric changes were statistically significant.

Conclusions: within the limitation of this study it may be concluded that adding a titanium mesh as a new floor of the sinus helps limiting the shrinkage of both the linear dimensions and overall graft volume.

Key Words: Cone-beam computed tomography, deproteinized bovine bone matrix, lateral sinus lift, sinus augmentation, titanium mesh.

Received : 23.06.2020
Accepted : 21.10.2020

How to Cite: Fattouh SJ, Dayoub ST, AL-Manadili AI. The Role of Titanium Mesh in Limiting Dimensional Changes of Maxillary Sinus Graft: Randomized Clinical Trial. Cumhuriyet Dent J 2020;23:4: 300-307.
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INTRODUCTION

Placing dental implants in posterior Maxilla might be inapplicable due to sinus pneumatization and thus require sinus lift procedure to augment the bone in this area prior to implant placement.

Sinus lift technique was introduced by Tatum *et al.*[1] where he proposed creating a lateral window to gain access to the Schneiderian membrane and elevate it. Boyne and James[2] introduced the use of autogenous bone to Tatum original procedure but later several biomaterials were introduced to the technique including Deproteinized Bovine Bone Matrix (DBBM), Alloplasts, Allografts and even Platelet rich fibrin (PRF).[3,4] A healing time of 6-9 months is required depending on the biomaterial used.

During that time a dimensional change to the graft occur due to continuous pneumatization of the sinus and remodeling of the graft particles and the collapse of the gaps between these particles.[5] The percentage of the shrinkage both linear and volumetric varies depending on the biomaterial used. Zhang *et al.*[6] found a 16% linear and 22% volume shrinkage of DBBM. Another study reported 10% shrinkage of DBBM after 8-9 months[7], while this percentage rises to 17.65% when using calcium phosphate with DBBM mixture in 2:1 ratio[8], and 13% in hydroxyapatite and autogenous bone mixture.[9] Allografts seems to undergo more resorption as only 82% of allografts remained after 6 months and 60% after one year.[10] In a systematic review about dimensional changes after lateral sinus lift Shanbhag *et al.*[11] reported that graft resorption is inevitable especially with autogenous bone which reach 45% while when other biomaterials or the mixture of autogenous bone with biomaterials are used this percentages varies between 18-22% although this reduction in graft volume didn't seem to effect implant placement or survival.

Cone-Beam Computed Tomography (CBCT) is a reliable diagnostic tool and is widely used in the treatment planning of dental implants and in the diagnosis of several pathologies in the jaws.[12] CBCT was successfully used to follow the changes in graft dimension in several studies by using many computer softwares.[6–11,13–16]

Choukroun *et al.*[17] first introduced the concept of Platelet Rich Fibrin (PRF) and was under constant development so the same author introduced Advanced PRF Plus (A-PRF+) which is a more advanced form of PRF based on low centrifugation speed and less time thus trapping a higher number of leukocytes in the fibrin matrix and therefore increasing cytokines release from it.

PRF became widely used in regenerative dentistry because of its ability to promote angiogenesis and growth factors release[18], PRF was used in improving the healing of extraction sites[19], with immediate implant placement[20], in the treatment of Periimplantitis[21], in mucogingival surgeries[22,23], and in the treatment of periodontitis.[24-28]

PRF was used in sinus lifting in several studies either alone or in a mixture with DBBM. The benefit of this mixture was advocated by the authors who reported better new bone formation and faster healing[29,30], however this subject is still controversial as a recent meta-analysis showed no evidence on the necessity of adding PRF to the grafting material.[31]

In a case series, Atef *et al.*[32] used a titanium mesh as space maintainer in a lateral graftless sinus lift and found promising results so Bahaa-eldin *et al.*[33] performed a pilot study where they were able to place an implant in the newly formed bone under the titanium mesh but they reported that the density of this bone was far less than that of the bovine xenograft.

We proposed a modification to Atef *et al.*[32] technique by placing a titanium mesh on top of the graft material as a new floor of the elevated sinus and fixing this mesh with titanium screws to the lateral wall of the sinus to minimize the dimensional change of the graft material.

The aim of this study was to evaluate the dimensional changes in a grafted sinus with xenograft mixed with A-PRF+ compared to adding a titanium mesh on top of the same mixture as a new floor of the elevated sinus.

MATERIAL AND METHODS

Study Design

This research was approved by Damascus University ethical committee for scientific research ID#1926 dated 14/5/2018 and the study protocol adhere to the international agreements (Helsinki Declaration revised 2008)

The study is a split mouth design clinical trial where a standard sinus lift is performed on one side using DBBM mixed with A-PRF+ (Control Group – Group A) and on the other side the same mix was used in addition to a titanium mesh (Test Group – Group B).

Patient's recruitment

8 patients who visited Damascus University– faculty of dentistry– Department of Periodontology – post-graduate clinic and needed bilateral sinus lift procedure were invited to participate in the study.

Inclusion criteria included 6 months since the last extraction, no smoking, residual bone height less than 5mm while exclusion criteria included pregnancy, diabetes, current treatment with corticosteroids and smoking.

After explaining the procedure, a written consent was signed by each participant. The sides were allocated randomly using a coin toss.

Surgical Procedure

The sinus lift procedure was carried out following the protocol proposed by Tatum *et al.*[1], after flap elevation and the exposure of the lateral sinus wall a bony window was prepared using piezoelectric instrument, then the Schneiderian membrane was elevated by manual instruments. The bony window was left in place and elevated with the membrane.

After the completion of the elevation A-PRF+ was prepared following the original protocol by Choukroun[17] and then mixed with the DBBM biomaterial (Bonefill® Mix, Bionnovation, Bauru, Sao Paolo, Brazil) and the mix is used to fill the sinus and finally the osteotomy was covered by a collagen membrane (Biocollagen®, Bioteck S.p.A., Arcugnano, Vicenza, Italy).

In the test group the depth of the sinus was measured using a periodontal probe, then a piece of titanium mesh (Titanium Wire Mesh, Orthomax, Vadodara, Gujarat, India) was cropped to the desired dimensions and fixed on the lateral wall of the sinus using 4mm titanium screw (Mini Screw Ø1.5mm, Orthomax, Vadodara, Gujarat, India) (Figure 1B).

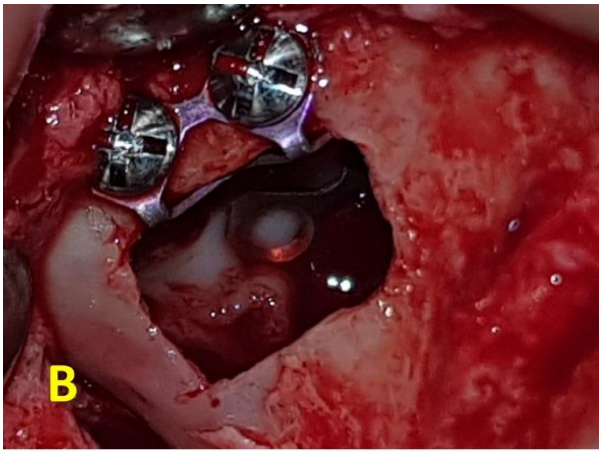

Figure 1B. The titanium mesh is fixed on the lateral wall of the sinus with titanium screws

Another modification was introduced to the original Atef *et al.*[32] technique where they cropped the mesh in an (L) shape while we cropped and then bend the mesh in a (√‾) shape (Figure 1A) which also provided additional 2-3 mm lift above the upper margin of the bony window.

Figure 1A. the bended titanium mesh,

We also used some A-PRF+ membranes as pads under the mesh to avoid any tear to the membrane while placing the mesh.

In case a membrane perforation occurred A-PRF+ membranes were used along with a piece of collagen membrane to cover the perforation.

Patients were informed that this mesh will not be removed in the future as it will be incorporated in the newly formed bone and in close contact with the Schneiderian membrane which will make its removal impossible without damaging the membrane.

Radiographic assessment

A cone beam CT radiograph was taken before (T0) and immediately after the operation (T2) and after 9 months (T3) (Figure 2).

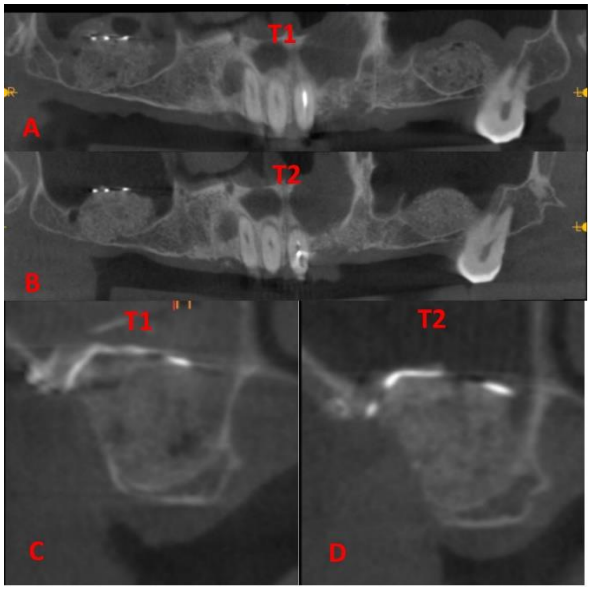

Figure 2: A) Panoramic reformatting of a patient directly after surgery, **B)** Panoramic reformatting of the same patient after 9 months, **C)** Oblique slice in the middle of the titanium mesh directly after surgery, **D)** Oblique slice in the same point after 9 months

Linear radiographic evaluation was carried out by taking measurements in the same points and directions in all three CBCT radiographs. These points were determined depending on fixed anatomical landmark to match all three radiographs like neighboring teeth and nasal septum based on the technique described by Anduze-Acher *et al.*[34] The distance between the nasal septum and the slice of interest was measured and applied in all three radiographs while the direction of measurement was determined by repeating an angel between the direction of measurement and a horizontal line tangent to the lowest point of the alveolar crest.

3d volumetric measurements were recorded using 3D Slicer software were the segment editor tool was used to mark the graft boundaries in all slices then the software calculates the volume using the segment statistics tool.[35]

Statistical Analysis

Data were analyzed using statistical software (IBM SPSS Statistics version 22). Unpaired Student t-Test was used to analyze the difference in bone dimensions during healing process between the two groups. Results were considered significant at $P < .05$ with 95% confidence intervals.

RESULTS

This study included 8 patients (5women, 3men) with mean age (52.13 years) who needed bilateral sinus lifting without implant placement.

The differences in mean bone height (BH) (Table 1) before the surgery and the graft height directly after surgery was not statistically significant between the two groups. While it was higher in the test group after 9 months with statistically significant results.

Table 1: Mean Bone Hight Value in the study groups compared before (T0), directly after surgery (T1) and after 9 months (T2)

	T0		T1		T2	
	BH	*p* value	BH	*p* value	BH	*p* value
Group A	2.41±1.06	0.613	13.08±1.21	0.389	11.39±1.65	0.006*
Group B	2.69±1.12		13.56±0.97		13.56±0.97	

BH: Bone Height (mm)
*: P<0.05 is considered statistically significant

The mean graft height (GH) (Table 2) directly after surgery was not statistically significant between the groups but after 9 months the mean graft height was 8.99±1.93 in group A and 10.88±0.58 in Group B and this difference between the groups was statistically significant.

Table 2: Mean Graft Hight and 2d Shrinkage compared between the study groups directly after surgery (T1) and after 9 months (T2).

	T1		T2		Graft Shrinkage	
	GH	*p* value	GH	*p* value	2D	*p* value
Group A	10.67±0.94	0.604	8.99±1.93	0.019	1.68±1.21 (15.74%)	0.001*
`	10.88±0.58		10.88±0.58		0.00	

GH: Graft Height (mm)
*: P<0.05 is considered statistically significant

The mean linear bone height reduction in the control group was 1.68 mm while in the test group when the measurements were made directly under the titanium mesh there was no reduction in bone height and these differences were statistically significant (Table 2).

The mean graft volume (GV) directly after surgery was not statically different between the two groups. At 9 months after surgery graft volume was significantly higher in the test group and the mean graft volume shrinkage was 0.29 and 0.10 cc in group A and group B respectively and there was a statistically significant difference between the groups. (Table 3)

Table 3: Mean Graft Volume and 3d Shrinkage compared between the study groups directly after surgery (T1) and after 9 months(T2).

	T1		T2		Graft Shrinkage	
	GV	*p* value	GV	*p* value	3D	*p* value
Group A	1.49±0.35	0.398	1.19±0.30	0.039	0.29±0.11 (20.01%)	0.000*
Group B	1.63±0.31		1.53±0.29		0.10±0.05 (5.93%)	

GV: Graft Volume (cc)

*: P<0.05 is considered statistically significant

DISCUSSION

As far to the author's knowledge this is the first split mouth randomized clinical trial that studies the use of titanium mesh as a space maintainer in two stages sinus lift surgery using DBBM mixed with A-PRF+ as grafting material.

The shrinkage of grafting material, both linear and volumetric is an inevitable outcome of the healing process of sinus grafts. The clinicians are advised to increase the amount of biomaterials introduced to the sinus to compensate for this remodeling process.[11]

In the present study we found 1.68mm (15.74%) linear shrinkage of the graft material in the control group between follow-ups, this reduction came in accordance with other similar studies in the literature that reported variable amount of shrinkage of the grafting material.[6,36–38]

This shrinkage is mainly due the continuous pneumatization of the sinus and the pressure it implicates on the grafting material during breathing and accelerate resorption of the grafting material especially in two stage sinus lift this process is also influenced by the properties of the biomaterial used.[8] Multiple tooth lost, the angel between the medial and lateral sinus wall, and large sinus may be associated with higher graft contraction.[6]

In the study group the installment of the titanium mesh as a new floor of the elevated sinus provided protection for the graft material from the continuous pressure thus allowed the biomaterial to be remodeled and replaced by new host bone without loss of height. This space maintenance effect of the mesh is one of the fundamental principles of guided bone regeneration[39], as this protected space is essential for bone graft healing by hard tissue cells which will regenerate the bone during the healing and maturation time.[40] The comparison between the two groups showed statistically significant difference in term of linear measurement reduction over time.

Volumetric analysis of the graft showed some shrinkage in both groups and this shrinkage was statistically significant between the two groups. In the control group the mean volume shrinkage was 0.29 cc (20%) which corelate to the previously established graft shrinkage in the literature.[6–8,10,11,16,36] In the test group the applied mesh covered most of the graft but on the sides the graft was still subject to some pressure thus explaining the minor reduction in volume with an average of 0.1cc (5.93%). As a study by Guo *et al.* [41] showed an overall reduction of DBBM graft volume by 19.4% and more specifically 0.29% in the bucco-palatal, 5.87% in the mesio-distal, and 14.32% in the vertical direction thus explaining the finding of our study where the titanium mesh offered protection

to the graft from the shrinkage in the vertical dimension while it was still under some pressure in the mesio-distal direction which resulted in the statically significant less mean graft volume reduction in the test group compared to the control group.

This study had some limitations, such as the width of the titanium mesh was limited to 10 mm or 15 mm due to its design so in all the cases the mesh's width was 10 mm and sometimes even if we had a wider osteotomy/Sinus we were not able to place wider mesh and as mentioned before the effect of the mesh application was at its best directly under the mesh so for future study we recommend using a more flexible mesh design that allows to perfectly crop the mesh to the width of the osteotomy.

We also believe that the real benefit of the protection offered by the mesh application is when using other than the bovine biomaterial e.g.: Allograft/autogenous alone bone or mixed with DBBM which are more prone to resorb during the healing time thus we recommend to study the application of the titanium mesh above these biomaterials.

CONCLUSIONS

Within the limitation of this study it may be concluded that adding a titanium mesh as a new floor of the elevated sinus helped in protecting the underling graft from forces applied from continuous pneumatization thus eliminating linear shrinkage directly under the mesh and minimizing the overall shrinkage of the graft.

This technique may be applied to improve the final outcome of sinus lift procedure and make it more predictable and guarantee a sufficient graft volume after healing time thus facilitate implant placement.

ACKNOWLEDGMENTS

The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.773890

Original research

SATISFACTION WITH DENTAL AND MEDICAL EDUCATION QUALITY AMONG FOREIGN STUDENTS CONSIDERING NON-NATIVE ENGLISH-SPEAKING ORIGIN OF THE TEACHERS

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ABSTRACT

Objectives: To evaluate satisfaction with dental and medical education quality among foreign students considering non-native English-speaking origin of the teachers.

Materials and Methods: This research was conducted in the form of a questionnaire survey among foreign students of Dental Faculty and Medical Faculty during 2019-2020. The questionnaire was presented in the format of Google-form, published in faculties' social media group as well as was sent to the e-mail of each student registered via Moodle system. The survey was held considering the anonymity of each respondent and personal willingness of each student to take part in the research. One hundred and sixty-seven students took part in the survey, among which only 82 answered all the questions.

Results: 60 (73.2%) respondents rated their satisfaction with the quality of provided educational process as excellent, while 5 (6.1%) and 17 (20.7%) as satisfactory and good, respectively. Such factors as teacher's/lecturer's knowledge of the subject (r=0.72), teacher's/lecturer's knowledge of English (r=0.60), personal motivation (r=0.75), and educational organization considering the aspect of language adaptation (r=0.65) were found to be statistically related to the quality of obtained dental and medical education, subjectively evaluated by the foreign students.

Conclusions: Considering limitations of the performed study, it could be resumed that foreign students are generally highly satisfied with provided level of dental and medical education, even though such was carried out by the lecturers for whom English isn't the native language. Structured interactive sessions, direct-feedback lectures, and additional use of video-podcast are not only preferable among students, but also provide sufficient opportunities for educational process improvement, especially in the settings when both student and teacher are non-native English speakers, and when stationary education is limited due to the COVID-19 pandemic situation.

Keywords: Foreign medical graduates, education, dental, medical, communication barriers.

Received : 26.07.2020
Accepted : 28.09.2020

How to Cite: Goncharuk-Khomyn M, Cavalcanti AL, Stoika O, Hliudzyk-Shemota M, Deviatko Y, Boykiv A. Satisfaction with Dental and Medical Education Quality Among Foreign Students Considering Non-Native English-Speaking Origin of the Teachers. Cumhuriyet Dent J 2020;23:4: 308-317.
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INTRODUCTION

Medical and dental education of foreign students represents a complex system of students-teachers interactions, characterized with formation of further academical and professional networks worldwide.[1-5] Based on the numbers of graduated specialists and those who had nostrificate their original diplomas in other countries, process of dental and medical education of foreign students seems to progress and improve gradually over past decade, and it could be defined as internationally successful process. Considering such tendency, the convergence of European dental educational models have been described previously.[6-9] Latter included usage of the same educational protocols with translations into different languages at universities and further discussion on aspects related to the general and subject-specific competences, ECTS-mode as accumulation mechanism and educational process quality assessment.[9]

As reported by the State Enterprise "Ukrainian State Center for International Education", number of foreign students in Ukraine reached 80,470 in 2019 compared to 53,664 in 2011, while 87.85% of them are seeking bachelor's and master's degree education.[10] Based on the previous study, selection of an international institution for degree education was associated with the influence of such factors as academic quality and reputation, internationalization, amount of tuition fee, student-oriented educational process and availability of scholarships.[11] Language factor also remains one of the most important to choose among universities abroad, while English is considered as an international language for providing an educational process among foreign students. Foreign languages courses within medical curriculum tend to help foreign students integrate into social network not only among academic environment, but also beyond it.[11]

The number of foreign students tends to increase in Ukraine and that is related to such factors as a progression of academic mobility possibilities, seeking for a degree education at a foreign country, development of exchange educational programs, and optimization of student enrollment process for university education worldwide. Even though most of the academic staff not only in Ukraine but worldwide, has high English language fluency skills, it is still important to notice that educational process for foreign dental and medical students in many cases is provided by the teachers of non-native English-speaking origin.

Considering all above-mentioned information, it is reasonable to resume that the research of specific aspects during dental and medical education of foreign students proceeds relevant and actual, while working out in detail of such could improve overall quality of provided academic activity.

OBJECTIVE

To evaluate satisfaction with dental and medical education quality among foreign students considering non-native English-speaking origin of the teachers.

MATERIALS AND METHODS

The research was provided in the form of a questionnaire survey among foreign students of Dental Faculty and Medical Faculty #2 at Uzhhorod National University (Ukraine) during 2019-2020 years. The questionnaire was presented in the format of Google-form, published in faculties social media group, and/or sent to the e-mail of each student registered via Moodle system. Students without Moodle-account couldn't take part in the survey, which was limited by access algorithm. Before getting access to the questionnaire, student should mention the year of study in corresponding text box without indication his/her name or other personal information. Using of Google-form as a questionnaire optimize the possibilities for further data collection and analysis of obtained results.

The questionnaire used for grading satisfaction with medical and dental education was modified compared to the analogical versions provided in previous publications and was adapted considering the aspects of students and teachers language origin impact.[12,13] The questionnaire consisted of 11 questions, representing three different domains. The first domain included questions from 1 to 5, and was aimed at subjective evaluation of overall satisfaction with provided

education quality, and teachers'/students' knowledge of English. Respondents could choose one of the 5 proposed possible answers, represented in percentage meanings from 0% to 100% with each interval of 20% interpreted in following manner: 0-20% - poor, 21-40% - weak, 41-60% - satisfactory, 61-80% - good, 81-100% - excellent. The second domain presented by questions 6-10 was related to the subjective evaluation of possible connections between the quality of provided education and related factors, considering the impact of English fluency. In this domain, respondents also could choose one of the 5 proposed possible answers, represented in the meanings of absolute values from 1 to 5, which was interpreted in the following fashion: 1 – no connection, 2 – weak connection, 3 – moderate connection, 4 – good connection, 5 – strong connection. The third and last domain was presented only by question number 11 aimed at the verification of the most effective and motivational type of lesson considering its' possible subjective impact on the knowledge of English language. Respondents could choose one of the proposed 5 possible answers or propose their own one. Five options for answering on the question number 11 included the following: 1) lecture with no student's interaction; 2) direct-feedback lectures; 3) seminar; 4) interactive sessions with a small group of students; 5) distance (remote) learning using podcasts and provided material for preparation.

The above stated 11 questions within questionnaire were formulated as follows:

1. What is your overall satisfaction with the quality of obtained education?
2. What is your overall satisfaction with organization of educational process, considering aspects of language adaptation?
3. How do you evaluate the overall English fluency among teachers/lecturers you have worked with?
4. Grade your own knowledge of English before you attempt the university.
5. Grade your own knowledge of English after finishing the previous study year at university.
6. Rate the connection between teacher's/lecturer's knowledge of subject and quality of provided education.
7. Rate the connection between teacher's/lecturer's knowledge of English and quality of provided education.
8. Rate the connection between your own knowledge of English and quality of provided education.
9. Rate the connection between your own impact and motivation on the outcome of provided education.
10. Rate the connection between length of studying at university and improvement of your knowledge including improvement of your English language.
11. Identify the most motivational and effective type of lesson considering their positive influence on knowledge of English.

Survey was held considering the anonymity of each respondent and personal willingness of each student to take part in the research. One hundred sixty-seven students took part in the survey, among which only 82 answered all the questions. Due to the need for correct interpretation of all obtained results, answer's forms with incomplete information were not considered for further analysis.

The study was approved by the Ethic Committee of Medical Faculty #2 (Uzhhorod National University) within Group of studies aimed at educational process optimization and designated with the number of № 22092019 (22/09/2019).

All data obtained via Google-form (questionnaire) was transferred to the Microsoft Excel 2019 software (Microsoft Office 2019, Microsoft), where further data structuring and tabulation were performed. Descriptive statistics methods were used for obtained data summarization (distribution of obtained results, mean values, absolute levels of respondents' answers). Correlation between factors related to knowledge of English and quality of obtained education was evaluated using correlation function (Pearson's r) with $p<0.05$ considered as adequate level of probability.[14]

RESULTS

Among 82 fully completed forms, 18 (21.9%) were provided by 2nd year students, 39 (47.6%) – by 3rd year students, and 25 (30.5%) – by 4th years students. Even though some freshmen, seniors (5th year) and final year (6th year) students submitted answer forms, but none of them have completed it to the full extent, without missing or ignoring some of the provided questions. The most frequently missed question (57.6%) was one from the third domain, considering type of lesson that could have the greatest impact on knowledge of English language. Nevertheless, obtained distribution of the answers among representatives of different years of studying was sufficient to provide further analysis of the data.

Considering obtained answers on first domain's questions (1-5), it was found that 60 (73.2%) respondents rated their satisfaction with the quality of provided educational process as excellent, while 5 (6.1%) and 17 (20.7%) as satisfactory and good, respectively. Similar distribution was noted regarding answers on overall satisfaction with obtained educational organization, considering aspects of language, with 61 (74.4%), 18 (21.9%) and 3 (3.7%) respondents demonstrating excellent, good and satisfactory levels, respectively. Students evaluated the overall level of teacher's/lecturer's knowledge of English as good (by 37.8% respondents) and excellent (by 53.7% respondents). On the other hand, 16 students (19.5%) graded their level of English as weak before entering the university, while 31 (37.8%) – as satisfactory, 23 (28.1%) – as good, and 12 (14.6%) – as excellent. Provided analyses also revealed that students subjectively noted the improvement of their knowledge of English during studying dentistry and medicine in the university: 33 (40.2%) respondents graded their English knowledge as excellent, while 37 (45.1%) as good after finishing previous academic year.

Based on the answers received within the second domain's questions, it was found that all respondents linked the quality of provided dental and medical education with the teacher's/lecturer's knowledge of the subject, while only 50% of them indicated the correlation between quality of provided education and teacher's/lecturer's knowledge of English. It was interesting that 16 students (19.5%) didn't recognize any connection or identify just weak matching between educational outcome and their own knowledge of English. On the other hand, only 14 students (17.1%) mentioned that there is a strong connection between the above-mentioned parameters. Still, 29 respondents (35.4%) agreed that their personal impact and motivation had a strong influence on the outcome of provided dental and medical education, while 26 (31.7%) and 27 (32.9%) evaluated such connection as moderate and good, respectively. 45 (54.9%) of surveyed students also strongly linked together the improvement of English and the duration of their study at university.

Among all proposed options for answering within the third domain's question, 37 (45.1%) of the dental and medical students preferred interactive sessions with a small group of participants as the most efficient lessons considering their positive influence on knowledge of English language, while 23 (28.1%) have chosen "direct-feedback lecture" answer for the same question. Lectures with no student's interaction, seminars and distance (remote) learning using podcasts and provided material for preparation were chosen as those accompanying learning of English only by 7 (8.5%), 10 (12.2%), and 5 (6.1%) respondents, respectively.

Due to the all above obtained data and provided statistical analysis such factors as teacher's/lecturer's knowledge of the subject (r=0.72), teacher's/lecturer's knowledge of English (r=0.60), personal motivation (r=0.75), and educational organization, considering aspect of language adaptation (r=0.65) were found to be statistically related to the quality of obtained dental and medical education, subjectively evaluated by the foreign students; student's primary English knowledge and duration of studying demonstrated statistically lower levels of correlation. On the other hand, the outcome level of foreign students' knowledge of English was influenced in relatively the same manner by all of the above-mentioned factors, except teacher's/lecturer's knowledge of

the subject. Also, duration of studying dental and medical subjects at university demonstrated a positive impact on resulted knowledge of English among foreign students (r=0.59) (Table 1).

Table 1. Correspondence between studied parameters, obtained quality of education and outcome knowledge of English

Factors	Quality of education		Outcome knowledge of English	
	r	p	r	p
Teacher's/Lecturer's knowledge of the subject	0.72	p<0.05	0.21	p<0.05
Teacher's/Lecturer's knowledge of English	0.60	p<0.05	0.52	p<0.05
Student's knowledge of English at baseline	0.36	p<0.05	0.61	p<0.05
Educational organization considering the aspect of language adaptation	0.65	p<0.05	0.76	p<0.05
Personal motivation and impact	0.75	p<0.05	0.59	p<0.05
Duration of education	0.41	p<0.05	0.59	p<0.05

*p<0.05 is statistically significant

DISCUSSION

Dental and medical education aimed at providing and developing competent knowledge, skills and abilities among students and postgraduates, thus guarantying ongoing improvements within the health care academical and professional community.[1-3,10] Educational process itself represents translational phase for a future specialist, while being more advanced life-experience for students attending universities abroad in comparison with those obtaining degree at resident country.[2,4,5] Foreign students who are not originally English-speaking faced challenges mastering not just English itself, but medical and dental English terminology during corresponding curriculums. In some countries, dental and medical education provided by academic staff for whom English is also not a native language, thus forming situations in which non-native English speakers teaching non-native English-speaking students. An interesting trend have been noted during the analyses of foreign students' enrollment in Ukrainian education system: the number of students from post-soviet countries demonstrated decreasing pattern from 29.47% in 2017-2018 to 21.52% in 2019-2020, while 22.90% and 10.23% of all students in 2019-2020 came from India and Morocco, respectively.[10]

Such a decreasing tendency of students' enrollment from post-soviet countries has also impacted the teaching process, considering the language aspect: English is becoming more requisite as teaching language considering the origin of foreign students.

Due to the results of our study, aimed at the assessment of subjective satisfaction with provided education among foreign students, it was found that the latter is predominantly dependent on such factors, as teacher's/lecturer's knowledge of the subject, teacher's/lecturer's knowledge of English, educational organization, considering the aspect of language adaptation, and personal motivation and impact. Among these factors, the influence of English knowledge among teachers/lecturers could not be categorized as being the most substantial, while personal motivation and teacher's/lecturer's knowledge of the subject demonstrated comparatively greater correlation with registered high satisfaction level with obtained education quality. Nevertheless, students evaluate the teacher's/lecturer's knowledge of English as good in 37.80% responses, and as excellent in 53.66% responses. Even though for the majority of academic staff providing dental and medical education at Ukrainian university English is not a native language, but such pattern of distribution could be related to the fact that all teachers providing educational process for the foreign students were previously certified with B2 (high intermediate) or C (advanced) levels of English by one of various grading tests (IELTS, FCE, TOEFL, APTIS, etc.).

Previous studies demonstrated that near 75% of analyzed US hospitals provided medical care for patients who are not native English speakers, while only 18% of surveyed medical facilities validated the level of foreign language proficiency among stuff.[15-19] Based on that, authors resumed that

assessment of foreign language skills should be categorized as a mandatory measure within medical educational system, while improvement of such should be provided by specifically design of language training courses.[19] Such English language mastering by individual or group patterns together with English language curriculum classes could improve the overall quality of dental and medical education among foreign students. Moreover, learning of foreign language in general supports dental competencies development related to the dental care of patients with different sorts of disabilities. Such educational approach could be realized through the specially developed elective courses.[20]

Mitchell C. *et al.*[21] noted the need for supportive English-speaking opportunities for the optimization of international nursing students' education. Such approach could reduce the risk of students being accused in deficiency of language or even in deficiency of clinical proficiency.[21] Mykhailenko L. categorized "foreign language professional competence" as an important part of general professional competence within condition of postgraduate medical education system.[22] Addition of new subject such as "Medical/Dental English for foreign students" within medical/dental core course curriculum supports an idea of future specialists' integration into professional field with relevant knowledge of active practical and theoretical trends. In the same manner, not only increasing the academic hours for English teaching but also modification of such in the way of its adaptation to clinical needs will help to enhance the general quality of medical and dental education.

The teacher guidance remains one of the factors associated with the success of education, especially in the settings of foreign students' educational process. Even though interaction and student inter-guidance are also the methods of teaching modes within some classes, but the dominant role of mentoring should be held by teacher/lecturer. Previously, it was established that student-made educational podcasts did not pronounce any improvement in foreign language vocabulary teaching, while teacher-produced ones support the increase of foreign language skills.[23] Results of systematic review demonstrated that none of the analyzed teaching methods demonstrated significantly greater effectiveness compared to others, but still video-based ones characterized with greater positive impact on average knowledge scores.[24] Usage of virtual classrooms supported better educational outcomes among Chinese and foreign students in China in three medical schools.[25] Such outcome is associated with great relevant importance considering the need for distant education implementation due to the COVID-19 pandemic conditions.

However, due to the results obtained considering teaching and learning modes, it was found that such methodologies as interactive lectures, case-based lectures and problem-based learning approaches demonstrated statistically significant positive impact on reaching educational objectives, while structured interactive session were characterized by greater ability to solve complicated aspects.[26] Considering such outcomes, it is reasonable to recommend the usage of structured interactive sessions for English learning during dental and medical education, since concept of such is also more adapted for clinical orientation of language skills among future medical specialists. On the other hand, team-based learning was associated with greater examination scores, higher levels of learning attitude and learning skills compared to lecture-based learning due to the results of provided meta-analysis among medical educational environment in China.[26] Team-based learning also provides possibilities of medical English language skills improvement in terms of student and teacher both-way interaction, while just classical lecturing itself restricts such options.

In our study, students have chosen interactive sessions with a small group of participants and direct-feedback lectures as the most efficient types of lessons considering their positive influence on knowledge of English language during dental and medical studying. Despite all the analyzed modifications of dental and medical educational models, the principle of blended mode of learning, consisting of various teaching methodologies, seems to provide the most effective results on

overall educational quality regardless of students' and lecturers' language origin.[28,29]

Analysis of international medical students' expectations provided within the focus group, revealed that social exclusion because of foreign language knowledge deficiency is one of the most reported students' worries, which could be compensated by corresponding language training courses.[2] Results obtained in nationwide survey have shown that even though medical education for foreign students were provided via English or French in general, but 88.5% were confident in providing medical history in their native language.[30] In previous study, foreign language was categorized as an obstacle for medical students during the 1st year, but after that, the language barrier was not considered as a problem.[31] In our study, we have found out that educational process organization, considering the aspect of language adaptation, seems to be an influential factor both on students' satisfaction regarding the quality of education, and on the outcome of English knowledge among dental and medical students. Moreover, it was noted that the duration of study has a positive influence on outcome of English knowledge among dental and medical foreign students. Educational process organization, considering the aspect of language adaptation includes additional pre-entry English courses, activities involving students' interaction, teachers' support while dealing not only with educational, but also with usual life situations, and full language support provided by translators or senior students. Such approach is organized at the faculty level and could be effectively implemented as an integrated educational strategy.

The problem of insufficient English language skills among foreign students is also critical during classical lecturing since the absence of response restricts the chances of complete and adequate knowledge sharing.[32] On the other hand, students themselves preferred active learning methodologies, which included conductive learning function, motivational aspects and repetitive feedback[32], which was also registered in our study.

In research provided by Hanna, foreign students demonstrated a higher level of satisfaction while studying medical behavior disciplines compared to their German colleagues.[12] Such results were associated with a component of generally deeper experience of foreign students studying abroad considering their "broader life situation".[12] Answers received from foreign students enrolled in our survey also demonstrated the high level of satisfaction with obtained education quality at the excellent level among 73.17% of respondents.

Understanding of foreign students' present and further needs considering English language impact helps to organize highly qualitative system of language training activities oriented toward real situation conditions.[34] As it has been shown in previous research, dental and medical students mostly use the next major strategies for vocabulary learning: guessing, selective attention, study preference, dictionary use and memory.[13] Also, supportive English lessons could promote deeper cross-disciplinary integration, as it has been shown previously with other subjects.[35] Another perspective of medical sciences education improvement considering English language communication skills represented by exchange programs, forming the chances for teachers and for students to be involved in the educational process within a totally English-speaking environment.

Despite the high number of foreign students not only in Ukraine, but worldwide, it could be resumed that educational system while still developing going through total globalization and integration processes.[1-3,36] New challenges occur considering not only academically associated changes, but also influence of "out of educational field" factors, such as COVID-19 pandemic and limited territorial migrations related to sanitary restrictions.[37]

This study characterized with a few limitations, which are following: 1) provided questionnaire itself was composed in English, and there were no alternatives questionnaires presented in respondents' native languages; 2) students background was not considered as a potential factor of influence on the subjective assessment of

education satisfaction; 3) evaluation of satisfaction with education quality was provided considering students' general impression among all studied subjects, while some of them could be related to greater intellectual endeavors and more complicated vocabulary content, and other could be characterized as less complex. All the above-mentioned limitations associated with pilot design of the study. Even though used questionnaire was composed just in English version, but it was represented in the simplest way possible with minimal chances of some aspects misinterpretations. Students' background is a valuable factor of influence during research of satisfaction with an educational program, but such will be differentiated during further in-depth analysis, and will be considered as a perspective of future research. The same way differentiation of various subjects' learning aspects on overall satisfaction with educational process will be studied in the future, but for such research the new groundbase should be prepared considering the need in pre-analysis of objectified academic load difference between various kinds of disciplines.

CONCLUSIONS

Considering the limitations of conducted study, it could be resumed that foreign students are generally highly satisfied with provided level of dental and medical education at Ukrainian university, even though such was carried out by the lecturers for whom English isn't a native language. Such outcome could be argumented by the fact that most of the lecturers certified with English knowledge at the levels of B2 (high intermediate) or C (advanced). Moreover, students' satisfaction with the quality of provided education was statistically associated with educational organization, considering the aspect of language adaptation and personal motivation. The same factors relatively similar have influenced on the outcome knowledge of English among foreign students who attended dental and medical courses at Ukrainian university. Structured interactive sessions, direct-feedback lectures and additional use of video-podcast are not only preferable among foreign dental and medical students, but also provide sufficient opportunities for educational process improvement, especially due to the conditions, when both student and teacher are non-native English speakers, and when stationary education is limited due to the COVID-19 pandemic situation.

ACKNOWLEDGEMENTS

Presented study was not supported by a grant or any other kind of funding.

CONFLICTS OF INTEREST STATEMENT

Authors declare no conflicts of interest

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.777057

Original research

SUCCESS OF ARTIFICIAL INTELLIGENCE SYSTEM IN DETERMINING ALVEOLAR BONE LOSS FROM DENTAL PANORAMIC RADIOGRAPHY IMAGES

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ABSTRACT

Objectives: This study aims to detect alveolar bone loss from dental panoramic radiography images by using an artificial intelligence (AI) system.

Materials and Methods: A total of 2276 panoramic radiography images were evaluated. Of these, 1137 were of bone loss cases and 1139 were of periodontally healthy cases. This dataset is divided into training (n = 1856), validation (n = 210), and testing (n = 210) sets. All images were resized to 1472x718 pixels before training. A random sequence was created using the open-source Python programming language and OpenCV, NumPy, Pandas, and Matplotlib libraries. A pretrained Google Net Inception v3 convolutional neural network (CNN) was used for preprocessing, and the datasets were trained using transfer learning. The diagnostic performance was evaluated using a confusion matrix in terms of the sensitivity, specificity, precision, accuracy, and F1 score.

Results: Of 105 cases with bone loss, the CNN system detected 99 with sensitivity, specificity, precision, accuracy, and F1 score of 0.94, 0.88, 0.89, 0.91, and 0.91, respectively.

Conclusions: The CNN system successfully determines periodontal bone loss. Therefore, it can be used to facilitate diagnosis and treatment planning by oral physicians in the future.

Keywords: Panoramic radiography, artificial intelligence (AI), alveolar bone loss, periodontitis.

Received : 04.08.2020
Accepted : 06.10.2020

How to Cite: Kurt Bayrakdar S, Celik O, Bayrakdar IS, Orhan K, Bilgir E, Odabas A, Aslan AF. Success of Artificial Intelligence System in Determining Alveolar Bone Loss from Dental Panoramic Radiography Images. Cumhuriyet Dent J 2020;23:4:318-324.
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INTRODUCTION

Periodontitis is a chronic inflammatory disease that affects the supporting tissues of the teeth, and it has clinical findings such as attachment loss and probing pocket depth.[1] Clinical periodontal evaluations and various radiographs are used for managing this common disease.[1-3] Radiography images enable observing the alveolar bone condition and determining any alveolar bone loss and bone resorption patterns.[2,4,5]

Various intraoral and extraoral imaging methods such as periapical, bitewing, panoramic radiographs, and cone-beam computed tomography (CBCT) are used for periodontal evaluations.[2,5] Panoramic radiographs are a very fast and easy imaging method that enable imaging the teeth and edentulous alveolar bone area by using low-dose radiation.[2,6] They also provide oral physicians with general information about the periodontal status of patients and assist them in determining the extent of alveolar bone loss.[4,6,7]

Artificial intelligence (AI) deals with machines capable of learning, problem-solving, and analysis in a manner similar to human intelligence.[8] Many studies have used convolutional neural networks (CNNs), a type of artificial neural network, for image interpretation, diagnosis, and treatment planning in dental radiology.[9-12] Dental radiographs are a diagnostic tool that can be effectively used to evaluate the condition of periodontal hard tissues and to analyze the success of periodontal treatment.[2,5] However, only a few studies have used CNN systems to determine periodontal disease[13,14] and alveolar bone loss[15-17] from radiography images.

Applying a CNN system to periodontal radiography images as a decision-support mechanism for oral physicians in diagnosis and treatment planning seems promising. The most important clinical benefit of using CNN systems in dental diagnosis and treatment planning is that it allows oral physicians to reduce diagnostic mistakes arising from strain or fatigue. The CNN system can also capture details overlooked by oral physicians in radiographic diagnoses, and its radiographic examinations can be recorded to build a database for oral physicians. In this light, the present study uses a CNN system to determine alveolar bone loss and periodontal disease/health status from dental panoramic radiography images.

MATERIALS AND METHODS

Patient selection and imaging

This study was conducted with a dataset of panoramic images obtained from the Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Eskişehir Osmangazi University. This study was approved by the Noninterventional Clinical Research Ethics Committee (decision date and number: 08.07.2019/ 2019-227), and it followed the principles of the Declaration of Helsinki.

Panoramic radiographs of patients with a large number of teeth missing (i.e., patients with less than 20 teeth), patients younger than 18 years, and patients with excessive crown-damaged teeth as well as images with artifacts or partial/severe distortion were excluded from the dataset. Further, all radiographs used in this study were taken with the same device (Planmeca Promax 2D, Planmeca, Helsinki, Finland), and only one radiograph of each patient was used. The final dataset contained 2276 panoramic images, of which 1137 were of bone loss cases and 1139 were of periodontally healthy cases, regardless of gender. All images were evaluated by an oral and maxillofacial radiologist and a periodontologist (İŞB and SKB, with at least 9 years of professional experience) for determining bone loss. The presence/absence of resorption at the bone crest was recorded in consideration of the distance between the enamel-cement junctions of the teeth and the alveolar bone crest. Radiographs showing bone resorption with a horizontal/vertical shape or bone defects were included in the bone loss group. Radiographs with no loss of bone crests or with the alveolar bone completely covering the root surfaces of the teeth (normal anatomical structure) were included in the periodontally healthy group.

Evaluation of panoramic radiography images

All images in the dataset were resized to 1472x718 pixels before training. A random sequence was created by using the open-source Python programming language and OpenCV, NumPy, Pandas, and Matplotlib libraries. The dataset is

divided into training, validation, and testing sets. The training set consisted of 1856 images (927 with bone loss and 929 with good periodontal health), validation set consisted of 210 images (105 with bone loss and 105 with good periodontal health), and testing set consisted of 210 images (105 with bone loss and 105 with good periodontal health). Table 1 shows the details of these datasets.

Table 1. Details of study datasets.

Dataset	Bone destruction	Periodontally healthy	Total
Training	927	929	1856
Validation	105	105	210
Testing	105	105	210

A pretrained Google Net Inception v3 CNN network was used for preprocessing, and the datasets were trained using transfer learning. This CNN performed perfectly in the 2014 ImageNet Large Scale Visual Recognition Contest, and it initially learned ~1.28 million images consisting of 1000 object categories. This CNN consists of 22 deep layers, and it can obtain features of different scales by applying convolutional filters of different sizes in the same layer.

The training and validation datasets were used to predict and generate optimal weight factors for this CNN. In this study, all CNNs used the InceptionV3 architecture with the TensorFlow library in Python, and they were trained using 20000 steps (Figures 1 and 2) (CranioCatch, Eskisehir, Turkey).

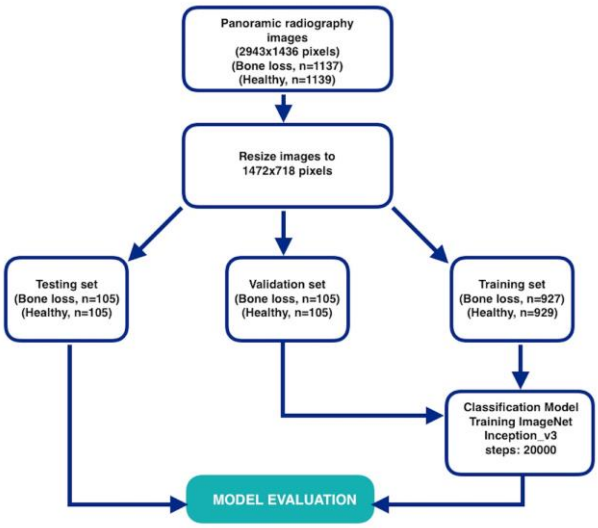

Figure 1. Evaluation of panoramic radiography data

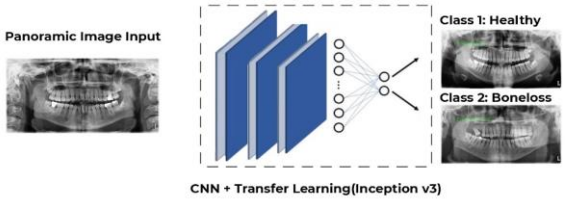

Figure 2. Evaluation of panoramic radiography data using CNN system

Statistical analyses

Statistical analyses were performed using a confusion matrix, a meaningful table that summarizes predicted and actual situations as metrics.

RESULTS

Table 2 shows the number of true positive and true negative cases determined using the CNN system. Of 105 bone loss cases, the CNN system evaluated 99 correctly and 6 incorrectly. Further, of 105 periodontally healthy cases, it evaluated 93 correctly and 12 incorrectly.

Table 2. Number of cases determined by the AI model as true positive and true negative.

	True Positive	True Negative
Predicted Positive	99	12
Predicted Negative	6	93
TOTAL	**105**	**105**

Table 3 shows the performance of the CNN system as calculated using the confusion matrix. The sensitivity, specificity, precision, accuracy, and F1 score were 0.9429, 0.8857, 0.8919, 0.9143, and 0.9167, respectively.

Table 3. AI performance calculated using the confusion matrix. TPR, true positive rate; TP, true positive; FN, false negative; SPC, specificity; TN, true negative; FP, false positive; PPV, positive predictive value; ACC, accuracy; P, positive; N, negative.

	Value	Derivations
Sensivitiy	0.9429	TPR = TP / (TP + FN)
Specificity	0.8857	SPC = TN / (FP + TN)
Precision	0.8919	PPV = TP / (TP + FP)
Accuracy	0.9143	ACC = (TP + TN) / (P + N)
F1 Score	0.9167	F1 = 2TP / (2TP + FP + FN)

DISCUSSION

Panoramic radiographs are extraoral radiographs that are commonly used in dentistry to provide a complete perspective through full mouth imaging of patients.[2,4] Studies are increasingly investigating the use of computer-aided systems for interpreting dental radiography images.[9-12,14-25] In this light, the present study used a CNN system to determine common periodontal diseases including alveolar bone loss from panoramic radiography images.

Panoramic radiographs are preferably not used alone for diagnosis; serial intraoral radiographs are used instead.[2,4] For performing periodontal diagnosis and treatment planning, it is recommended that panoramic radiographs be used with other radiographic techniques (e.g., periapical, bitewing).[2,4] Panoramic radiographs, despite their relatively low radiation dose compared to the intraoral mouth series, can give a general idea of both the teeth and the jaws of the patient, and panoramic radiography is inexpensive and easy to use compared to other extraoral imaging methods. Panoramic radiographs are one of the most preferred methods for routine evaluations in clinical dentistry.[26]

The use of AI systems for interpreting dental radiography images shows promise for diagnosis and treatment planning. When diagnosis is difficult owing to a lack of experienced oral or specialist physicians in small clinics/hospitals, AI systems could be used as a decision-support mechanism for oral physicians. Further, they could capture details overlooked by oral physicians owing to strain or fatigue. Diagnostic decisions made by oral physicians/dental students are subjective and could be wrong; AI systems will enable standardizing such decisions.[27] AI systems can also easily record the radiography images of all patients in a dental clinic/hospital with high workload in a database. Therefore, applying an AI system for analyzing periodontal diseases and other dental conditions from panoramic radiographs, which are frequently used in dentistry, could facilitate the early diagnosis, treatment planning, and archiving of information.

Studies have successfully used CNN systems for detecting sinus pathologies[16], vertical root fractures[10], mandibular canals[28], jaw tumors[22], first molar tooth root morphology[11], and teeth and tooth numbers[25] from panoramic radiographs. Other studies have used CNN systems for analyzing dental radiography images obtained by techniques such as periapical, bitewing, and CBCT.[12,24,29] However, few studies have used CNN systems for evaluating the alveolar bone status in clinical dentistry, and literature reviews have noted the importance of this issue.[13-17,30]

Aberin and Goma[13] used a CNN system for determining periodontal diseases from dental plaque microscopy images. Specifically, they used the CNN system to analyze images of periodontally healthy and unhealthy patients to match them to periodontally healthy and unhealthy conditions and achieved an accuracy of 75.5%. Similarly, Balaei *et al.*[30] used a CNN system to determine periodontal disease from intraoral images and achieved an accuracy of 66.7% for disease detection and 91.6% for pretreatment evaluation. These two studies show that CNN systems can be used successfully to determine the periodontal status.

Lee *et al.*[14] used a CNN system to determine periodontally risky teeth from 1044 periapical radiography images and classified the teeth as healthy, moderate, and severe. They calculated the accuracy separately for the mandible and maxilla and achieved a lowest and highest accuracy of 73.4% and 82.8%, respectively. They stated that their CNN system seemed promising in that it provided good predictions in the diagnosis of teeth with periodontal insufficiency. Their study could be considered more comprehensive than our study because it made clinical evaluations. In future studies, we could obtain more accurate diagnostic results by reporting clinical evaluations using a CNN system for determining the periodontal status from radiography images.

Krois *et al.*[15] reported a sensitivity, specificity, and accuracy of 0.81, 0.81, and 0.81, respectively; these results were similar to those of our study (sensitivity, specificity, precision, accuracy, and F1 score of 0.94, 0.88, 0.89, 0.91, and 0.91, respectively). However, Krois *et al*. evaluated 2001 radiography images, whereas we evaluated 2276 images. At the same time, Krois *et al.*[15] recorded and statistically compared the evaluation results of six dentists by using their CNN system; although our study achieved higher accuracy, its interpretability could be increased if it were performed with more dentists. Similarly, Kim *et al.*[16] used a CNN system to successfully evaluate periodontal bone loss from panoramic radiographs and stated that this system could reduce the image interpretation workload of dental radiologists.[16]

Chang *et al.*[17] reported high accuracy and reliability when using a CNN system for determining bone loss and periodontitis staging in line with the criteria of the "2017 World Workshop on the classification of periodontal and peri-implant diseases and conditions." By contrast, a limitation of our study was that it used only a classification model and did not perform evaluations through segmentation. Further, instead of performing disease classification, we used our CNN system only to determine the presence of bone resorption and to determine healthy and bone resorption radiographs. Therefore, our study cannot be compared with the study by Chang *et al*.

Thanathornwong and Suebnukarn[31] used a faster regional CNN system to analyze periodontal destroyed teeth from 100 panoramic radiographs. They reported that their system could be used to quickly detect periodontal destroyed teeth and to provide automatic documentation. They reported a sensitivity, specificity, precision, and F1 score of 0.84, 0.88, 0.81, and 0.81, respectively; however, they used a smaller dataset compared with our study.

CONCLUSIONS

Our study uses a CNN system to determine alveolar bone loss and periodontal disease from panoramic radiographs, and its results are comparable with those of other studies that have successfully used different CNN systems to determine the periodontal health status. Training such CNN systems with more cases will increase the accuracy rates of periodontal diagnosis. Studies investigating the use of CNN systems for the evaluation of periodontal status from intraoral photographs and intraoral and extraoral radiography images will open up new possibilities in clinical dentistry. Future studies should focus on the presence of bone loss and the staging of the disease and thereby observe the severity of the periodontal condition causing bone loss. In addition, more comprehensive studies comparing the radiographic interpretations of many oral physicians and CNN systems will increase the interpretability of the success of these systems. We believe that the promising results of our study on using a CNN system for interpreting dental radiography images will encourage further developments in this area.

ACKNOWLEDGEMENTS

The authors declare that they have no acknowledgments.

CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

Dental Panoramik Radyografi Görüntülerinden Alveolar Kemik Kaybını Belirlemede Yapay Zeka Sisteminin Başarısı

ÖZ

Amaç: *Bu çalışma, yapay zeka (Artificial Intelligence) (AI) sistemi kullanarak dental panoramik radyografi görüntülerden alveoler kemik kaybını tespit etmeyi amaçlamaktadır.* ***Gereç ve Yöntem:*** *Toplam 2276 panoramik radyografik görüntüsü değerlendirildi. Bunlardan 1137'si kemik kaybı olan vakalardı, 1139'u periodontal olarak sağlıklı vakalardı. Bu veri seti eğitim (n = 1856), doğrulama (n = 210) ve test (n = 210) setlerine ayrıldı. Eğitimden önce tüm görüntüler 1472x718 piksel olarak yeniden boyutlandırıldı. Açık kaynaklı Python programlama dili ve OpenCV, NumPy, Pandas ve Matplotlib kütüphaneleri kullanılarak rastgele bir dizi oluşturuldu. Ön işleme için önceden eğitilmiş bir Google Net Inception v3 konvolüsyonel nöral ağı (CNN) kullanıldı ve veri setleri aktarım öğrenimi kullanılarak eğitildi. Tanısal performans, duyarlılık, özgüllük, kesinlik, doğruluk ve F1 skoru bir konfüzyon matris kullanılarak değerlendirildi.* ***Bulgular:*** *Kemik kaybı olan 105 olgunun 99'u CNN sistemi ile sırasıyla 0.94, 0.88, 0.89, 0.91 ve 0.91'lik duyarlılık, özgüllük, kesinlik, doğruluk ve F1 skoru ile tespit edildi.* ***Sonuç:*** *CNN sistemi periodontal kemik kayıplarını başarıyla belirlemektedir. Bu nedenle, gelecekte diş hekimleri tarafından tanı ve tedavi planlamasını kolaylaştırmak için kullanılabilir.*

Anahtar Kelimeler: *Panoramik radyografi, yapay zeka, alveoler kemik kaybı, periodontit.*

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.779519

Original research

CLINICAL ANALYSIS OF DESQUAMATIVE GINGIVITIS RELATED ORAL MUCOCUTANEOUS DISEASES

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ABSTRACT

Objectives: Several mucocutaneous diseases are responsible for desquamative gingivitis (DG) and diagnostic delays are common in these diseases due to non-specific clinical oral presentations. The present study aimed to analyze the clinical features of DG-related mucocutaneous diseases with oral manifestations and compare the findings with previous reports.

Materials and Methods: Thirty newly diagnosed patients with oral mucocutaneous disease in the Dermatology and Venereology Department of Akdeniz University Medical School, between January 2018 and January 2020, and referred to the Periodontology Department for periodontal treatment were evaluated retrospectively. Patients' demographic data (age and gender), medical comorbidities, primary lesion site (oral or skin), and clinical features of gingival and oral lesions (location and symptoms) were examined.

Results: There were 18 oral lichen planus (OLP), 8 pemphigus vulgaris (PV), and 4 mucous membrane pemphigoid (MMP) patients with oral involvements. The mean age of the patients was 50.03 ± 9.71 years with a female predominance (male to female ratio 1:1.7). All patients suffered from chronic burning sensation and oral pain. DG was detected in all three mucocutaneous diseases but OLP was the main disease associated with DG (60%). However other oral mucosal lesions and extraoral involvements were more frequent in PV patients.

Conclusions: According to these results, OLP, MMP, and PV are the mucocutaneous diseases responsible for DG, and OLP is the most common cause of DG. Clinicians should be familiar with the clinical presentations and signs (patient complaints) of DG-related mucocutaneous disease for early diagnosis, proper treatment, and quality of life of the patient.

Keywords: Desquamative gingivitis, diagnosis, mucous membrane pemphigoid, oral lichen planus, pemphigus vulgaris.

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Received : 12.08.2020
Accepted : 06.10.2020

How to Cite: Daltaban Ö, Özçentik A, Akman Karakaş A, Üstün K, Hatipoğlu M, Uzun S. Clinical Analysis of Desquamative Gingivitis Related Oral Mucocutaneous Diseases. Cumhuriyet Dent J 2020;23:4: 325-330.
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INTRUDUCTION

Desquamative gingivitis (DG) is a clinical term to describe the presence of areas of smooth erythema, desquamation, and erosion of the attacehed and marginal gingiva.[1] Clinically, the involved gingival epithelium is very fragile, there is a loss of stippling, and the surface may desquamate easily in response to minor trauma.[2]

Several mucocutaneous diseases, such as oral lichen planus (OLP), mucous membrane pemphigoid (MMP), pemphigus vulgaris (PV), lupus erythematosus, graft versus host disease, erythema multiforme, epidermolysis bullosa acquisita, chronic ulcerative stomatitis, dermatitis herpatiformis and linear IgA disease, may possess DG.[1-3]

Adequate knowledge of disease associated with DG is mandatory in daily clinical practice because the oral cavity can be the only site of involvement or as the first manifestation of the disease.[3]

A careful medical history and intraoral examination for erythematous gingiva and other oral lesions with specific appearances are extremely important in establishing a clinical diagnosis.[2,4] However, DG-related mucocutaneous diseases frequently share similar clinical manifestations, so, further diagnostic methods, based on biopsy for histopathologic and direct immunofluorescence analysis (DIF), are considered to be the gold standard in diagnosis.[1,5,6]

Previous studies demonstrated that patients with oral mucosal diseases are widely misdiagnosed, leading to multiple consultations and insufficient treatments.[7,8] Delays in the diagnosis and treatment of DG-related mucocutaneous disease may adversely affect the disease course, treatment response, and prognosis.[9] Besides painful erosive or ulcerative gingival and oral lesions can significantly limits the patient's normal oral functions and interpersonal relationships.[10]

A lack of knowledge of primary health care practitioners for clinical features of oral mucosal diseases has been well documented in the literature.[11-13] Therefore, this study aimed to examine the clinical features of patients with DG-related mucocutaneous diseases, and compare the findings with previous reports.

MATERIALS AND METHODS

Study Design

This study protocol was approved by the Ethics Committee of the Medical Faculty of Akdeniz University Antalya, Turkey according to the Helsinki declaration (70904504\332-13.05.2020).

The present study is based on a retrospective review of patients with oral mucosa lesions who were newly diagnosed with DG-related mucocutaneous disease at the department of Dermatology and Venereology and were referred to the Periodontology department for periodontal treatment from January 2018 to January 2020. Patients who had non-specific histopathology and/or DIF or had previously been managed with systemic immunosuppressive agents were excluded. Diagnosis of DG-related mucocutaneous disease was established in all cases by clinical, histologic, and immunologic criteria described previously.[14,15]

Demographic data including age, gender, medical comorbidities, along with the primary site of lesions (oral or skin) and clinical presentations of the gingival and oral lesions were retrieved from patient records previously drafted for this study. The oral presentations for each disease were recorded according to the type of lesion (DG, non-gingival erosions and/ ulcers) and involved sites. Symptoms in oral lesions were defined as pain or burning/itching sensation. The involvement of skin and/or other mucosal membranes was also documented.

Statistical analysis

Descriptive statistics for frequencies and percentages were calculated. The results were expressed as the mean±standard deviation and range. Mann Whitney U test was used to detect any significant differences between groups for age. All calculations were undertaken using a statistical software package SPSS (version 23 Chicago, IL, USA). A p value <0.05 was considered statistically significant.

RESULTS

A total of 40 patients have been initially selected, but 10 were excluded because these patients were

on follow-up. Thirty patients fulfilled the inclusion criteria: 18 had OLP (60%), 8 had PV (27%), and 4 MMP (13%). Demographic data and clinical presentations of patients are detailed in Table 1.

Table 1: Demographic Data and Clinical Presentations of Patients

Clinical Features	OLP (n=18)	PV (n=8)	MMP (n=4)
% for total	60%	27%	13%
Mean age at onset	51.17 ± 10.97	47.01 ± 8.60	51.00 ± 4.76
Gender			
Female to male ratio	10\8	5\3	4\0
Oral Lesion Location (%)			
Desquamative gingivitis	83%	25%	100%
Non-gingival erosions and ulcerations			
Buccal mucosa	44%	88%	25%
Labial mucosa	0%	50%	0%
Palate	0%	50%	25%
Tongue	22%	63%	0%
Floor of the mouth	0%	13%	0%
Extra-oral involvement (%)			
Pharynx	0%	63%	50%
Larynx	0%	38%	0%
Nasal mucosa	0%	13%	0%
Conjunctiva	0%	13%	0%
Vaginal	6%	25%	25%
Skin	0%	75%	0%

OLP: Oral lichen planus, PV: Pemphigus vulgaris, MMP: Mucous membrane pemphigoid

The mean age of the patients was 50.03 ± 9.71 years, varying from 32 to 68 years (48.54 ± 10.20 for males and 51.11 ± 11.26 for females) with a female predominance (male to female ratio 1:1.7). The medical comorbidities of the patients included essential hypertension (4 patients), diabetes mellitus (2 patients), and hypothyroidism (2 patients).

There were 18 OLP patients: 10 female and 8 male patients. The mean age at diagnosis was 51.17 ± 10.97 years. The gingiva was the most affected site of involvement (83%), followed by the buccal mucosa (44%), and tongue (22%). The erosive gingival lesions involved attached and marginal gingiva with 8 of them in a mixed form (erosive and reticular). DG was the sole manifestation in 10 cases (56%). Only one female patient had extraoral involvement (genital mucosa).

There were 8 PV patients with oral mucosal involvement, comprising 5 (63%) females and 3 males (37%) with a female predominance. The mean age at the onset of the disease was 47.01 ± 8.60 years (45.67 ± 13.5 for males and 47.80 ± 6.01 for females). Two of 8 PV patients (25%) had oral mucosal lesions only. In 6 patients (75%), the disease initially began as persistent oral mucosal lesions, and then extended to involve the skin. All patients had erosive and/or ulcerative oral lesions. In the intraoral examination, the most affected anatomical site was the buccal mucosa (88%), followed by the tongue (63%), palate (50%), lips (50%), gingiva (25%) and floor of the mouth (13%). The involvement of other mucosal lesions was also observed as the pharynx (63%) larynx (38%), nasal mucosa (13%), vaginal (25%), and conjunctiva (13%) as well as skin and/or oral mucosa involvements.

There were 4 MMP patients. All of them were female and the mean age at diagnosis was 51.00 ± 4.76 years. DG was evident in all MMP cases and was the sole manifestation in 50% cases. Oral mucosa lesions were buccal mucosa (25%) and palate (50%). Pharynx was involved in two patients and vaginal mucosa in one patient. None of the patients developed cutaneous and ocular lesions. The clinical characters of gingival lesions were diffuse erythematous lesions with ulcers and erosions on the attached gingiva.

Symptoms

All patients complained of mild or moderate oral discomfort, and reported chronic soreness and burning sensation (93% n=28) particularly when

eating acidic foods, and pain (60% n=18) with oral function. One MMP patient had sore throat. In PV patients, the persistent painful ulcerations of the pharynx and larynx produced dysphagia (n=5, 63%), and odynophagia (n=2, 25%).

DISCUSSION

The analysis of this report revealed that OLP, MMP, and PV are responsible for DG. A common feature seen in all three diseases is that females are more frequently affected than males. Another common characteristic is that OLP, MMP and PV are diseases of older ages. There were no notable differences in the age distribution of patients with different diseases ($p>0.05$). Similar observations have been reported by other investigators.[16, 17]

OLP is a common chronic immunologically cell-mediated mucocutaneous disease in which host T lymphocytes play a key role in the development of the lesions.[3,18] In the present study, OLP was the primary disease associated with DG (60%). This observation is in accordance with most previous reports.[2,16] Further analysis of our clinical data showed that the majority of OLP lesions in the gingivae are of the erosive or ulcerative form with 8 of them in a mixed form (erosive and reticular). Mignogna *et al*.[19] presented a large series of patients with gingival OLP and found that the mixed forms were the most frequent.

PV, is a potentially life-threatening, rare, autoimmune mucocutaneous disease whose early diagnosis in the oral cavity and timely referral may significantly change the course and progression of the disease.[1,20] The pathogenesis of the disease is characterized by acantholysis and intraepithelial blister formation, resulting from IgG autoantibodies directed against desmosomal intercellular adhesion molecules (desmogleins) present on keratinocytes, mainly desmoglein 3.[2,6] In this study, oral involvement is the first sign of PV, and skin lesions manifest in 75% of our patients. All of our patients with PV have painful, hemorrhagic irregular, long-persisting multiple erosions, and ulcers on the oral mucosa. Compared to MMP and OLP patients, DG was evident in 25% of our PV patients, but other oral mucosa involvements were more frequent, and the most common site of disease presentation was the buccal mucosa (88%). This result confirms the findings of Leao *et al*.[16] that PV account for the minority of DG lesions. Mignogna *et al*.[21] also reported a retrospective study of all cases of oral PV revealing gingival involvement in 8 of eighteen patients (44%) with DG representing the sole oral presentation in 2 of eighteen patients (11%).

MMP, is a subepithelial blistering autoimmune disease that predominantly manifests in the oral cavity.[4,5] The pathogenesis of the disease is characterized by autoantibodies, predominantly, IgG with complement factor C3, directed against epithelial basement membrane adhesion proteins, BP180, BP230 and laminin 332.[6,22] In this study, all MMP patients had DG and it was the sole manifestation in 50% cases. In a cohort of 26 MMP patients and 31 PV patients with oral presentations, Sultan *et al*.[23] showed that gingival lesions were significantly more common in MMP patients (84%) as compared to PV patients (28%).

All of the patients in the present study suffered from chronic burning sensation and oral pain. However, patients with PV have a more severe disease progression, and pain was the main complaint with dysphagia (63%), and odynophagia (25%). These symptoms may have additional information for clinicians when diagnostic possibilities are being considered in a patient with erosive and or ulcerative oral lesions.

DG can be easily misdiagnosed for plaque-induced gingivitis, but specific clinical appearances of oral and gingival lesions may help to narrow the diagnosis, such as small blisters/vesicles, which have a relatively thick roof than PV and may be blood-filled in MMP[3,22], and the presence of white plaques or striae at the periphery of the erosive lesions in OLP.[1,18] Additionally, Nikolsky's sign should be an integral part of the oral mucosal examination.[24] However, a definitive diagnosis may be possible only with microscopic examination.[2,4-6]

Once a definitive diagnosis of the cause of DG has been established, special multidisciplinary coordinated approaches should be taken because; MMP can affect conjunctiva which may result in subconjunctival scarring (symblepharon) and

eventually permanent blindness[22,25], PV is a serious mucocutaneous disease which can be fatal if untreated,[20,26] and OLP has a risk for malignant transformation.[18]

The main limitation of this study was the limited sample size and relatively short observation period. But performing this type of study is very important as the studied diseases are rare and often difficult to diagnose in their initial stages. Further multicenter, long-term prospective studies with larger patient groups will probably give more valuable information.

CONCLUSIONS

Within the limits of the present study, it is concluded that OLP, MMP and PV are the mucocutaneous diseases associated with DG. As the initial presentations of these diseases frequently occur in the oral cavity, general practitioners (medical and dental) should become familiar with the clinical presentations of DG-related mucocutaneous diseases, for providing proper management of these conditions.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Deskuamatif Gingivitis ile İlişkili Oral Mukokutanöz Hastalıkların Klinik Analizi

ÖZ

Amaç: *Birçok mukokutanöz hastalık, deskuamatif gingivitis lezyonlarından (DG) sorumludur ve bu hastalıklarda, spesifik olmayan ağız bulguları nedeniyle, tanısal gecikmeler oldukça yaygındır. Bu çalışmada DG ile ilişkili mukokutanöz hastalıkların klinik özelliklerinin incelenmesi ve bulguların daha önceki çalşmalarla karşılaştırılması amaçlanmıştır.* ***Gereçler ve Yöntem:*** *Ocak 2018 ve Ocak 2020 tarihleri arası Akdeniz Üniversitesi Dermatoloji bölümünde DG ile ilişkili mukokutanöz hastalık tanısı alan ve periodontal tedavi amacıyla Periodontoloji bölümüne yönlendirilen 30 yeni teşhis hastası retrospektif olarak değerlendirildi. Hastaların demografik verileri (yaş ve cinsiyet), sistemik hastalıkları, ilk lezyon bölgesi (oral veya deri) ve dişeti ve oral mukoza lezyonlarının klinik özellikleri (lokalizasyon ve semptomları) değerlendirildi.* ***Bulgular:*** *Oral tutulumu olan 18 oral liken planus (OLP), 8 pemfigus vulgaris (PV) ve 4 mukoz membran pemfigoid (MMP) hastası vardı. Hastaların yaş ortalaması 50.03 ± 9.71 ve kadın oranı daha yüksekti (erkek/kadın oranı 1: 1.7). Tüm hastalarda ağız içerisinde kronik yanma hissi ve ağrı şikâyeti vardı. Her üç mukokutanöz hastalıkta da DG bulgusuna rastlandı ancak OLP, DG bulgusuna en sık rastlanan mukokutanöz hastalıktı (%60). PV hastalarında ise diğer oral mukozal lezyonlar ve ekstraoral tutulumlara daha sık rastlandı.* ***Sonuçlar:*** *Bu sonuçlara göre, OLP, MMP ve PV deskuamatif gingivitisten sorumlu mukokutanöz hastalıklardır ve OLP, deskuamatif gingivitisin en yaygın nedenidir. Klinisyenler, erken teşhis, uygun tedavi ve hastaların yaşam kalitesinin arttırılması açısından DG ile ilişkili mukokutanöz hastalıkların klinik özellikleri ve belirtilerine karşı daha fazla bilgi sahibi olmalıdır.*

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.793013

Original research

IDEAL SPONTANEOUS SPACE CLOSURE AFTER LATE EXTRACTION OF PERMANENT FIRST MOLAR TEETH

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ABSTRACT

Objectives: Before they haven't completed its development, permanent first molar (PFM) teeth which have been decayed and treated with root canal therapy is mostly extracted before the child even reaches adult hood. With correct evaluation of patient, these extracted teeth's space can be ideally closed by permanent second molars (PSM). The aim of the study is to learn under which conditions we can provide ideal closure and occlusion in patients who refer to us in a late stage.

Materials and Methods: 695 patients aged 6-16 years who had PFM tooth extracted at a Faculty of Dentistry were retrospectively evaluated from the system. 117 patients had their PFM tooth extracted after the ideal age range and 15 of them were found to meet the inclusion criteria. Panoramic films of 21 teeth of 15 patients with spontaneous ideal closure were evaluated before extraction and after eruption of PSM teeth. The contact between the PSM and the second premolar was recorded and intraoral photographs were taken. Rotations in the PSM and second premolars, axis disturbances towards the lingual/buccal, midline deviations in both jaws, TMJ dysfunctions and chewing habits were evaluated.

Results: When PFM extraction was performed, PSM development stage was determined as Demirjian G in 19 teeth and H stage in 2 teeth. Tipping was observed in 80%, and rotation in 9.5% of the PSM teeth. Clinically, it has been determined that 85% of the patients don't notice the PFM deficiency, don't complain about their teeth and don't consider orthodontic treatment.

Conclusions: Within the limits of this study, it has been observed that ideal spontaneous space closure and ideal occlusion can be achieved, although it was extracted in late period. In ideal occlusion, rotation or tipping after extraction in late period; permanent third molar presence and eruption pressure were more effective than the eruption direction and angle of the PSM tooth.

Keywords: Molar, first, tooth extraction, pediatric dentistry, malocclusion, extraction timing, orthodontic space closure.

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Received : 10.09.2020
Accepted : 07.10.2020

How to Cite: Demir P, Aydoğdu H. Ideal Spontaneous Space Closure After Late Extraction of Permanent First Molar Teeth. Cumhuriyet Dent J 2020;23:4: 331-339.
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INTRODUCTION

Permanent first molar (PFM) teeth are more susceptible to coronal defects such as MIH and hypoplasia caused by metabolic or physiological disorders in early infancy, as they begin to develop from pregnancy and calcify at birth.[30] It is the first permanent tooth in the mouth, and since it is thought to be a primary tooth by families and not maintained properly[29] and has a fissured anatomy[32], it is prone to decay. Furthermore, limited hand skills of children under the age of 8 also prevent them from performing effective teeth brushing.[26,40] With the addition of poor oral hygiene to these coronal defects and predispositions, permanent first molars become the teeth with the worst prognosis[30] and decay and extraction are inevitable in a very short time.[5,24,36]

When performing root canal treatment in pediatric patients, the desired cooperation may not be achieved. In cases where the prognosis of the tooth is doubtful in the long term, the fact that keeping these teeth in the mouth with root canal treatment is seen as a temporary solution, which creates a dilemma between extraction and root canal treatment for the physician.[2,25] In PFM teeth with deep caries and doubtful long-term prognosis, extraction is a saving treatment option.[1,2,7,13,27]

When the prognosis of the tooth is poor and extraction is inevitable; with planned and timed extractions, it can be ensured that the remaining teeth of the patient are directed and the occlusion is not deteriorated due to missing teeth and is minimally affected. In the literature, it has been reported that an optimum occlusion can be provided for the child with the appropriate timing and case selection for PFM tooth extraction.[16,37,38] The ideal time varies according to the physical and dental development of the child. The ideal time for the extraction of a PFM is the time when calcification begins to form in the bifurcation area of permanent second molars (PSM) and usually corresponds to the age range of 8-10 years.[1,20,38] These ages correspond to “E” in the Demirjian classification which is widely used, and 6th and 7th stages in the Nolla classification.[10,21] However, patients who present for PFM tooth extraction are generally older than this ideal age range.1 Studies in the literature indicate that the extraction area will not be completely closed[13] and that weak contacts will form between teeth in case the teeth extraction is delayed.[6,11] However, it has been reported in recent studies that successful results can be obtained, especially in the upper jaw, up to the age of 11.5 years.[12] Although the results of extraction of PFM teeth can be clinically indicated, there is little scientific evidence as to which factors are effective other than the ideal extraction timing to minimize unwanted negative consequences such as the tipping and/or rotation of the second premolars or PSM teeth.[37,38]

There are controversial opinions about the effects of some criteria other than timing on the prognosis of the extraction space to ensure ideal occlusion.[37,38] Many researchers find that the correct timing is not enough alone for successful spontaneous closure of the extraction gap. They argued that the determination of the germ of the permanent third molar and the second premolars radiographically, the patient's current occlusion type, the initial eruption angle of the permanent second molar tooth, and the compensation or balance extractions are at least as important as timing.[1,7,13,23,24,35]

The idea that only extractions made at the ideal time will be successful has changed. The desire is, of course, the ideal treatment at the ideal time, but even when the patient arrives very late, optimum occlusion can be achieved with correct planning. Clinical studies conducted by evaluating factors more effective than the extraction time will contribute to the literature on this subject. In this case series, cases showing ideal closure despite being extracted after the ideal time were examined. This study evaluates which factors affect the occlusion and ideal closure place after extraction of PFM teeth, based on historical clinical data. The aim of the study is to learn under which conditions we can provide ideal closure and occlusion in patients who present to us in a late stage.

MATERIALS AND METHODS

In this study, the occlusion of 695 patients between the ages of 6 and 16 who came to the

Department of Pedodontics of a university hospital and who had their PFM teeth extracted in this clinic between 2015 and 2018 was evaluated. The pre- and post-extraction films of 695 patients were retrospectively evaluated within the scope of a pilot study (Ethics number: 2019/338). 117 patients were detected who extracted DBM tooth after the tooth development stage Demirjian E, which is shown as the ideal time. Among them; 38 patients with spontaneous ideal space closure[36] on radiography were contacted by phone and informed about the study. The ideal space closure was determined by modifying the scale created by Teo in his study.[36] 21 teeth of 15 of the patients who agreed to come for examination and intraoral photographs were found to meet the inclusion criteria. Ethics committee approval for this prospective clinical study, which includes patient examinations and intraoral photographs, was obtained from the Health Sciences Clinical Research Ethics Committee affiliated to the Scientific Research and Publication Ethics Committee of our university (Decision No: 2020/113).

For the inclusion of patients in the study, the conditions sought for were;

* Having a panoramic film before PFM tooth extraction,

* Having the PFM tooth extracted in our clinic and after the Demirjian stage E,

* Spontaneous space closure of the PSM tooth to the extraction gap of the PFM tooth and ideal contact with the distal of the second premolar tooth,

* Having a panoramic film after the PSM tooth erupts and contacts with antagonist,

* The parents' permission for the child's intraoral photos to be taken and used in the study by signing the consent form.

Patients who received orthodontic treatment after PFM extraction were excluded from the study.

During the clinical examination of the patients, the closure relationships of the teeth were evaluated. The contact between PSM and the second premolar was recorded and intraoral photographs were taken. Rotations in PSM and second premolars, axis abnormalities towards the linguale/ buccal, midline deviations in both jaws, TMJ dysfunctions and chewing habits were evaluated. Radiographic examination was performed in two panoramic radiographs before and after extraction. In the panoramic radiography before extraction; the radiographic development stage of the tooth, the eruption angle of the PSM tooth and the presence/absence of permanent third molar were evaluated. In pre-extraction radiographic examination; a line was drawn passing through the bifurcation perpendicular to the line passing through the cusp tips, as described by Heckmann *et al.*[15] The angle between this reference line and the line passing through the right and left gonion was calculated.[17,41] After determining reference lines, the program automatically took the angular measurements (Figure 1).

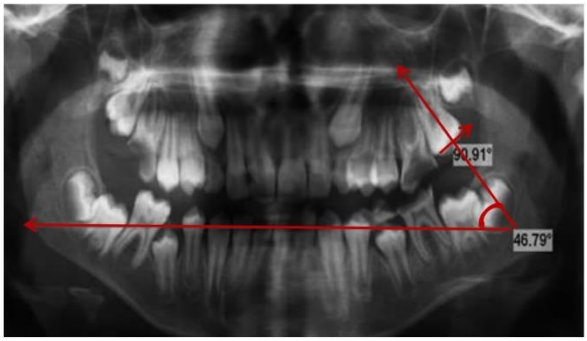

Figure 1: Angular Measurements for the left upper PFM

In cases where PFM deficiency in the mouth was not noticed and SPM was showed ideal contact in the clinical control after extraction; Radiography was taken to evaluate the PSM/second premolar long axis and the presence of tipping. In the post-extraction radiography, the parallelism of the long axes of the PSM and the second premolar and the presence of tipping were evaluated.

RESULTS

Of the 15 patients included in the study, 7 were girls and 8 were boys, with an average age 12.8 ± 1.03. A total of 21 teeth were evaluated, of which were 9 mandibular and 12 maxillar molar. When PFM extraction was performed, PSM development stage was determined as Demirjian G in 19 teeth and H stage in 2 teeth. All patients have a permanent third molar tooth. Midline

deviation was detected in only 1 patient. TMJ dysfunction was detected in 1 patient and unilateral chewing habit in 3 patients. Postextraction control radiographs of all patients were taken at least 3 years later. Tipping was observed in 80%, and rotation in 9.5% of the PSM teeth. PSM angles are as in Table 1.

Table 1: Clinical and Radiographical Evaluation

Patent's Age At Extraction	Demirjian Stage At Extraction	Teeth Number	Permanent Third Molar Presence	PSM Angulation	Tipping		Rotation		Midline Deviation	Patient' s Age at Postextraction Evaluation
					Second Premolar	PSM	Second Premolar	PSM		
13	G	16	+	131.57^0	-	-	-	+	-	19
13	G	26	+	133.21^0	-	-	-	-	-	19
12	G	46	+	60.46^0	+	+	+	-	-	17
13	G	16	+	114.79^0	-	+	-	-	-	17
13	G	26	+	118.65^0	-	+	-	-	-	17
12	G	46	+	46.81^0	-	+	-	-	-	17
13	G	26	+	127.05^0	-	+	+	-	-	19
13	G	46	+	63.77^0	-	+	+	-	-	19
11	G	16	+	101.05^0	+	+	-	-	-	18
11	G	26	+	116.91^0	+	-	+	-	-	18
11	G	36	+	65.16^0	-	+	+	-	-	16
13	G	16	+	135.74^0	-	+	+	-	-	16
13	G	36	+	65.82^0	-	+	-	-	-	16
15	G	36	+	48.03^0	-	+	-	-	+	18
12	G	36	+	71.47^0	-	+	+	-	-	17
12	G	26	+	58.05^0	-	+	+	+	-	17
14	G	46	+	66.45^0	-	+	-	-	-	17
13	H	16	+	64.41^0	-	+	-	-	-	17
13	G	26	+	57.73^0	-	+	-	-	-	17
14	H	36	+	70.91^0	-	+	-	-	-	17
11	F	16	+	132.64^0	-	-	-	-	-	14

Tipping was detected in 14.2% and rotation in 38% of the second premolar teeth.

The parents of the patients who accepted to participate in our study were informed again when they came to our clinic, and the consent form was signed. Clinically, it has been determined that 85% of the patients do not notice the PFM deficiency, don't complain about their teeth and don't consider orthodontic treatment. Some of the panoramic films and intraoral photographs taken from the patients are shown below (Figure 2,3,4,5).

Figure 2: 2a: Pre-extraction radiography of the patient whose upper left PFM tooth was extracted while the PSM tooth was in the G stage **2b:** Radiography taken 6 years after PFM extraction **2c:** Intraoral photo 6 years after PFM extraction

Figure 3: 3a: Pre-extraction radiography of the patient whose lower left PFM tooth was extracted while the PSM tooth was in the G stage **3b:** Radiography taken 5 years after PFM extraction **3c:** Intraoral photo 5 years after PFM extraction

Figure 4: 4a: Pre-extraction radiography of the patient whose upper right PFM tooth was extracted while the PSM tooth was in the H stage **4b:** Radiography taken 4 years after PFM extraction **4c:** Intraoral photo 4 years after PFM extraction

Figure 5: 5a: Pre-extraction radiography of the patient whose lower right PFM tooth was extracted while the PSM tooth was in the G stage **5b:** Radiography taken 5 years after PFM extraction **5c:** Intraoral photo 5 years after PFM extraction

DISCUSSION

It is thought that the treatment success of PFM teeth with large restorations or excessive crown destruction will be low.[1,22,31] When the prognosis of the tooth is not good, keeping these teeth in the mouth with root canal treatment will be a temporary solution.[2,25] In these teeth with deep caries, extraction appears as a saving treatment option.[2] Unfortunately, no randomized controlled clinical study evaluating the extraction timing of non-restorative PFM teeth has been found in the literature[12]. Although prevention of complications is the most important issue in cases of PFM extraction, there is little scientific evidence about the timing of extraction to minimize unwanted adverse effects.[12] Extraction at ideal time or early extraction are mostly mentioned in literature.[1,9,13,18,24,34] There are very few clinical studies about late extractions.[12]

It has been reported that late extractions in the mandible will cause cross-bite and non-working side conflicts with the mesiolingual tipping of the PSM tooth, the second premolars to tip distally, and the development of periodontal diseases and alveolar bone atrophy due to poor contact relations between the PSM and the second premolars.[2,28,34] It has been reported that the second molar teeth can be drifted into the mesial, or even tipped and cause rotation as a result of late extractions in the maxilla.[2,28]

In the literature, there are cases that are extracted at the ideal time and do not show ideal closure, and cases that show ideal closure extracted after the ideal time. In the meta-analysis study conducted by Eichenberger *et al.*[12], it was reported that the late extractions showed less satisfactory but still good clinical results. According to the results of this study, it was shown that 50% of the extracted teeth had good results between the ages of 8-10.5, while this rate was found to be 59% between the ages of 10.5-11.5.[12,31] It has been reported that the clinical results in children younger than 8 years or older than 11.5 years are 34% and 44% respectively, and they give less satisfactory results. As a result of the studies, it has been observed that there is a heterogeneous situation, especially in the mandible. All these data show that factors other than age are also effective in spontaneous space closure.

It was stated that there are factors more important than time in Teo *et al.*[36] clinical study done with 236 teeth, most of which are in the G stage. According to this study, they reported that the mesial angulation of the PSM tooth and the presence of a permanent third molar is a stronger indicator of spontaneous closure of the extraction space compared to the developmental stage of the PSM tooth. Again, according to the study of Teo *et al.*, 92% of the upper extractions caused a good closure regardless of the timing, while only 66% of the lower PFM teeth showed positive results despite the 'ideal time' extraction. Jälevik and Möller also obtained similar results in their study evaluating the spontaneous closure of the space after extraction of hypomineralized PFM teeth.[18,36]

No statistically significant difference was found between extraction times in the maxilla.[12] However, timing is more critical in the mandible than in the maxilla.[12,13] The reason for this, is that the maxilla is more spongy and tooth movement is easier.[38] In addition, the PSM apex in the maxilla has a mesial angle with respect to the crown; the tendency of the crown to be closed by drafting mesially due to the more distal angle of the PSM apex of the mandible[8,12] is also an important factor. In this study, all of the cases (14%) who showed an ideal contact despite being extracted in

the late period and who did not show tipping and rotation in the PSM and second premolar teeth, were in the upper jaw. In accordance with the literature; in this study, even though clinical contact was observed when PFM extraction was performed while PSM teeth were in Demirjian G or H stage in the mandible, radiographic tipping was detected in all PFM teeth.

Although it was in contact with the antagonist teeth in such a way that the patient does not notice the missing tooth, it was determined radiographically that this result was achieved by PSM tipping. As a result of the late extractions performed in this study, it was observed that the patients did not complain about the lack of PFM, and they even did not feel the deficiency due to the place closed by the pressure of the third molar. Even if the deficiency is not noticed in the mouth, it has been determined as a result of the radiographs taken, that in some cases, closure occurs by the rotation of the premolars and/or the tipping of the PSM.

Panoramic radiographs have been used around the world for a long time and play an important role in dental radiology. Therefore, similar to the opinion of Bayram *et al.*[4,28], it is thought that there is no need for other radiographs to evaluate the eruption of PSM after PFM extraction. Although panoramic radiography has disadvantages such as image magnification and distortion, the consistency between angle measurements and measurements can be assessed by continuous use of the same device and settings.[14,19] Larheim and Svanaes[19] reported that horizontal dimensions can be measured with acceptable reliability if the patient is properly stabilized during irradiation.[31] In this study, measurements were made on the radiographs taken using the same equipment and settings, and the margin of error was minimized.

PFMs have been found to be decayed in more than 50% of children over the age of 11.[39] In a study, it was found that the most common age of PFM extraction was 11, followed by 10 and 12 years respectively, and 14 was the most common evaluation age.[28] It was stated that the reason why the patients present to the clinic in a late period, was that parents think that PFM is a primary tooth and permanent teeth will replace PFM teeth. During the eruption of a PFM tooth, neither the child nor the family are usually aware of the eruption of the permanent teeth in the mouth.[33] As a result, they think that PFM teeth are also primary teeth, and that it is unnecessary to spend time/money to provide adequate oral care for children[29] and because only 13.4% have an idea about PFM eruption[42], it is too late to treat PFM teeth.

Extracting the teeth always in their ideal developmental stage is the least risky, and enables the most ideal results possible for that patient. However, what is important here is to make an indication by evaluating each patient's own specific occlusion. The late arrival of patients does not mean that dentists still cannot achieve the ideal occlusion. Especially for PFM teeth in the upper jaw, extraction can be applied without any hesitation. In the lower jaw, extraction can be done considering;

- Long-term prognosis of restored PFM tooth
- The dental age of the patient
- Distal inclined eruption angle of the PFM
- Type of malocclusion
- Current degree of crowding
- The presence of the permanent third molar tooth.

Failure in the extraction timing causes vertical resorption of the alveolar bone, promoting tipping and rotation of neighboring teeth. As a result, it causes occlusal conflicts, TMJ dysfunctions and skeletal asymmetry in the anterior region[28]. When early extraction is made, this non-compensatory path is entered, but relatively less and local damage (vertical bone loss in PSM teeth) occurs in late extractions. This is caused by the eruption direction of the PSM teeth being distal, the presence of permanent third molars and the eruption pressure. If the patient meets these conditions, even applied in a late period, ideal closure is observed in extractions made before the PSM teeth eruption, and the patient's future graft + implant necessity are eliminated. By evaluating special conditions for each patient, it is possible to

support the development of the jaw and occlusion with the patient's own healthy teeth.

CONCLUSIONS

It is anticipated that this study will contribute to the limited literature available and will provide a much better clinical picture about treatment in such cases. In patients with accompanied orthodontic problems, it is difficult to make correct recommendations and make evidence-based decisions about prognosis, and long-term vision is essential when planning.[3] Within the limits of this study, it has been observed that ideal spontaneous space closure and ideal occlusion can be achieved, although it was extracted in the late period. In ideal occlusion, rotation or tipping after extraction in a late period; permanent third molar presence and eruption pressure were more effective than the eruption direction and angle of the PSM tooth. Clinical studies done with more patients are needed to make clear decisions on this issue.

DECLARATIONS

- **Availability of data and material:** The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request. We guarantee that the data and the the patients' informed consent will be shared if requested by your journal.

CONFLICTS OF INTEREST STATEMENT

All authors declare that they have no competing interests that may have influenced either the conduct or the presentation of the research.

ACKNOWLEDGEMENTS

Not applicable.

FUNDING

The authors declare that there have no any financial relationships that need to be known regarding the study.

Daimi Birinci Molar Dişin Geç Dönemde Çekiminden Sonra İdeal Spontan Yer Kapanması: Bir Olgu Serisi

ÖZ

Amaç: *Henüz gelişimini tamamlamadan çürüyen ve kök kanal tedavisi yapılan daimi birinci molar dişler, çocuk erişkinliğe bile ulaşamadan kaybedilmektedir. Doğru değerlendirme ile çekilen bu dişlerin yeri daimi ikinci molarlar tarafından ideal bir şekilde kapatılabilir. Çalışmanın amacı geç dönemde bize başvuran hastalarda da ideal yer kapanması ve oklüzyonu hangi koşulda sağlayabileceğimizi öğrenmektir.* ***Gereç ve Yöntemler:*** *Bir üniversiteye bağlı Diş Hekimliği Fakültesinde DBM dişini çektirmiş 6-16 yaş arası 695 hasta retrospektif olarak sistemden taranmıştır. 117 hasta ideal yaş aralığından sonra daimî birinci molar dişini çektirmiş ve 15 tanesi dahil edilme kriterlerine uygun bulunmuştur. Radyografide spontan ideal yer kapanması gözlenen 15 hastanın 21 dişinin çekim öncesi ve daimi ikinci molar dişi sürdükten sonra panaromik filmi değerlendirilmiştir. Daimi ikinci molar ile 2. premolar arasındaki kontakt kaydedilmiş ve ağız içi fotoğraf kayıtları alınmıştır. Daimi ikinci molar ve 2. premolardaki rotasyonlar, linguale/bukkale doğru aks bozuklukları, her iki çenede orta hat sapmaları, TME disfonksiyonları ve çiğneme alışkanlıkları değerlendirilmiştir.* ***Bulgular:*** *DBM çekimi yapıldığında daimi ikinci molar gelişim evresi 19 dişte Demirjian G, 2 dişte Demirjian H evresi olarak tespit edilmiştir. Radyografik olarak daimi ikinci molar dişlerinin %80'inde devrilme, %9.5'inde rotasyon görülmüştür. Klinik olarak hastaların %85'inin diş eksikliğini fark etmediği, tamamının dişlerinden şikayetçi olmadığı ve ortodontik tedavi düşünmediği tespit edilmiştir.* ***Sonuçlar:*** *Bu çalışmanın sınırları dahilinde, geç dönemde çekilmesine rağmen ideal spontan yer kapanması ve ideal oklüzyonun oluşturulabileceği gözlenmiştir. Geç dönemde çekim sonrası ideal kapanma, rotasyon veya devrilmede; DİM dişin sürme yönü ve açısından ziyade daimi üçüncü molar varlığı ve sürme baskısı daha etkili olmuştur.* ***Anahtar Kelimeler:*** *Birinci molar, diş çekimi, çocuk diş hekimliği, maloklüzyon, çekim zamanlaması, ortodontik yer kapanması.*

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.796823

Original research

REMINERALIZATION OF PRIMARY MOLAR DENTINE WITH SILVER DIAMINE FLUORIDE AND SODIUM FLUORIDE: AN *IN VITRO* STUDY

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Mert Ocak[2]
Zeynep Ökte[3]

ABSTRACT

Objectives: Silver diamine fluoride (SDF) offers a minimally invasive and inexpensive alternative to traditional restorative caries treatment. Aim of this study was to evaluate the mineral density value and remineralization effect of 38% SDF solution and 5% sodium fluoride (NaF) varnish on artificial primary tooth dentin caries lesions.

Materials and Methods: 54 extracted sound primary molars were demineralized and allocated into two groups: SDF and NaF groups. After pH-cycling, mineral density values (MDV) were assessed with micro computed tomography (micro-CT) and surface morphology were studied *via* scanning electron microscopy (SEM).

Results: After remineralization agents were apllied and specimens pH-cycled, the MDVs of the Region of Interest (ROI) in lesions increased in both NaF and SDF group. The mean MDV of SDF applied specimens was found to be higher than NaF applied specimens at statistically significant level. In the SEM images, it was observed that the dentinal tubules orifices were occluded almost completely in SDF group while the orifices in NaF group were partially occluded.

Conclusions: The use of 38% SDF demonstrated a great remineralization effect on primary molar dentin than 5% NaF varnish. Further clinical studies are needed to investigate the effect of 38% SDF on primary molars which remains in the mouth longer than incisors and have a great function on chewing.

Key words: Topical fluorides, silver diamine fluoride, microcomputed tomography, tooth remineralization.

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Received : 18.09.2020
Accepted : 22.09.2020

How to Cite: Yilmaz N, Ocak M, Okte Z. Remineralization of Primary Molar Dentine with Silver Diamine Fluoride and Sodium Fluoride: An In Vitro Study. Cumhuriyet Dent J 2020;23:4: 340-347.
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INTRODUCTION

Despite the advance in dental health care in the past few decades, early childhood caries (ECC) is a still widespread condition seen among children throughout the world.[1,2] It is essential to diagnose and treat ECC in early stage, because untreated cavities can lead to severe toothache and development of dental abscesses.[3] Conventional caries treatment involves mechanical removal of the carious lesion by rotary instruments and restoration of the cavity by filling material. However, in most cases it may not be possible to treat ECC with standard methods due to cooperation problems in children and expensive options such as general anesthesia or sedation are needed.[4-6] Therefore alternative treatments for dental caries are neccessary and professionally applied fluoride therapy has been proposed not only to prevent but also to arrest caries.[7] Compared to traditional restorative treatment, arresting caries with fluoride agents is non-invasive, simple to apply and low cost.[8,9]

A number of remineralization agents have been studied in clinical trials or *in vitro* trials to arrest caries lesions.[10] Fluoride varnishes are one of the most widely used topical fluoride treatments. Varnishes are supposed to act as slow-release fluoride reservoirs due to extended contact time between tooth surface and fluoride.[11] One of the most common used varnishes contains 5% sodium fluoride (NaF) and the fluoride concentration is 22.600 parts per million (ppm).[12] However, in recent years the anticaries effects of various formulations have mostly been investigated on initial (enamel) caries but not on dentin.[13]

Silver diamine fluoride (SDF) which has become increasingly popular, is a practical and affordable treatment option for arresting carious lesions.[14] SDF is a translucent solution and the most commonly used SDF concentration is 38% which contains 44.800 ppm fluoride and 255.000 ppm silver ions. Although SDF has been used in some countries in Asia and South America for many years, it has gained considerable interest in the United States after the approval by the Food and Drug Administration (FDA) in 2014.[15,16] However, FDA only approved its use for the management of dental hypersensitivity therefore more evidence is needed for the use of SDF in caries management.

Micro computed tomography (Micro-CT) is a high resolution 3D X-ray imaging tehnique and has been used to determine the mineral density in dental hard tissues, recently. Comparing to transverse microradiography (TMR), Micro-CT is a non-destructive method and does not require complicated and time-consuming specimen preparation procedures.[17,18] In the literature most of the *in vitro* studies evaluating the efficacy of SDF were performed with permanent teeth and in the majority of these studies, mineral density values were not given. Therefore the aim of this study was to evaluate the dentin remineralization of 38% SDF and 5% NaF by comparing mineral density values in primary teeth in *in vitro* conditions. The null hypothesis was that there is no difference between 38% SDF solution and 5% NaF varnish in primary teeth dentin remineralization.

MATERIALS AND METHODS

Preparing specimens with artificial dentine caries

The study protocols were approved by the Ethics Committee of the Faculty of Dentistry, Ankara University (36290600;01). The flow chart in Figure 1 summarises the protocol of this study.

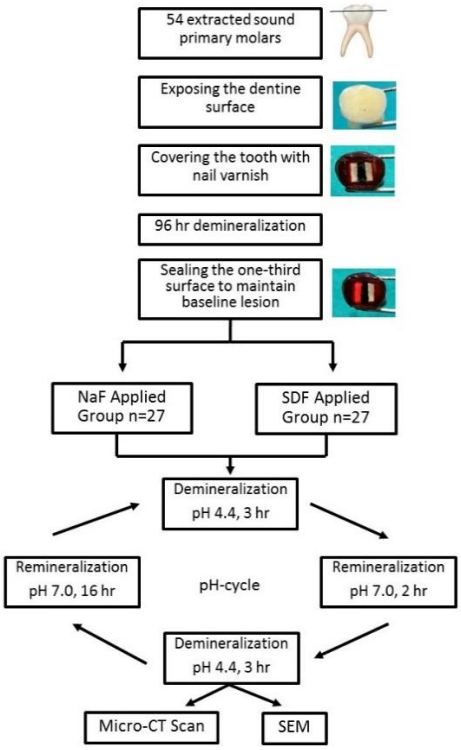

Figure 1. Flowchart of the experiment design.

Extracted sound human primary molars were collected with patient consent. The teeth were thoroughly cleaned and then inspected under a stereomicroscope for visibly observable cracks, hypoplasia and white spot lesions. Gingival tissues at the neck of the tooth were removed manually using hand instruments. Teeth were stored in 0.1% thymol solution at 4 ^{0}C until use and were used within two months of extraction.

Assuming a common standard deviation of 0,1 gHApcm^{-3} and with power at 0.80 and α=0.05, the sample size was at least 27 in each group. 54 extracted sound primary molars were abraded with diamond bur from occlusal surface and dentin was exposed. Then occlusal side was polished by sand papers (600, 800, 1200, 2000 grades). All surfaces except the area of 3x3 mm window on the occlusal surface were sealed with acid resistant nail polish (Revlon Corp., NY, USA). Middle 1x3 mm window of each specimens surface was covered with acid-resistant nail varnish to serve as sound control.

After the varnish had dried completely, each specimen was immersed in 20 mL of demineralizing solution in a small container for 96 hours to produce demineralized lesions approximately 100 microns deep. The solution was changed every 24 hours to ensure the tooth surfaces were in contact with fresh solution. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid and the pH was adjusted to 4.4 with 1 M KOH.[19] Following lesion development, the samples were rinsed thoroughly with deionized water, and the other 1x3 mm window were covered with an acid-resistant nail varnish to maintain the baseline lesion.

Remineralization regimen

The demineralized samples were randomly divided into two groups (n=27) based on treatments: 38% SDF solution (FAgamin, Tedequim S.R.L., Argentina) and 5% NaF varnish (Duraphat; Colgate-Palmolive Co., New York City, NY, USA). A microbrush was used to apply SDF solution and NaF varnish to the uncovered one-third of the dentin surface (demineralized dentin). In the SDF treated group, 2-3 drops of the solution were applied 2-3 minutes to dentin surface according to the manufacturer's instructions. After application, the samples were washed with a flow of deionized water for approximately 30 seconds. In the NaF treated group a thin layer of the varnish was applied using microbrush on demineralized dentin suface and then the samples were immersed in artificial saliva (.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2.2H_2O$, 3 mMNH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mMK_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4, and traces of ascorbic acid, pH 6.8) at 25°C for 6 hours.[20] Then varnishes were carefully removed using a surgical blade.

pH-cycling model

To assess the effect of the remineralization agents on dentine demineralization in the treatment groups, the protocol used by Ekambaram *et al.* was employed.[21] The specimens were placed in the pH-cycling system for a period of 7 days. Each cycle involved 3 h of demineralization (2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4 and 0.05 M acetic acid, pH: 4.5) twice daily, with 2 h of remineralization (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4 and 0.15 M KCl, pH: 7) between the periods of demineralization. Samples were then placed in the remineralizing solution over-night (16 hours). All solutions were freshly prepared for each cycle; separate containers were used for each specimen. The pH level of the demineralizing and remineralizing solutions was measured before every cycle.

Microcomputed Tomography (Micro-CT) Measurements

The specimens (n=27 for each group) were scanned by a Skyscan 1172 micro-CT (Bruker, Kontich, Belgium) for mineral density assessment. The X-ray source voltage and current were set at 80 kV and 100 μA, respectively. Specimens were rotated through 360º at 0.6º steps. 0.5 mm aluminium-copper filter used to cut off the softest X-rays. The image pixel size was 13.68 μm. During the scanning, a wet sponge was put into the tube to fix the position of the tooth and to keep the tooth moist.

Two standard mineral cylindrical phantoms (Bruker, Kontich, Belgium) with mineral density values (MDVs) of 0.25 gHApcm^{-3} and 0.75 gHApcm^{-3} were scanned for calibration of the greyscale of the specimens. Phantoms were scanned with the same settings used for the test specimens.

The image analysis software (CTAn, Skyscan NV, Kontich, Belgium) was used to calibrate the greyscale value and calculate it into the mineral density value (MDV, gHApcm−3) using the same software. The MDV of the ROI in each one-third surface of groups were measured. Manually determined ROIs were placed only in the dentin. The ROI was a 1 mm diameter circle in the middle part of the measured surface 50 μm below the exposed tooth surface. Measurements were taken on three locations in each one-third surface and the mean of these measurements were used as the MDV of the related surface.

Surface Morphology

Two specimens in each group were chosen randomly to assess surface morphology. After removing the nail varnish, specimens were dehydrated in a series of ethanol solutions and coated with gold and palladium. The surface morphology of specimens were then observed under scanning electron microscopy (SEM) (FEI Quanta 200, Scanning Electron Microscope, FEI Company, OR, USA).

Data analysis

All of the data were assessed using the 'Kolmogorov-Smirnov (K-S)' test for normality ($p>0.05$). Paired t-test was applied to compare the MDVs of experimental groups. All of the analyses were performed with the software SPSS for Windows version 22.0 (IBM Corporation, Armonk, NewYork, USA). The significance level was set at 5%.

RESULTS

The MDVs of groups are shown in Table 1.

Table 1: MDVs and Standard Deviations of CS, DMS and RMS of Groups

Groups	MDV of CS $g_{HAp}cm^{-3}$	MDV of DMS $g_{HAp}cm^{-3}$	MDV of RMS $g_{HAp}cm^{-3}$
NaF (n=27)	1.361 (0.062) [a1]	0.910 (0.166) [b1]	1.145 (0.120) [c1]
SDF (n=27)	1.376 (0.070) [a1]	0.961 (0.221) [b1]	1.623 (0.171) [c2]

*For each horizontal row: values with identical letters indicate no statistically significance ($p>0.05$)
**For each vertical column: values with identical numbers indicate no statistically significance ($p>0.05$)

In both NaF and SDF groups, the surface covered with nail varnish at the beginning of the experiment was used as a control surface (CS). The surface covered with nail polish after demineralization process was used as demineralization surface (DMS) and the surface that remineralization agents were applied was used as remineralization surface (RMS).

The MDV of CS in NaF and SDF was 1.361 $g_{HAp}cm^{-3}$ and 1.376 $g_{HAp}cm^{-3}$ respectively with no statistically significant difference ($p>0.05$). The MDVs of the DMS were 0.910 $g_{HAp}cm^{-3}$ in NaF group and 0.961 $g_{HAp}cm^{-3}$ in SDF group and there were no statistically significant difference between them ($p>0.05$). After remineralization agents were applied and specimens pH-cycled, the MDVs of the ROI in lesions increased in both NaF and SDF group. The mean MDV of SDF applied specimens were found to be higher than NaF applied specimens at statistically significant level ($p<0.05$).

In NaF group, the MDV of CS was higher than both DMS and RMS at statistically significant level ($p<0.05$). MDV of RMS was also found to be statistically significantly higher than demineralization group ($p<0.05$). Unlike the NaF group, in SDF group the MDV of RMS was found to be statistically significantly higher than the CS, ie the sound dentin surface ($p<0.05$).

SEM images of the groups are displayed in Figure 2a, 2b, 2c, 2d. The CS was covered with dense smear layer with no visible dentinal tubules.

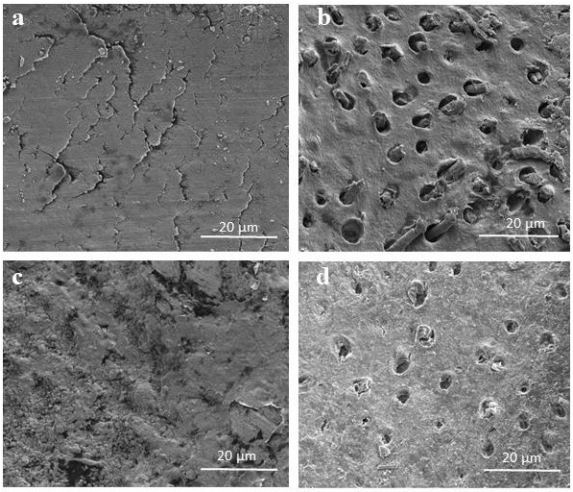

Figure 2. SEM images (x5000 magnification) of surface morphology of CS with smear layer (a), DMS with visible dentinal tubules (b), RMS with SDF (c) and RMS with NaF (d).

In the DMS the dentinal tubules were visibly exposed. The SDF applied surface remained relatively dense and intact compared with NaF applied surface.

DISCUSSION

This study was conducted to compare the effect of SDF and NaF on mineral content of demineralized primary molar dentine. It provides useful information about changes in the minerals of demineralized primary tooth dentine. According to the results of this study, the null hypothesis was rejected. SDF solution showed higher remineralization effect on primary molar dentin than NaF varnish.

In both SDF and NaF groups mean MDV of DMS was found to be lower than CS at statistically significant level which means the demineralization solution was effective to produce artificial dentin caries. However, the MDVs of DMSs in this study were 20–30% higher than natural dentine caries reported in other studies .[18,20] The reason for that may be the MDV of DMS in this study were taken from the superficial layers of the teeth which had a higher mineral density than deeper surfaces of the teeth. It should be noted that natural carious lesions greatly vary in depth depending on the activity of the lesion.[22,23] Additionally, the natural carious lesions occur in a long time period in contrast to artificial caries where the lesion was created in a short time period.

The mean MDV of CS 50 μm below the surface in the varnished area was around 1.36 $g_{HAp}cm^{-3}$ and 1.37 $g_{HAp}cm^{-3}$ in NaF and SDF groups, respectively. These values are similar to other studies with deciduous molars.[18,22,24] The mean MDV of RMSs in both groups were found to be higher than DMS at statistically significant level. So that both SDF solution and NaF varnish showed effective remineralization. Comparing SDF and NaF each other showed that SDF applied RMS presented statistically significantly superior remineralization than NaF group and even CS ie sound dentine. This finding corroborated with Mei *et al.*'s study which reported that density of SDF arrested dentinal lesion was higher than unaffected dentine.[25] It is noteworthy that the fluoride concentrations in 5% NaF and 38% SDF are 22.600 ppm and 44.800 ppm, respectively.[26] The high remineralization effect of SDF on dentine could be explained by its high fluoride concentration and the presence of silver.[27] However, an *in vitro* study reported that the use of 38% SDF demonstared a greater inhibitory effect on dentine demineralization and collagen degradation than 10% NaF and 42% $AgNO_3$ solutions which involves equal amount of fluoride and silver ions, respectively.[10] Similarly, another study compared 38% SDF, 38%SDF + 5% NaF, 5% NaF and water on dentin remineralization and reported that lesion depth in the SDF groups with or without NaF treatment did not show significant differences.[28] SDF and NaF both promote remineralization by formation of insoluble calcium fluoride which dissolves in a salivary environment to realese calcium and fluoride ions. Those released fluoride ions replace with hydroxyl ions of hydroxyapatite to form acid-resistant fluoroapatite. SDF is an alkaline solution and an alkaline environment provides ideal conditions for ion exchange so that formation of fluoroapatite can be promoted.[29,30] Therefore, it should be kept in the mind that the reason for superior remineralization effect of SDF is not only the high fluoride concentration but also the alkalinity of the solution. In this study, greyish black stains were observed on the tooth surfaces treated with SDF even after washing the specimens with deionozied water for 30 seconds according to manifacturer's advisement. In *in vitro* studies, it was determined that silver chloride was formed as a result of chemical reaction between SDF and hydroxyapatite. Also it was concluded that this insoluble silver chloride works as a protective layer on the dentine surface so that the loss of calcium and phosphate from dentine could be decreased.[10,28] In the current study, this silver chloride deposition on the surface was not included in MDV measurements to prevent the high radioopacity effect.

In the SEM images it was observed that the dentin tubules, which were exposed after demineralization, were partially occluded and the diameter of the exposed dentinal tubule orifices were decreased in the NaF treated group. On the other hand, in SDF treated group most dentinal

tubule orifices were occluded and dense granular structures were observed. Transmission electron microscopy can be performed to investigate the mineral content of the surfaces.

In the present study, single dentine surface was used for the control group, demineralization group and remineralization group. So that the effect of substrate variability on the hypotheses tested was reduced. Additionally, random allocation of the specimen between the experimental groups further avoided such an effect. To simulate dentine caries, specimens were initially demineralized before SDF and NaF application. After NaF varnish was applied specimens were immersed into artificial saliva for 6 hours. After this period, fluoride varnishes were removed with the help of a surgical blade without touching the dentine surface directly. Thus, this procedure simulated the clinical conditions in which the varnish would probably be removed after some hours via toothbrushing or mastication.[13,20] Since SDF is liquid material and was already washed with a flow of deionized water as recommended by the manufacturer, surgical blade did not used in SDF applied group.

After the demineralization process and application of remineralization agents, there are different studies in which samples are immersed in remineralization solution or subjected to pH-cycle. In daily life, due to the nutrition the pH level never remains constant in oral cavity, therefore it is important to reflect the pH changes to the experimental environment. So that the present study used a pH-cycling model involving combination of demineralization and remineralization on the dentine surface. Although this model is rapid and inexpensive, it was unable to entirely simulate the complex oral conditions such as bacterial biofilm, quality and composition of saliva, oral hygiene practises, eating habits and fluoride uptake.

Many different methods have been used to evaluate the remineralization effect of various agents in enamel and dentin. Chemical analysis, cross-section microhardness testing and transverse microradiography techniques have been widely used. However, these methods are destructive and require complicated and time-consuming specimen preparation procedures.[17,18] Lately, micro-CT has been used to determine the mineral density in dental hard tissue without damaging the tooth specimens. Therefore, to assess the mineral content of primary molar dentin, micro-CT was preferred in the present study.

CONCLUSIONS

Within the limitations of this *in vitro* study, it is concluded that the use 38% SDF demonstrated a great remineralization effect on primary molar dentin than 5% NaF varnish. Further clinical studies are needed to investigate the effect of 38% SDF on primary molars which remains in the mouth longer than incisors and have a great function on chewing.

ACKNOWLEDGEMENTS

This study was supported by Ankara University Scientific Research Projects Funds (No: 16B0234001).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

Gümüş Diamin Florid ve Sodyum Florürün Dentin Remineralizasyonundaki Etkinliğinin In-Vitro Koşullarda Değerlendirilmesi

ÖZ

Amaç: *Bu çalışmada, yapay süt dişi dentin çürük lezyonlarında %38'lik gümüş diamin florid (GDF) çözeltisinin ve %5'lik sodyum florür (NaF) verniğinin remineralize edici etkisi ve mineral yoğunluk değerlerinin karşılaştırılması amaçlanmıştır.* ***Gereç ve Yöntemler:*** *58 adet çekilmiş süt azı dişi okluzal yüzeyden aşındırılarak dentin yüzeyi açığa çıkarılmış ve aside dayanıklı tırnak cilasıyla 1×3 mm'lik 3 adet pencere oluşturulmuştur. Orta üçlüde olan pencere kontrol grubu olarak kullanılmak üzere hiçbir işlem yapılmadan tırnak cilası ile kapatılmış, diğerlerine ise demineralizasyon solüsyonu kullanılarak yapay dentin çürüğü oluşturulmuştur. Daha sonra bir taraftaki pencere, lezyonun ilk halini değerlendirebilmek amacıyla tırnak cilasıyla kapatılmış, diğer taraftaki ise remineralizasyon materyallerine tabi tutulmuştur. Remineralizasyon ajanı olarak sırasıyla; GDF ve NaF uygulanmıştır. Ardından örnekler 7 günlük pH döngüsüne sokulmuştur. Örnek yüzeylerindeki mineral değişimi ölçümleri Mikro-BT ile; yüzey morfolojisindeki değişimler ise SEM ile değerlendirilmiştir.* ***Bulgular:*** *Demineralizasyon*

yüzeyine göre mineral yoğunluk değerleri hem NaF hem de SDF grubunda artmıştır. SDF uygulanan örneklerin ortalama mineral yoğunluk değerleri, istatistiksel olarak anlamlı düzeyde NaF uygulanan örneklerden daha yüksek olduğu bulunmuştur. SEM görüntülerinde, dentin tübüllerinin GDF grubunda neredeyse tamamen tıkalı olduğu, NaF grubunda ise kısmen tıkalı olduğu gözlenmiştir. ***Sonuçlar:*** *GDF'nin organik içeriği yüksek süt dişi dentininde etkili bir remineralizasyon gösterdiği ve bu nedenle uzun yıllardır altın standart olarak kabul edilen floride alternatif olabileceği düşünülmektedir. Kesici dişlerden daha uzun süre ağızda kalan ve çiğneme üzerinde büyük bir işlevi olan süt azı dişleri üzerinde %38'lik GDF'nin etkisini araştırmak için daha fazla klinik çalışmaya ihtiyaç vardır.* ***Anahtar Kelimeler:*** *Topikal florür, mikrobilgisayarlı tomografi, diş remineralizasyonu.*

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.795870

Original research

ARE MAXILLARY AND SPHENOID SINUS VOLUMES PREDICTORS OF GENDER AND AGE? A CONE BEAM COMPUTED TOMOGRAPHY STUDY

*Alaettin Koç[1]

ABSTRACT

Objectives: This study aimed to analyze the applicability of maxillary sinus (MS) and sphenoid sinus (SS) volumes in determining gender and age.

Materials and Methods: This retrospective study included cone beam computed tomography images of 164 MSs and 164 SSs of 82 patients (40 females and 42 males). Right, left, and total MS and SS volumes were estimated. The independent samples t-test, logistic regression, and Pearson correlation coefficient were used to explore their accuracy in determining age and gender.

Results: Males had greater right SS, average, and total SS volumes than females (p=0.007, p=0.013, and p=0.013, respectively). The right SS volume had the best predictive power (65.9%) for gender. A regression formula using this parameter alone showed satisfactory accuracy. No significant correlations were found between age and MS or SS volumes (p>0.05).

Conclusions: The SS volume is a valuable tool in determining gender, whereas neither MS nor SS are reliable predictors of age. A simple gender determination is possible with our regression formula using the right SS volume. Further studies are needed to investigate the role of paranasal sinus volumes with a view to develop formulations in determining age and gender in different populations.

Keywords: Maxillary sinus, sphenoid sinus, cone beam computed tomography, forensic sciences, forensic dentistry.

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Received : 16.09.2020
Accepted : 15.11.2020

How to Cite: Koç A. Are Maxillary and Sphenoid Sinus Volumes Predictors of Gender and Age? A Cone Beam Computed Tomography Study. Cumhuriyet Dent J 2020;23:4: -.
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INTRODUCTION

Genderual dimorphism can exist in nearly all skeletal parts of adults. It may be determined by environmental factors such as genetic factors, nutritional status, and lifestyle, but ethnic background may also play a role. Thanks to morphological and metric properties of cranial structures, a person's gender can be determined with an accuracy rate of over 60%.[1]

Prior to the advent of computed tomography (CT), cadaveric studies and two-dimensional radiographs were used to measure paranasal sinus size. The use of CT has greatly contributed to the diagnosis of paranasal sinus diseases. For instance, studying the variations of certain anatomical structures, such as the sinus septa, pneumatization, and ostia, has now made it possible to perform detailed analyses of those structures.[2]

Age determination is often of critical importance.[3] For example, determination of legal or illegal immigrants may directly affect the legal procedures followed. In autopsies, the paranasal sinuses can be used to determine a person's gender. Even if body parts lose their anatomical properties in an accident or disaster, the paranasal sinuses may remain intact by virtue of their sheltered position within the cranium, where anthropometric measurements play an important role.[4]

The genderual uniqueness of anatomic structures can be clearly viewed on tomographic images. Several studies have reported the usefulness of the paranasal sinuses in gender determination, particularly in postmortem examinations.[3,5,6] Other studies, however, have argued that the paranasal sinuses do not possess a dimorphic character.[7-9] Thus, no clear consensus exists in this regard.

The sphenoid bone, which neighbors the optic nerve, cavernous sinus, and pituitary gland, is a unique and strategically located cranial bone. The sphenoid sinuses (SS) are located inside that bone. The development of the SSs begins in intrauterine life and is completed by the age of 14 years. Their size may vary according to age, gender, and ethnicity.[10] The development of the maxillary sinus (MS), on the other hand, begins in the prenatal period and continues until the age of 18 years.[8]

In this study, we investigated the applicability of SS and MS volumes in determining gender and age using cone beam CT (CBCT) images of an Eastern Turkish population.

MATERIALS AND METHODS

Study Sample

This retrospective study used CBCT images of SSs and MSs of female and male residents of Eastern Turkey. A power analysis was conducted using G*power version 3.1.9.2 (Franz Faul, University of Kiel, Germany). The analysis showed that a minimum sample size of 68 patients would be required to determine statistical significance with 95% confidence, 95% test power, and an effect size of $d = 0.88$.

According to the power analysis results, the study included 82 patients, 40 females and 42 males, and examined the CBCT images of 164 SSs and 164 MSs. The mean age of the patients was 37.3 years (range: 14–71 years). The patients had undergone tomography imaging for dental implant planning, impacted teeth, dental anomalies, odontogenic or non-odontogenic lesions, and root fractures. The inclusion criteria were a patient age of 14 years or older, adequate image quality, and absence of craniofacial abnormalities. Patients who had a history of trauma to or any lesions in the sinus region and patients whose CBCT images were of poor quality were excluded. This study was approved by the Ethics Committee of Van Yüzüncü Yıl University (approval no. YYU-2018/12/07-10).

CBCT Scanning

The CBCT images were acquired using a 3D eXam device (KaVo, Biberach, Germany). The scan parameters were as follows: 18.54 mAs, 120 kVp, 8.9 s scan time, 160 × 130 field of view (FOV), and 0.3 mm voxel size.

Sinus Volume Measurements

The CBCT images were converted to DICOM format and transferred to 3D-DOCTOR (Able Software Corp., Lexington, MA, USA) to estimate the right, left, and total SS and MS

volumes. The program's segmentation function allows the coloring of sinuses according to their density in axial sections. Manual correction was performed when the color-tagged areas did not accurately match the borders of the sinuses. After the sinus borders were drawn, the software combined the segmented areas in each section to obtain a three-dimensional (3D) model (Figure 1).

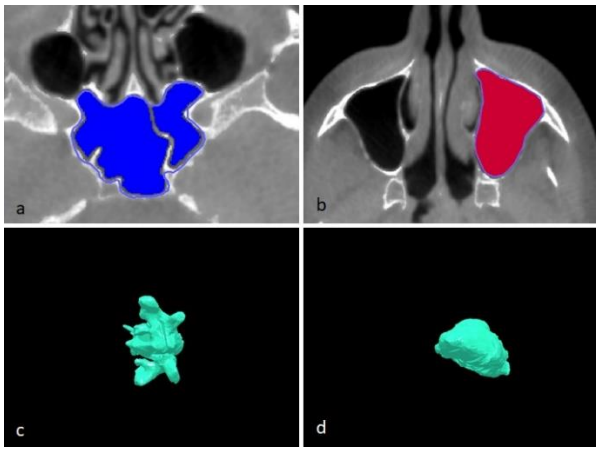

Fig. 1. Segmentation of maxillary and sphenoid sinuses on sectional cone-beam computed tomography images (a, b). Three-dimensional model of maxillary and sphenoid sinuses after volume estimation using semiautomatic segmentation (c, d).

The volumes were then estimated automatically. All measurements were performed by a single oral and maxillofacial radiologist with five years' experience in CBCT and volumetric measurements. Repeated measurements on the images of 20 patients were performed to estimate intra-reader reliability.

Statistical Analysis

Statistical analyses were performed using NCSS (Number Cruncher Statistical System) 2007 (NCSS, LLC., Kaysville, UT, USA). Descriptive statistics (means and standard deviations, medians, frequencies, and ranges) were used to analyze the study data. The normality of the distribution of quantitative variables was evaluated with the Shapiro-Wilk test and graphical analyses. The independent samples t-test was used to compare normally distributed variables between males and females. Logistic regression analysis was used to determine the patients' gender using the volume variables. Correlations between age and volumes were assessed using Pearson's correlation analysis. The level of statistical significance was set to $p < 0.05$. The intraclass correlation coefficient (ICC) was used to evaluate intra-examiner reliability.

RESULTS

Males had greater right, mean, and total SS volumes than females ($p = 0.007$, $p = 0.013$, and $p = 0.013$, respectively). No significant differences between males and females were found with respect to right, left, and mean MS and left SS volumes (Table 1).

Table 1. Gender-based comparison of paranasal sinus volumes (mm^3)

	Male		Female		p
	Mean±SD	Min-Max	Mean±SD	Min-Max	
Right MS	13248.08±5747.61	2341.78-24711.99	12063.93±4608.12	4270.37-22847.94	0.308
Left MS	14059.72±5494.37	3076.72-23975.23	12228.41±4633.38	3415.72-21165.49	0.108
MS average	13653.9±5387.16	2709.25-23227.47	12146.17±4500.29	3943.43-20310.36	0.174
Right SS	5160.94±2603.3	953.39-12319.08	3727.46±2037.65	483.91-8464.45	0.007*
Left SS	4555.36±2867.1	602.98-13109.32	4129.63±1977.14	537.81-9839.21	0.438
SS average	4858.15±1801.13	1367.98-9409.9	3928.55±1481.49	1218.89-7257.99	0.013*
Total SS	9859.26±3655.25	2776.22-19096.69	7972.7±3006.57	2473.64-14729.56	0.013*

SD standard deviation, Min minimum, Max maximum

A logistic regression model using the right MS volume accurately predicted 66.7% of males, 42.5% of females, and 54.9% of total patients. A model using the left MS volume accurately predicted 64.3% of males, 50% of females, and 57.3% of total patients. A model using the mean MS volume accurately predicted 64.3% of males, 45% of females, and 54.9% of total patients. A model using the right SS volume accurately predicted 64.3% of males, 67.5% of females, and 65.9% of total patients. A model using the left SS volume accurately predicted 54.8% of males, 37.5% of females, and 46.3% of total patients. A model using the mean SS volume accurately predicted 61.9% of males, 62.5% of females, and 62.2% of total patients. A model using the total SS volume accurately predicted 61.9% of

males, 62.5% of females, and 62.2% of total patients. A model using all sinus volumes accurately predicted 64.3% of males, 65% of females, and 64.6% of total patients (Table 2).

Table 2. Results of the logistic regression analysis of the studied volumes to predict gender

Correctly classified; n (%)

	Male	Female	Total
Right MS	28 (66.7)	17 (42.5)	45 (54.9)
Left MS	27 (64.3)	20 (50)	47 (57.3)
MS average	27 (64.3)	18 (45)	45 (54.9)
Right SS	27 (64.3)	27 (67.5)	54 (65.9)
Left SS	23 (54.8)	15 (37.5)	38 (46.3)
SS average	26 (61.9)	25 (62.5)	51 (62.2)
Total SS	26 (61.9)	25 (62.5)	51 (62.2)
All sinuses	27 (64.3)	26 (65)	53 (64.6)

n number

All variables were included as independent variables, and logistic regression analysis with the backward elimination method was performed. The resulting model identified only the right SS volume as an independent predictor. In a regression model using the right SS, no improvement in accuracy was observed when any other variable was included. In the regression formula (Eq. 1), male = 0 and female = 1. Values of 0.5 or greater were considered as indicating females, and those below 0.5 were considered as indicating males.

$$Gender = 1.142 - 2.71 \times 10^{-4} \times right\ SS\ volume\ (\mathrm{mm}^3)\ (1)$$

No significant correlation was found between age and any sinus volume (Table 3). Thus, no reliable age-based model could be designed. Excellent intra-examiner reliability (ICC=0.988) was estimated for repeated measurements.

Table 3. Coefficients of the correlations between age and paranasal sinus volumes

	Age	
	r	p
Right MS	-0.018	0.870
Left MS	-0.091	0.417
MS average	-0.057	0.615
Right SS	-0.115	0.305
Left SS	0.053	0.639
SS average	-0.043	0.702
Total SS	-0.043	0.702

DISCUSSION

In this study, we investigated the applicability of MS and SS volumes to determine age and gender in an Eastern Turkish population, aiming to develop predictive models. Of all the MS parameters, the left MS volume had the highest prediction accuracy. The SS parameters had a higher prediction accuracy; among them, the right SS volume showed the highest accuracy. The model using all sinus volumes was less accurate than the model using the right SS alone. These results suggest that the right SS volume alone can accurately predict gender, obviating the need for including all sinus volumes.

Technological advances have made CBCT imaging a more accessible technique. Besides its clinical applications, it has now become a tool of forensic medicine. For instance, if previously acquired CBCT images of a person are available, they can be used to identify that person in cases of mass deaths, such as those occurring during wars, natural disasters, or airplane crashes. Registries of postmortem personal identification show that the frontal bone and sinus remain unaffected in only one-tenth of the cases, whereas the cranial region involving the SS is nine times less affected.[5] This suggests that it is reasonable to use more sheltered anatomic structures, such as the SS, for postmortem personal identification instead of

anatomical structures like the frontal sinus and MS, which are close to the body surface and thus more susceptible to trauma. Furthermore, the greater degree of anatomical variation compared to the MS, such as asymmetric right and left SSs, and the absence of a septum dividing the two sides or accessory septa in some individuals, make the SS unique among anatomical structures. Both SS morphological examinations and dimensional measurements enable prompt personal identification.

Several studies have attempted to predict age and gender using MS and SS volumes of patients of various populations.[1-6,9] Previous studies have utilized two-dimensional imaging techniques to determine the applicability of paranasal sinuses to determine gender and age. Luo *et al*.[11] explored gender determination by estimating the area of the frontal sinus on lateral cephalograms of 475 patients and found that, when combined, the parameters of the frontal sinus index and area predicted gender in 76.6% of the cases. Sidhu *et al*.[12] measured the area of the MS and its perimeter using lateral cephalograms of 50 patients, reporting a predictive accuracy of 76%.

Other studies have attempted to predict age and gender based on linear measurements using 3D techniques. Rani *et al*.[3] reported that MS length, width, and height on MR images can be used to determine age and gender. Uthman *et al*.[4] analyzed the helical CT images of 88 patients to determine gender. They found that MS length was the best parameter and reported an accuracy rate of 73.9% when all MS parameters were combined. Bangi *et al*.[13] performed linear measurements on CT images of 100 patients' MSs to determine gender, reporting an average accuracy rate of 88%. Conversely, in our study, the MS volume was not a reliable predictor of gender. In line with our results, Barghouth *et al*.[7], Degirmenci *et al*.[8], Ariji *et al*.[14], and Koppe *et al*.[15] found that the MS volume did not show dimorphism. Kajoak *et al*.[16] performed linear measurements on CT images of SSs in a Sudanese population and reported that the studied parameters showed significant differences not only between genders but also from other populations. Other studies, in line with our findings, have reported significantly greater SS volumes in males.[2,17] Previous studies have reported conflicting results regarding the applicability of SS volumes to gender predictions. Some studies found that SS volumes did not show genderual dimorphism.[9,18,19]

The development of 3D modeling software for 3D imaging techniques has enabled gender predictions based not only on linear measurements but also on volume estimations of paranasal sinuses. Farias Gomes *et al*.[20] performed linear measurements and volumetric estimations of MSs in 60 patients. They found that MS length was the most dimorphic parameter (77.7% accuracy) and reported that their regression model accurately predicted the gender of 84% of the patients. Oliveira *et al*.[9], on the other hand, measured SS volumes on the CT images of 47 patients and found no significant differences according to gender. Similarly, measuring SS volumes on the CBCT images of 172 patients, Nejaim *et al*.[18] failed to detect a significant correlation between gender and SS volume.

Regarding age prediction based on paranasal sinus volumes, the results reported in the literature are also contradictory. Yonetsu *et al*.[19] found no correlation between age and SS size calculated on CT images. Similarly, Radulesco *et al*.[21] and Jun *et al*.[22] found no correlations between age and MS volumes on CT images. Likewise, Oliveira *et al*.[9] reported that SS volume did not significantly correlate with age. In line with these findings, we found no correlations between age and SS or MS volumes. Conversely, Cohen *et al*.[2] reported a significant age-dependent volume reduction in MS and SS, although they found no correlation between the frontal sinus volume and age.

As can be seen, previous studies have reported varying predictive accuracies of paranasal sinus measurements in determining gender and age. These inconsistencies are possibly due to differences in patient populations, sample sizes, radiographic techniques, and data analyses. It is also possible that the volume measurement method and the software used may affect the results. For

instance, Möhlhenrich *et al*.[23] suggested that paranasal sinus volume estimation using formulas obtained from linear measurements does not provide accurate results. Moreover, whereas some studies used stereological methods applied to tomographic sections to estimate the volumes of sinuses and various organs, others have used 3D models to estimate the volumes of certain anatomical structures.[2,24] It has been reported that semiautomatic segmentation, by which three-dimensional modeling can be performed, is superior to stereological methods for volume estimation on CBCT images and can provide up to three times more practical volumetric estimations.[25]

CONCLUSIONS

This study found no correlations between age and MS or SS volumes. Conversely, MS and SS volumes were more reliable predictors of gender. However, it is reasonable to use SS rather than MS parameters, as they show higher accuracy in gender predictions. The right SS volume can be used alone. Our regression model shows acceptable accuracy in predicting gender. Thus, CBCT with a relatively lower dose, a smaller FOV, and high precision is preferable for performing all measurements and evaluations.

ACKNOWLEDGEMENTS

None

CONFLICT OF INTEREST STATEMENT

The author declare no conflict of interest.

Maksiller ve Sfenoid Sinüs Hacimleri Cinsiyet ve Yaş Hesaplamada Belirleyici midir? Bir Konik Işınlı Bilgisayarlı Tomografi Çalışması

ÖZ

Amaç: *Bu çalışmanın amacı; maksiller sinüs (MS) ve sfenoid sinüs (SS) hacimlerinin cinsiyet ve yaş tahminindeki rolünü analiz etmektir.* ***Gereç ve Yöntemler:*** *Bu retrospektif çalışmada 82 hastaya (40 kadın, 42 erkek) ait 164 MS ve 164 SS'ün konik ışınlı bilgisayarlı tomografi (KIBT) görüntüleri kullanılmıştır. Sağ MS, sol MS, sağ SS, sol SS, total SS ve total MS hacimleri hesaplanmıştır. Hacimlerin cinsiyet ve yaş tayinindedi rolünü analiz etmek için bağımsız örneklem testi, lojistik regresyon analizi ve Pearson korelasyon analizi kullanılmıştır.* ***Bulgular:*** *Erkekler daha büyük sag SS, ortalama SS, ve total SS hacimlerine sahiptir (p=0,007, p=0,013, p=0,013, sırasıyla). Sağ SS hacmi, cinsiyeti belirlemede en yüksek tahmin gücüne (%65,9) sahiptir. Tek başına bu parametrenin kullanıldığı regresyon modelinde, tatmin edici doğruluk elde edilmiştir. Yaş ve herhangi bir hacim değeri arasında korelasyon bulunmamıştır (p>0,05).* ***Sonuçlar:*** *SS hacminin cinsiyet belirlemede değerli bir araç olduğu sonucuna varılmıştır, buna rağmen, ne MS ne de SS yaş tayininde belirleyici değildir. Daha basit bir cinsiyet tayini, bizim regresyon formülünde sağ SS boyutları kullanılarak yapılabilir. Farklı hasta populasyonlarındaki hastaların paranazal sinüs hacimlerini kullanarak, yaş ve cinsiyet tayinini formüllerle belirleyecek ileri çalışmalara ihtiyaç duyulmaktadır.* ***Anahtar Kelimeler:*** *maksiller sinüs, sfenoid sinüs, konik ışınlı bilgisayarlı tomografi, adli bilimler, adli diş hekimliği.*

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.779686

Original research

ASSOCIATION BETWEEN MANDIBULAR CANAL COURSE AND INTERFORAMINAL AREA IN PANORAMIC RADIOGRAPHS FOR IMPLANT PLACEMENT

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ABSTRACT

Objective: The aim of this study was to evaluate the relationship of mandibular canal course with mental foramen localization in panoramic radiographs on edentulous patients' for implant placement evaluation.

Material and Methods: This retrospective study was carried out on the panoramic radiographs of 788 edentulous patients. Horizontal measurements L (left mental foramen to the left mandibular ramus), R (right mental foramens to the right mandibular ramus), M (between the mental foramens) and vertical measurements D1 (mental foramen's inferior border to the mandibular basis), D2 (mental foramen's superior border to the alveolar crest) were digitally evaluated. Mandibular canals were classified into two types as linear and elliptic. Analysis of demographic data and correlations between canal course and linear measurements were carried out. Kolmogorov-Smirnov and Shapiro Wilks tests, Kruskal Wallis test, and Chi-squared test were used to compare the qualitative data ($p < 0.05$).

Results: There was a statistically significant difference between the D1/D2 mean values and the canal course according to the age groups. The rate of the elliptic canal course in the men (41.6%) was significantly higher than that in the women (26.6%). There was no statistically significant difference between the mean D1/D2, (R + L)/M and L/M values according to the canal course.

Conclusions: Both elliptical and linear canal courses do not affect the interforaminal distances, resulting ineffective in the anteroposterior spread of implants in cases of interforaminal implant placement.

Key Words: Mandibular canal, canal course, interforaminal area, canal anatomy, implant.

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Received : 12.08.2020
Accepted : 17.11.2020

How to Cite: Burdurlu MÇ, Dagasan V, Cabbar F. Association Between Mandibular Canal Course and Interforaminal Area in Panoramic Radiographs for Implant Placement. Cumhuriyet Dent J 2020;23:4: 356-363.
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INTRODUCTION

Edentulous mandibulas with advanced alveolar bone atrophy present cases of complex augmentation procedures especially in the posterior regions for implant-supported prosthetic rehabilitation such as grafting or nerve lateralization/transposition.[1] A solution for these cases is to have implant support between the mental foramens.[1,2] For the compromised biomechanical position, the anteroposterior spread of implants becomes a clinical success parameter.[2]

The mental foramen and mandibular canal are strategically important landmarks during implant surgery procedures. Therefore, prior to the any surgical procedures in the vicinity of the mandibular canal, it is important to conduct a thorough evaluation, involving a meticulous radiographic examination.[3] Although panoramic radiography is not completely adequate to identify the mental foramen compared to computerized tomography, it is the most frequently used diagnostic imaging in routine clinical practice.[4]

There has been no study evaluating the emergence location of the mental foramen in comparison to the mandibular canal course in the literature. The aim of this study was to evaluate the relationship of mandibular canal course with mental foramen localization in vertical and horizontal planes on edentulous patients' panoramic radiographs for implant placement.

MATERIALS AND METHODS

This study was approved by Yeditepe University Ethical Committee, Istanbul, Turkey in accordance with the principles of the Declaration of Helsinki (Research no. 1752-1110). The study was carried out on the panoramic radiographs of 788 edentulous patients who attended Yeditepe University Faculty of Dentistry in Istanbul, Turkey. The inclusion criteria were: patients above 30 years old of both sexes, presence of edentulous mandibula, high quality imaged radiographs in which reference points are clearly visible for measurement of distances. The exclusion criteria were: trauma, pathology, surgery or congenital deformity history which might affect radiograph interpretation, anterior loop detected mental foramens and indistinct mandibular canal borders.

Panoramic radiographs taken with an Orthopantomograph model X-550 (J. MoritaMfg Coro., Kyoto, Japan) were included. Horizontal and vertical measurements were made in each patient's digital panoramic radiograph with the Sectra PACS IDS7 (Sectra AB, Sweden) software.

Linear measurements

The following measurements were recorded in millimeters (mm) using specific reference points on the mandibula by the same operatorRe (Figure 1).

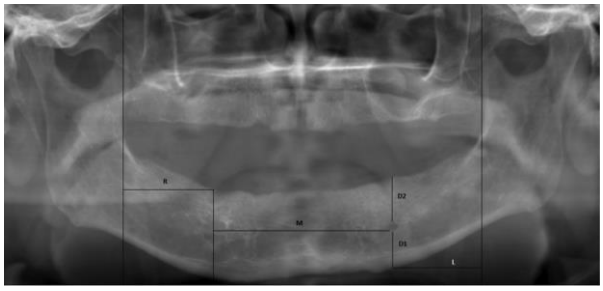

Figure 1. Vertical and horizontal distances measured on panoramic radiographs.

D1 – Vertical distance from the mental foramen's inferior border to the mandibular basis.

D2 – Vertical distance from the mental foramen's superior border to the alveolar crest.

L – Horizontal distance from the left mental foramen's posterior border to the vertical line that passes through the anterior border of the mandibular ramus in the left mandibula.

R – Horizontal distance from the right mental foramen's posterior border to the vertical line that passes through the anterior border of the mandibular ramus in right mandibula.

M – Horizontal distance between the anterior borders of the mental foramens.

Mandibular canal course

The mandibular canals seen on the panoramic radiographs were classified into two types: linear and elliptic.[5] Visual evaluation of the canal anterior to the vertical line that passes through the anterior border of the mandibular ramus was made by 2 clinicians (Fig. 2).

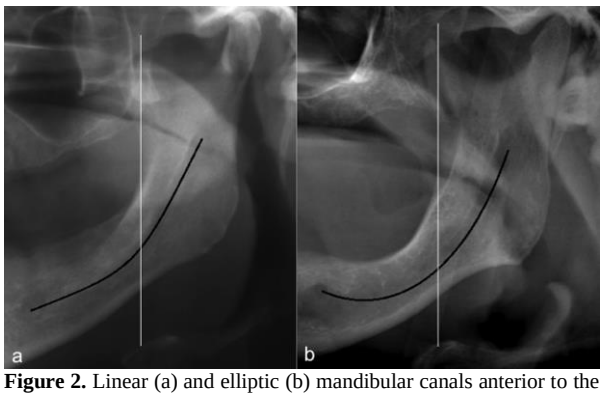

Figure 2. Linear (a) and elliptic (b) mandibular canals anterior to the vertical line that passes through the anterior border of the mandibular ramus.

Analysis of the demographic data and correlations between canal course, linear measurements, D1/D2, L/M, (R+L)/M were carried out.

Statistical analysis

The data were analyzed by descriptive statistical methods (mean ± standard deviation). Statistical analysis was carried out using IBM SPSS Statistics 22 (SPSS IBM, Turkey). The suitability of the parameters to normal distribution was evaluated by Kolmogorov-Smirnov and Shapiro Wilks tests, and it was determined that the parameters did not show a normal distribution. Kruskal Wallis test was used for comparison of the quantitative data, and Mann Whitney U test was used for determination of the group causing the difference. Mann Whitney U test was used to compare the parameters between two groups. Chi-squared test was used to compare the qualitative data. The significance threshold was set at $p<0.05$.

RESULTS

The study was conducted on a total of 788 patients, of which 399 (50.6%) were female, and 389 (49.4%) were male. The mean age of the patients was 61.82 ± 11.40 years (Table 1).

Table 1. Demographic data of the participants

		n	%
Age	30-40	18	2.3
	41-50	111	14.1
	51-60	247	31.3
	61-70	224	28.4
	71-80	141	17.9
	81 ≤	47	6
Sex	Female	399	50.6
	Male	389	49.4
Canal Course	Elliptic	268	34
	Linear	520	66

n: number

The distribution of the descriptive data for the measurements is shown in Table 2.

Table 2. Evaluation of the measurements

	Minimum	Maximum	Mean ± SD	Median
D1 (mm)	4.1	30.3	11.9±2.2	11.7
D2 (mm)	0.4	37	10.8±4.4	10.8
R (mm)	20.3	70.2	33.8±5.7	33.3
L (mm)	20.1	64.3	34.6±5.6	34.1
M (mm)	35.4	125.8	58.3±9.5	57.6
D1/D2	0.4	18.75	1.4±1.2	1.1
(R+L)/M	0.65	2.69	1.2±0.3	1.2
L/M	0.31	1.73	0.6±0.1	0.6

SD: standart deviation.
Distances in mm; L: left mental foramen to the left mandibular ramus, R: right mental foramen to the right mandibular ramus, M: between the mental foramens, D1: mental foramen's inferior border to the mandibular basis, D2: mental foramen's superior border to the alveolar crest.

There was a statistically significant difference in the canal course according to the age groups (P: 0.003; $P<0.05$) (Table 3). In the 71-80 years (22.7%) and 81 and older (%19.1) age groups, the

rate of elliptic course was significantly lower than those in the 30-40 years (50%), 41-50 years (39.6%), 51-60 years (37.2%) and 61-70 years (36.6%) groups (*P*1:0.02; *P*2:0.004; *P*3:0.003; *P*4:0.005; *P*<0.05), (*P*1:0.027; *P*2:0.013; *P*3:0.017; *P*4:0.026; *P*<0.05). There was no statistically significant difference between other age groups in terms of canal course (*P*>0.05). In addition, the rate of elliptic canal course in the men (41.6%) was significantly higher than that in the women (26.6%) (*P*<0.001) (Table 3).

Table 3. Canal course evaluation in relation to age and sex

		Canal Course		
		Elliptic	**Linear**	***P***
		n (%)	**n (%)**	
Age	30-40	9 (50%)	9 (50%)	0.003*
	41-50	44 (39.6%)	67 (60.4%)	
	51-60	92 (37.2%)	155 (62.8%)	
	61-70	82 (36.6%)	142 (63.4%)	
	71-80	32 (22.7%)	109 (77.3%)	
	81≤	9 (19.1%)	38 (80.9%)	
Sex	Female	106 (26.6%)	293 (73.4%)	0,000*
	Male	162 (41.6%)	227 (58.4%)	

Chi-squared test ** P<0.05*
n: number.

Statistically significant difference was found in the D1/D2 mean values according to the age groups (*P*<0.001) (Table 4). The D1 / D2 mean value of the 71-80 age group was significantly higher than the 30-40 years, 41-50 years and 51-60 years groups (*P*1:0.025; *P*2<0.001; *P*3:0.001; *P*<0.05). The D1/D2 mean value of the 61-70 age group was significantly higher than the 30-40 years, 41-50 years and 51-60 years groups (*P*1:0.044; *P*2:0.001; *P*3:0.043; *P*<0.05). The mean D1/D2 value of the 81 and older age group was significantly higher than the 30-40 years, 41-50 years and 51-60 years groups (*P*1: 0.034; *P*2: 0.001; *P*3: 0.048; *P*<0.05). There was no statistically significant difference between the other age groups in terms of D1/D2 (*P*>0.05). Furthermore, no statistically significant difference was found in the mean (R+L)/M and L/M values according to the age groups (*P*:0.551; *P*>0.05), (*P*:0.381; *P*>0.05) (Table 4).

Table 4. D1/D2, (R+L)/M and L/M evaluation in relation to age groups

	D1/D2	**(R+L)/M**	**L/M**
Age	**Mean ± SD (median)**	**Mean ± SD (median)**	**Mean ± SD (median)**
30-40	1.11±0.49 (0.96)	1.17±0.23 (1.12)	0.59±0.11 (0.56)
41-50	1.28±1.77 (0.97)	1.18±0.23 (1.17)	0.59±0.11 (0.58)
51-60	1.30±0.83 (1.03)	1.21±0.26 (1.18)	0.61±0.13 (0.59)
61-70	1.52±1.28 (1.09)	1.2±0.26 (1.16)	0.61±0.14 (0.59)
71-80	1.65±1.26 (1.28)	1.19±0.24 (1.16)	0.60±0.13 (0.58)
81≤	1.35±0.59 (1.15)	1.25±0.23 (1.22)	0.63±0.12 (0.61)
p	0.000*	0.551	0.381

Kruskal Wallis Test **P<0.05*
SD: standart deviation
Distances in mm; L: left mental foramen to the left mandibular ramus, R: right mental foramen to the right mandibular ramus, M: between the mental foramens, D1: mental foramen's inferior border to the mandibular basis, D2: mental foramen's superior border to the alveolar crest.

The mean D1/D2 value of the women was significantly higher than that of the men (*P*: 0.022; *P*<0.05) (Table 5). There was no statistically significant difference in the mean (R+L)/M and L/M values according to sex (*P*>0.05). There was no statistically significant difference in the mean D1/D2, (R + L)/M and L/M values according to the canal course (*P*> 0.05) (Table 5).

Table 5. D1/D2, (R+L)/M and L/M evaluation in relation to sex and canal course

	D1/D2 Mean ± SD (median)	(R+L)/M Mean ± SD (median)	L/M Mean ± SD (median)
Sex			
Female	1.54±1.47 (1.11)	1.21±0.24 (1.18)	0.61±0.12 (0.6)
Male	1.29±0.84 (1.04)	1.19±0.26 (1.17)	0.60±0.14 (0.58)
P	0.022*	0.349	0.295
Canal Course			
Elliptic	1.25±0.69 (1.04)	1.21±0.26 (1.19)	0.61±0.14 (0.60)
Linear	1.51±1.39 (1.08)	1.20±0.24 (1.17)	0.60±0.12 (0.59)
P	0.103	0.482	.337
Mann Whitney U Test	**P<0.05*		

SD: standart deviation

Distances in mm; L: left mental foramen to the left mandibular ramus, R: right mental foramen to the right mandibular ramus, M: between the mental foramens, D1: mental foramen's inferior border to the mandibular basis, D2: mental foramen's superior border to the alveolar crest.

DISCUSSION

In this study, the mandibular canal course and the vertical-horizontal localization of the mental foramens were analyzed in edentulous patients for evaluation of interforaminal implant placement. The mandibular canal contains the inferior alveolar neurovascular bundle, and therefore, it is a crucial anatomical landmark in lower jaw surgery.[6,7] Its morphology and position are important for preservation of the neurovascular structures within it.[8,9] Aging and loss of teeth in combination results in alveolar bone atrophy which usually limits the amount of bone required for implant placement in the posterior regions of edentulous mandibulas.[5] In such cases, implant-supported prosthetic rehabilitation without bone augmentation is obtained from the interforaminal area.[2] Therefore, increased horizontal distance between the mental foramens provide the anteroposterior spread of implants which becomes a clinical success parameter.[2]

Wical *et al.*[10] mentioned in their study that alveolar bone atrophy observed superior to the mental foramen does not change the distance from the foramen to the mandibular basis, and throughout life, it remains relatively constant. Lindh *et al.*[11] also stated that the stability of the inferior region of the mental foramen does not depend on the superior region in which alveolar bone atrophy is observed. Therefore, due to the stable positions of the mandibular basis and the mental foramen, the mental foramen was used as a reference point for the linear measurements in the panoramic radiographs in this study.

Craniofacial dimensions differ between individuals, especially in men being 5% to 9% larger in comparison to women.[12] On this basis, rather than evaluating D1, D2, L, R, M measurements alone, their ratios were evaluated. In all age groups, D1/D2 was higher than 1, meaning the height of the residual alveolar bone superior to the mental foramen was lower in comparison to the inferior region. This corroborated earlier studies that evaluated edentulous patients, whereas it was in contrast to dentulous patients in which the mental foramen is located closer to the mandibular basis rather than the alveolar ridge.[12,15] There was a statistically significant difference in the D1/D2 value according to the age groups. The mean D1/D2 value in the 61-70 and 71-80 age groups was significantly higher than the 30-40, 41-50 and 51-60 age groups. Differences in these findings were mainly attributed to the increased alveolar bone resorption with age and period of edentulism.

The mean D1/D2 was significantly higher in the women than in the men. Sex hormones were thought to be the reason. Following menopause, reduction in estrogen levels results in increased rate of bone resorption, which cannot be compensated with bone deposition.[15] As a result, alveolar process atrophy is observed more in women, making the mental foramen closer to the alveolar crest. There was no statistically significant difference in the L/M and (R+L)/M ratios between the age groups and sexes, confirming the stability of this relationship throughout adult life regardless of sex.

There are several studies that have classified the mandibular course as straight, symmetrical elliptic, asymmetrical elliptic, catenary and spoon shaped.[5,16-20] In this study, it was reduced to linear and elliptic to evaluate the relationship between the canal course and the vertical location of mental foramen emergence. Difference was not statistically significant in the mean D1/D2, (R + L)/M and L/M values according to the canal course. This study's results showed that canal course does not cause any significant difference in the emergence of the mental foramen either vertically or horizontally. Vertical location is clinically important in dental implant surgery for the presence of enough alveolar process height above the mental foramen, whereas the horizontal position is important for the interforaminal distance, which may be used for implant placement in the presence of posterior alveolar atrophy for an implant-supported prosthetic rehabilitation without additional bone augmentation surgeries. Although there was no difference in the mental foramens' vertical position, an elliptical course provides more space for implant placement in the posterior regions than linear ones.

The rate of linear canal course in the women was significantly higher than that in the men. This result may have been based on the increased dimensions of the cranio-facial structures in men. On the other hand, in the 71-80 and 81 and older age groups, the rate of elliptic course was significantly lower than the others. These results were in contrast with previous studies that analyzed the mandibular canal course in which an elliptic course was the most commonly observed.[16-20] The reason for the difference was thought to be from evaluation of the canal course anterior to the vertical line that passes through the anterior mandibular ramus. The gradual decrease in the elliptical course of the canal as the age progresses may be analyzed with more detailed reference points to conclude on the relationship between age and mandibular canal course.

One of the limitations of the present study is that it was conducted via panoramic radiographs that allow only the mesial and distal sides of the image to be evaluated. Although panoramic radiographs represent two-dimensional view, the majority of the clinicians worldwide are still using these images for implant placement. Moreover, this study was based on the mandibular canal and mental foramen, which are the most clearly observed mandibular anatomical formations from panoramic radiography, not on implant planning. Further studies with higher accuracy can be designed with computerized tomography images.

CONCLUSIONS

In conclusion, the results of this study showed that there was no statistically significant relationship between mandibular canal course and the vertical-horizontal localization of the mental foramen. Both elliptical and linear canal courses did not affect interforaminal distances, resulting ineffective in the anteroposterior spread of implants in cases of interforaminal implant placement.

ACKNOWLEDGEMENTS

The authors would like to thank to Yeditepe University Department of Oral and Maxillofacial Radiology.

AUTHORS' CONTRIBUTION

M.C.B. participated in conceptualizing, study design, data collecting and supervision of manuscript. V.D. took part in data collecting and writing. F.C. did correction and revision of manuscript.

ETHICS APPROVAL AND CONSENT TO PARCITIPATE

This study was approved by Yeditepe University Ethical Committee (Research no. 1752-1110) and the informed consent was waived.

CONFLICT OF INTEREST/SOURCE OF FUNDING

The authors claim to have no financial interests, either directly or indirectly, in the products or information listed in this article and also declare that there are no conflicts of interest related to this study.

İmplant Yerleşimi için Mandibular Kanal Seyri ve Interforaminal Bölge Arasındaki Ilişkinin Panoramik Radyografilerde Incelenmesi

ÖZ

Amaç: *Bu çalışmanın amacı dişsiz hastalarda implant uygulaması için mandibular kanal seyri ve mental foramen konumunun ilişkisinin panoramik radyografi üzerinden değerlendirilmesidir.* ***Gereç ve Yöntemler:*** *Bu retrospektif çalışma 788 dişsiz hastanın panoramik radyografileri ile yürütülmüştür. Ölçümler horizontal ve vertikal olarak dijital ortamda yapılmıştır. L (sol mental foramen ile sol ramus arası mesafe), R (sağ mental foramen ile sağ ramus arası mesafe), M (mental foramenler arası mesafe) horizontal ölçümler; D1 (mental foramenin alt sınırı ile basis mandibular arası mesafe) ve D2 (mental foramenin üst sınırı ile alveol kret tepesi arası mesafe) ise vertikal ölçümlerdir. Mandibular kanal seyri doğrusal ve eliptik olarak iki sınıfta incelenmiştir. Demografik veriler, kanal seyri ve dijital ölçümler arası ilişki değerlendirilmiştir. Kolmogorov-Smirnov ve Shapiro Wilks testleri, Kruskal Wallis testi, ve Ki-Kare testi niteliksel verilerin karşılaştırılmasında kullanılmıştır (p <0,05).* ***Bulgular:*** *Yaş grupları arasında D1/D2 oranları ve kanal seyri açısından istatistiksel olarak anlamlı farklılık tespit edilmiştir. Kanal seyrinin eliptik görülme oranı erkeklerde (%41,6) kadınlardan (%26,6) anlamlı derecede yüksek bulunmuştur. Kanal seyrine gore D1/D2, (R+L)/M ve L/M ortalamaları açısından istatistiksel olarak anlamlı farklılık yoktur.* ***Sonuç:*** *Eliptik ve doğrusal kanal seyrinin interforaminal mesafeye herhangi bir etkisi yoktur, bu durum antero-posterior yönde interforaminal bölgeye implant yerleştirilmesini etkilememektedir.* ***Anahtar Kelimeler:*** *Mandibular kanal, kanal seyri, interforaminal bölge, kanal anatomisi, implant.*

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.798591

Original research

THE EFFECT OF AUTOCLAVE POLYMERIZATION ON THE TENSILE STRENGTH OF VARIOUS DENTURE BASE MATERIALS

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ABSTRACT

Objectives: The aim of this *in vitro* study was to evaluate the effect of autoclave polymerization method on the tensile strength of the polymethylmethacrylate (PMMA) acrylic denture base resins.

Materials and Methods: Total of 60 specimens were fabricated from four different denture base materials such as three heat polymerized denture resins Meliodent, Paladent, and QC-20 and one microwave polymerized resin Acron MC. Specimens were divided into control (heat and microwave polymerization), short (130 °C 10 min) and long (130 °C 20 min) autoclave polymerization groups according to polymerization techniques (n=5). The tensile strength tests were performed. Data were analyzed by ANOVA followed by the Duncan test (α=0.01).

Results: In control group, Acron-MC showed the highest tensile strength value, QC-20, Paladent and Meliodent were followed. Autoclave polymerization 20 minutes Acron MC group was significantly different from the other acrylic resins for the control and autoclave polymerization 10 minutes groups ($p<0.01$). Autoclave polymerized acrylic resin specimens showed higher tensile strength values than the control groups ($p<0.01$). There was no significant difference between the increasing time of autoclave polymerization methods 10 minutes and 20 minutes ($p>0.01$).

Conclusion: Within the limitations of the study, autoclave polymerization method may provide a stronger alternative to conventional polymerization methods. Autoclave polymerization method provides high temperature that the activation ratio of cross-linking agents (glycoldimethacrylate) can be increased.

Key words: denture base materials, acrylic resins, tensile strength, autoclave, polymerization.

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Received : 22.09.2020
Accepted : 17.11.2020

How to Cite: Deste Gökah G, Durkan R, Oyar P. The Effect of Autoclave Polymerization on the Tensile Strength of Various Denture Base Materials. Cumhuriyet Dent J 2020;23:4: 364-370.
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INTRODUCTION

Poly (methyl methacrylate) (PMMA) polymers are the material of choice in dentistry to fabricate complete and partial removable dental prostheses, for use in provisional restorations and orthodontic applications and denture repair due to their low cost, relatively simple processing techniques with thermal energy, durability in oral fluids, the ease of manipulation and repair.[1]

However, PMMA has some disadvantages. During a three-year period, 68% failure was observed in removable prostheses, and 29% of them had fractures.[2] The most common problem for both patients and dentists is the fracture of denture bases.[1-3] The reason for denture fracture may be the denture function, fabrication process, handling, stress intensification, porosity, residual monomer, the presence of cracks, and poor adaptation.[4]

Fractures in dentures can be caused by impact and bending fatigue. Different methods have been tested to enhance the physical and mechanical features of PMMA, and alternative materials have been introduced. The addition of fibers (glass, polyamide, polyethylene, polypropylene), metal oxides (aluminum oxide, zirconia, titanium), noble metals (silver, nano gold, platinum, palladium), minerals (hydroxyapatite filler, silicon dioxide, silica-based filler, carbon-based filler, nanocarbon, nanodiamond) and hybrid boosters have been used to enhance the mechanical features of denture base resins.[5]

More attention has recently been paid to studies on microwave polymerization. Nevertheless, a lot of problems that should be explained for an effective application continue to exist.[6-8]

In case of their use as a denture base material, powder-like polymerized PMMA beads are mixed with methyl methacrylate liquid monomers that contain a small percentage of a dimethacrylate crosslinker. The polymerization of PMMA-powder and MMA-monomer liquid causes forming a semi-interpenetrating polymer (SIPN) network structure. The semi-IPN structure comprises one or more thermoplastic polymers that are mixed with a cross-linked polymer.[9]

A cross-linking agent represents a substance that promotes or irregulates intermolecular covalent bonding between polymer chains. The incorporation of cross-linking agents into the monomer leads to the formation of an insoluble cross-linked network in the course of polymerization and increases the glass transition of a polymer by introducing restrictions on the molecular motions of the chain. Therefore, many physical properties of the polymer may be improved. Furthermore, cross-linking agents, such as glycol methacrylate, provide increased resistance to crack formation and progression, impact and fatigue and create a stronger structure.[10]

An autoclave represents a pressurized device that is designed for heating aqueous solutions above their boiling point for the purpose of achieving sterilization and is commonly utilized in dentistry, microbiology, medicine, and metallurgy. Although the conventional method of heat polymerization is the most widely used method, different polymerization methods, such as injection molding, autopolymerization, light polymerization, microwave polymerization, and autoclave polymerization, have been used for the polymerization of denture bases. The autoclave polymerization method provides a high temperature at which the activation ratio of the cross-linking agent (glycol dimethacrylate) can be increased.[11,12]

Tensile strength, compressive strength, shear strength, impact strength, fatigue strength, proportional limit, flexural strength and modulus, and surface hardness methods can be used to investigate the mechanical properties of PMMA. Among the many desired mechanical properties of a denture base resin, high tensile strength is of particular practical importance to the final product. Tensile strength indicates the maximum tensile stress that can be applied in a uniform way over the cross-section of a test piece during stretching the test piece to failure.[12]

Various studies have reported the desirable features of polymer denture base materials, including biocompatibility, satisfactory durability in oral fluids, and the ease of handling.[7,13-15] Nevertheless, previous studies have not been

consistent concerning the mechanical features and behavior of the mentioned resins.[16,17] This *in vitro* study aimed to assess the impact of the autoclave polymerization method on the tensile strength of acrylic denture base resins. The null hypothesis stated that there would be a statistical difference when the tensile strength of the four denture base materials investigated was compared.

MATERIAL AND METHODS

Preparation of specimens

Four types of polymethyl methacrylate denture base materials were utilized in the research. The materials utilized in the present research are shown in Table 1.

Table 1. Denture base materials used in the present study

Materials	Polymerization Types	Chemical Compositions	
		Powder	Liquid
Meliodent	heat	Polymethylmethacrylate, ethyl hexyl acrylate, N-octyl methacrylate	methyl methacrylate, glycol dimethacrylate, dimethyl p-touludine
QC-20	heat	Copolymer (methyl-n-buthyl) methacrylate	methyl methacrylate, glycol dimethacrylate, N, N dimethyl p-touludine
Paladent	heat	Polymethylmethacryate, ethylmethacrytlate, N-octyl methacrylate	methyl methacrylate, glycol dimethacrylate, dimethyltouludine
Acron-MC	microwave	Polymethylmethacrylate, ethylmethacrytlate co-polymer	methyl methacrylate, glycol dimethacrylate, N-dimethyl p-touludine

Ethical approval was acquired from the Clinical Research Ethics Committee of Afyonkarahisar Health Science University (decision date: 3.04.2020, ID number: 2020/218). The samples that were prepared for tensile measurements were standard dumbbell-shaped test specimens in accordance with ASTM D 638M. A micrometer (Mitutoyo, Japan) was utilized for measuring the dimensions of the test specimens. A total of 60 specimens were fabricated from four different denture base materials: three heat-polymerized resins, Meliodent (Dental Bayer Ltd, Berkshire, UK), Paladent (Heraeus Kulzer GmbH & Co. KG, Wehrheim, Germany), and QC-20 (De Trey, Dentsply, Germany), and one microwave-polymerized resin Acron MC (GC Corp, Tokyo, Japan). The mixing of the powder (PMMA) and liquid (MMA) elements of the denture base materials was performed in a glass cup following the manufacturer's instructions for all groups. The mixture was allowed to stand until the formation of a dough stage, which was then packed into the flask and kept under pressure for a period of 10 min. The heat-polymerized resin specimens were manufactured by packing the acrylic resin into the stone molds present in denture flasks and curing for 30 min at 80 ºC in boiling water following the manufacturer's recommendations. For microwave-polymerized specimens, the dough formed from the powder/liquid mix was packed into the mold within a flask under pressure and subjected to microwave polymerization at 550 W for 3 min using a microwave oven (Vestel, Manisa, Turkey).

In accordance with polymerization techniques, specimens were divided into the control, short and long autoclave polymerization groups (n=5). Heat-polymerized acrylic resin specimens were processed by curing in a conventional water bath, while microwave-polymerized acrylic resins were processed in a microwave following the manufacturer's instructions for the control group. In the second group of acrylic resin specimens, a dough that was prepared from the powder/liquid mix was packed into the metal mold within the flask, and they were polymerized in an autoclave sterilization unit (OT 4060 Steam Sterilizer, Germany) at 60 °C for 30 minutes and at 130 °C for 10 minutes. In the third group, the resin specimens were polymerized

in the autoclave sterilization unit at 60 °C for 30 minutes and then at 130 °C for 20 minutes. There was no porosity problem. Then the flasks were cooled in the air for 30 min and then in the running water for 15 min. These temperature and time settings were identified according to a preliminary study.[18]

After polymerization, irregularities were removed from only one surface of each specimen by simulating oral conditions and utilizing a series of silicon carbide paper abrasive discs starting from 120 grit and ending with 320 grit for the purpose of acquiring a polished surface. The test specimens were stored in a water bath at a temperature of 37±2 °C for a period of 48 hours and kept in the open air for 1 hour before testing.

Tensile strength testing

The tensile test was conducted by utilizing the Llyod Universal Testing Machine (Llyod LRX, Llyod Instruments Ltd., Farehan, Hampshire, UK) at a crosshead speed of 1 mm/min. Tensile strength was calculated according to the following equation:

$Q=F/A$

Q: stress (N/mm^2)

F: maximum recorded force at failure (N)

A: the area of the specimen, expressed in mm^2

The data were obtained from the tests and analyzed by the analysis of variance (ANOVA). As a post hoc test, Duncan's HSD test was performed for the purpose of comparing the groups of means that could be attributed to the presence of interactions between the variables tested. All statistical testing was carried out at a 95% level of confidence.

RESULTS

Table 2 shows the mean and standard deviations of the tensile strength values of the four denture base materials tested.

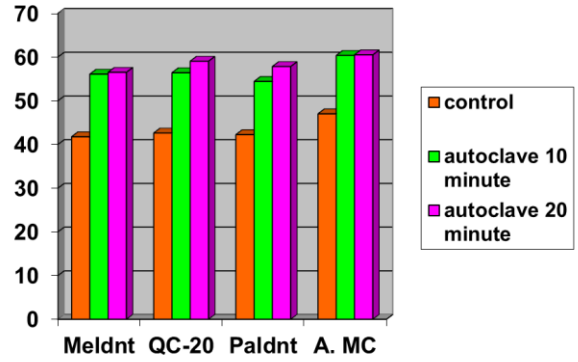
Table 2. Mean values and standart deviations of acrylic resins test specimens

Materials	Control groups	Autoclave polymerization 10 min	Autoclave polymerization 20 min
Meliodent	41.75 (1.17) **A**[a]	56.08 (2.99) **A**[b]	56.50 (2.96) **A**[b]
QC-20	42.61 (0.85) **A**[a]	56.40 (1.27) **A**[b]	59.04 (2.33) **A**[b]
Paladent	42.24 (2.04) **A**[a]	54.41 (0.73) **A**[b]	57.82 (1.43) **A**[b]
Acron- MC	47.00 (0.40) **B**[a]	60.36 (1.18) **B**[b]	60.50 (1.01) **A**[b]

Vertically, identical capital letters denote no significant differences among materials (p>0.01)
Horizontally, identical small letters denote no significant differences among materials (p>0.01)

Acron MC (47.0 MPa) exhibited the highest tensile strength value, followed by QC-20 (42.61 MPa), Paladent (42.24 MPa), and Meliodent (41.75 MPa) for the control group. Acron MC differed statistically significantly from the other acrylic resins for the control and 10-minute autoclave polymerization groups in terms of autoclave polymerization for 20 minutes (p<0.01).

Autoclave-polymerized acrylic resin specimens exhibited higher tensile strength values than the control group (p<0.01). No significant difference was found between the autoclave polymerization methods for 10 minutes and 20 minutes (p>0.01). No significant difference was determined between the tensile strength values of Meliodent, QC-20, and Paladent for three different polymerization methods (p>0.01). The increase in the tensile strength values of the autoclave polymerization method can be observed in the graph presented below (Graph 1).



Graphic 1. Tensile strength of different polymerization method and acrylic denture base resins

DISCUSSION

The complete polymerization of the polymer matrix is an important factor in the success of dentures. In the present study, the tensile strength of four different types of acrylic denture base resins was investigated using the autoclave polymerization method. The null hypothesis stated that there was a statistically significant difference among the groups.

The current *in vitro* study was designed for the purpose of comparing the tensile strength of four denture base resins obtained using a new curing method (autoclave polymerization method). Anusavice and Phillips[19] reported that dry heat-cured denture base PMMA exhibited a tensile strength of 52 MPa. The mentioned finding was not consistent with the current study, which revealed that Meliodent (56.08 MPa) and QC-20 (56.40 MPa) PMMA resin exhibited a higher tensile strength than Paladent 20 (54.41 MPa) under the 10 min autoclave polymerization condition.

In a study, Vertex acrylic resin specimens were divided into the heat, short and long autoclave polymerization groups in accordance with polymerization techniques. The groups were separated into three subgroups by weight based on the glass fiber (GF) concentration (0%, 2.5%, and 5% by weight). Autoclave polymerization caused a significant increase in the hardness of acrylic resin without the GF supplement, and it was reported that autoclave polymerization might be an alternative to the conventional heat polymerization method.[20] The results may differ from the present study due to the use of a reinforcement agent, different brands of PMMA, or different polymerization times.

Ayaz *et al*.[21] reported that autoclave polymerization increased the hardness of three acrylic resin groups (Meliodent, Paladent, and QC-20), but no significant differences were revealed between short (10 min) and long (20 min) autoclave polymerization. In the present study, while increasing the time of autoclave polymerization from 10 to 20 minutes caused no significant difference in tensile strength, it showed higher tensile strength values in autoclave polymerization compared to the heat-polymerized control groups. Similar to the findings of the current research, Gad *et al*.[22] assessed the impacts of autoclave polymerization on the elastic modulus and flexural strength of PMMA acrylic resins and reported no significant differences between the short and long-cycle autoclave-polymerized specimens. However, increased elastic modulus and flexural strength values were found compared to water bath-polymerized specimens.

Lai *et al.*[17] reported that microwave energy could efficiently polymerize denture base polymers. High statistical differences in mechanical properties were found in comparison with the water-bath method. The present research demonstrated that Acron MC resin exhibited the highest tensile strength in comparison with conventional heat curing denture base materials (Meliodent, Paladent, and QC-20). Moreover, autoclave polymerization was found to be stronger than microwave or heat curing methods. The underlying reason for this increase was composing a strong mesh structure with the autoclave polymerization method. Thus, it might be harder to break the connections of molecules between these materials. Therefore, owing to an ethylene glycoldimethacrylate structure in the composition of the microwave or heat-polymerized denture base material, they were readily curable by crosslinking at a high temperature and pressure, compared to the compression molding technique. However, the crosslinking of multifunctional monomers shows an abnormal kinetic behavior in the course of polymerization.[23]

Tensile strength indicates the maximum tensile stress that can be applied in a uniform way over the cross-section of a test piece during stretching the test piece to failure. Despite the fact that *in vitro* experiments may not always reflect intraoral conditions and predict clinical performance, they are valuable and applicable to clinical conditions.[24]

Factors such as the chemical composition of acrylic resin, polymerization time and type, the power of the microwave used in the studies should also be taken into consideration since they are

directly responsible for interchain force and polymer chain arrangements.[25]

Among the limitations of the present study, there are the usage of tensile strength testing in the air and testing only the denture base components of the Meliodent, Paladent, QC-20, and Acron-MC systems. Further studies are required to examine different resins, various polymerization durations, and temperature. Moreover, it is necessary to reveal the impacts of water absorption, staining, wear resistance, and other physical characteristics of the autoclave polymerization method and to find the possible alternative to the other methods used in prosthetic treatment.

CONCLUSIONS

The present study assessed the tensile strength of four different denture base materials: Meliodent, QC-20, Paladent, and Acron MC. When compared to the tensile strength of the four denture base resins, conventional heat curing, and microwave curing method, a statistically significant difference was found between Acron MC and the other acrylic denture base resins. Within the limitations of the current research, the microwave polymerization method showed significantly higher tensile strength when compared to the conventional water bath technique. The autoclave polymerization (130 ^{0}C, 20 min) method showed the highest tensile strength value, which was followed by the autoclave polymerization (130 ^{0}C, 10 min) and microwave polymerization method. The autoclave polymerization method may be a useful alternative to the conventional heat curing method.

ACKNOWLEDGEMENTS

There is no acknowledgement for this study.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

Otoklav Polimerizasyon Yönteminin Farklı Protez Kaide Materyallerinin Çekme Dayanımı Üzerine Etkisi

ÖZ

Amaç: *Bu in vitro çalışmanın amacı, otoklav polimerizasyon yönteminin polimetilmetakrilat (PMMA) protez kaide rezinlerinin çekme dayanımı üzerindeki etkisini değerlendirmektir.* ***Gereç ve Yöntemler:*** *Üç ısı ile polimerize olan akrilik rezin Meliodent, Paladent ve QC-20 ve bir mikrodalga polimerize rezin Acron MC olmak üzere dört farklı protez kaide materyalinden toplam 60 adet örnek üretildi. Örnekler, polimerizasyon tekniklerine göre kontrol (geleneksel ve mikrodalga polimerizasyon), kısa (130° C 10 dakika) ve uzun (130°C 20 dakika) otoklav polimerizasyon gruplarına ayrıldı (n=5). Çekme mukavemeti testleri yapıldı. Veriler ANOVA ve ardından Duncan testi (a=0,01) ile analiz edildi.* ***Bulgular:*** *Testler sonucunda çekme dayanımı değerleri sırasıyla en yüksek Acron MC, kontrol grubu için ise QC-20, Paladent ve Meliodent olarak sıralandı. Acron MC, kontrol ısı polimerizasyonu ve 10 dakika otoklav polimerizasyonu grupları için diğer akrilik rezinlerden önemli ölçüde farklı bulundu (p<0,01). Otoklavda polimerize akrilik rezin örnekleri, kontrol gruplarına göre daha yüksek çekme dayanımı değerleri gösterdi (p<0,01). Otoklav polimerizasyon yöntemlerinin artan süreleri arasında ise anlamlı bir fark bulunmadı (p>0,01).* ***Sonuç:*** *Çalışmanın sınırları dahilinde, otoklav polimerizasyon yöntemi geleneksel polimerizasyon yöntemlerine göre daha güçlü bir alternatif sağlayabilir. Otoklav polimerizasyon yöntemi, çapraz bağlama ajanlarının (glikoldimetakrilat) aktivasyon oranının artırılabileceği yüksek sıcaklık sağlar.* ***Anahtar kelimeler:*** *Protez kaideleri, akrilik rezinler, çekme dayanımı, polimerizasyon.*

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Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.792699

Original research

EVALUATION OF CYTOTOXICITY OF DIFFERENT UNIVERSAL BONDS USING THE XCELLIGENCE SYSTEM

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ABSTRACT

Objectives: The purpose of this study was to explore the cytotoxic effects of five different universal bonding agents on mouse fibroblast cell lines (L929).

Materials and Methods: Five different widely used universal adhesive systems were chosen that have different contents, pH levels, and polymerization methods. A real-time cell analyzer (RT-CES, xCELLigence; Roche Applied Science, Germany, and ACEA Biosciences, USA) was used for cytotoxic evaluation of light-cured polymerized G-Premio Bond (GC Europe, Belgium), Prime&Bond Universal (Dentsply Sirona, USA), Universal Bond Quick (Kuraray, USA), Single Bond Universal (3M ESPE, USA) and self-cured polymerized Tokuyama Universal Bond (Tokuyama, USA) experimental groups. L929 were cultured in Dulbecco's modified Eagle's medium and supplemented with 10% fetal bovine serum and 1 % antibiotics. The assay was performed E-plate-16 and monitored every 15 min for 72 h. Statistical analysis was performed using ANOVA and Tukey's posthoc tests.

Results: All tested universal adhesive systems showed a statistically significant difference in cytotoxicity values in different periods ($p<0.05$). Among the groups compared, G-Premio Bond showed the least cytotoxic effect; and Tokuyama Universal Bond showed the most cytotoxic effect. Different times of all universal adhesive systems significantly increased the count of viable cells compared to the control group ($p<0.05$).

Conclusions: In dentistry, universal adhesive systems can be observed cytotoxic effects to live cells. The evaluation of cytotoxicity with xCELLigence device is a reliable method and should be supported by new studies on this subject.

Keywords: xCELLigence, cytotoxicity, universal adhesive systems, L929.

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Received : 10.09.2020
Accepted : 25.11.2020

How to Cite: Atilan Yavuz S, Surmelioglu D. Evaluation of Cytotoxicity of Different Universal Bonds Using the Xcelligence System. Cumhuriyet Dent J 2020;23:4: -.
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INTRODUCTION

Currently, there is increasing social awareness around the importance of health and wellness. This situation allows improves physicians' working conditions while at the same time providing patients more aesthetic, functional, and longer-lasting restorations. As a result of these developments, new researches are added to the literature with the study of both clinicians and academicians on aesthetic materials.

Dentin bonding agents can improve bonding strength, increase the adhesion of restorations, reduce micro-leakage between the tooth-resin interface, and decrease occlusal stress.[1] Initially, in the clinical resin restorative system, bonding agents were applied in three steps. However, several manufacturers have reduced the bonding procedure to two steps by introducing both the adhesive and the primary solvent in a single bottle.[2] Furthermore, in some systems, the acid, primer, and adhesive are incorporated into the same bottle to make a single-step or self-etching primer bonding system.[3] More recently, single-step universal adhesive systems have been developed which can be used in three different modes such as self-etch, selective etch, and total-etch.[4]

Although these adhesive systems are similar in terms of their monomers' composition, universal adhesive systems differ from other adhesive systems by their monomers that can establish chemical and micromechanical bonds.[5] Another property of universal adhesive is strong adhesion to enamel due to unique COOH or PO_4 monomers that ionically bond to the calcium in the hydroxyapatite crystals of the enamel.[6] Additionally, the MDP monomer, which is only present in universal adhesives, allows universal systems to be used with three different etching techniques.[7]

All adhesive systems such as self-etch, total-etch, and universal adhesive systems have different compositions, pH levels, and polymerization techniques.[8,9] In several studies in the literature, it has been reported that all these parameters are associated with cytotoxicity of adhesive systems in teeth and living tissues.[10-12] Although the purpose of adhesive systems is to provide bonding of restorative materials to enamel/ dentin, it is also important to investigate the potential cytotoxic effects due to the various monomers contain in adhesive systems. Adhesive systems typically include monomers such as bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), hydroxy ethyl methacrylate (HEMA) and dipenta erythritol penta-acrylate monophosphate (PENTA). Additionally, some contain biphenyl dimethacrylate (BPDM) and polyalkenoic acid.[13,14]

Hydrophobic monomers such as Bis-GMA and UDMA show more cytotoxic effects compared to hydrophilic monomers such as HEMA and TEGDMA.[15] Progress of hydrophilic monomers in dentinal fluid and transporting hydrophobic monomers in dentin tubules can cause cytotoxic effects in pulp. Furthermore, the toxic effects of hydrophilic and hydrophobic groups together are greater than the toxic effects they produce alone.[16] While resin-based dental materials have a local cytotoxic effect on pulp, systemic toxicity has not been observed in previous studies.[17,18]

The acidic characteristic of monomers in universal adhesive systems allows for the simultaneous creation of diffusion channels and the infiltration of these channels. Recently, new universal adhesive systems have been marketed with pH values, but there is not enough search about exposing the dentin complex to these agents. However, it is generally known that due to the cytotoxicity of bonding agents[19-22], there is the possibility of pulpal damage in clinical applications. Therefore, it is recommended for clinicians to use etch-rinse systems in shallow cavities and self-etch systems in deep cavities.[23]

The polymerization of resin monomers is important in terms of biocompatibility and bonding strength in bonding systems. HEMA and UDMA, which are hydrophilic monomers, provide better resin infiltration, increase bond strength and provide sufficient polymerization of monomers.[24,25] The effect of different polymerization types on polymerization is also an important question since it is known that residual monomer release increases due to inadequate polymerization.[26,27] Researches

have been conducted on the effects of universal adhesives with either light-cured or self-cured polymerization types on cytotoxicity because, in the bonding process, unreacted resin monomers can damage the pulp tissue through dentine tubules.[12,28] Although there are significant developments and innovations about the physical and mechanical properties of adhesive systems day to day, the biocompatibility of these systems in tooth and living tissues has not yet been fully characterized. In this study, a real-time cell analyzer (xCELLigence) was used to investigate the time-dependent cytotoxic effects of five different universal dentin bonding agents on a mouse fibroblast cell line (L929). In addition, little is known about the cytotoxicity of universal adhesive systems. This research is the first study in the literature in which the cytotoxicity of different universal adhesive systems is evaluated by the xCELLigence method.

Our study aims to evaluate the cytotoxic effects of five different universal bonding agents on mouse fibroblast cell lines (L929). The null hypothesis was all five different universal adhesive systems with different monomer content, pH level, and polymerization technique will have different cytotoxic effects.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Gaziantep University (process no. 2018/374).

Experimental Groups

Five different universal dentin bonding agents were tested in this experiment: G-Premio Bond (GC Europe, Inc., Leuven, Belgium), Tokuyama Universal Bond (Tokuyama America, Inc., California, USA), Universal Bond Quick (Kuraray America, Inc., Texas, USA), Prime&Bond Universal (Dentsply Sirona, Inc., Pennsylvania, USA) and Single Bond Universal (3M/ESPE, Inc., Minnesota, USA). Materials and ingredients are provided in *Table 1*.

Table 1. Materials used in this study

Adhesive Systems	Components	Manufacturer	pH
G-Premio Bond	MDP, 4-MET, Methacrylate monomer, acetone, water, silane	GC Europe (Leuven, Belgium)	1.5
Tokuyama Universal Bond	Bis-GMA, TEGDMA, HEMA, isopropanol, acetone, water	Tokuyama (California, USA)	2.2
Universal Bond Quick	Bis-GMA, HEMA, MDP, hydrophilic amide monomer, ethanol, water, silane	Kuraray (Texas, USA)	2.3
Prime&Bond Universal	PENTA,10-MDP, isopropanol, acetone, water	Dentsply Sirona (Pennsylvania, USA)	~ 2.5
Single Bond Universal	2-HEMA, 10-MDP, dimethacrylate resins, VitrebondTM copolymer, silane, filler, ethanol, water, initiators.	3M ESPE (Minnesota, USA)	2.7

MDP: 10-methacryloyloxydecyl dihydrogen phosphate, 4-META: 4-methacryloyloxyethy trimellitate anhydride, PENTA: Dipentaerythritol pentaacrylate phosphate, TEGDMA: triethylene glycol dimethacrylate, Bis-GMA: bisphenol A-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate.

Test specimens were prepared according to the manufacturers' instructions. In addition to the five different experimental groups, a control group containing only the L929 fibroblast cell line (ATCC®CRL-6364) without any adhesive material was added.

In order to obtain the cytotoxic values of the adhesive systems used, preparation of test samples, sterilization, preparation of cell culture, and evaluation with the xCELLigence method were performed. All processes were accomplished in accordance with the ISO 10993-5 protocol to ensure standardization.[10] Polymerization of universal dentin bonding systems was achieved by using a LED (Valo Led, Ultradent) light device at times recommended according to the manufacturer's instructions (with the exception of self-cured Tokuyama Universal Bond).

Cell Culture

An established cell lines, mouse fibroblasts L929 (American Type Culture Collection CCL 1), was cultivated in Dulbecco's Modified Eagle's

Medium (DMEM; Invitrogen, Carlsbad, CA, United States) including 10% fetal bovine serum (FBS) and 1% antibiotics (100 IU/ mL penicillin-streptomycin). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

Cells grown in flasks were passaged twice a week until the required cell density for the cytotoxicity test was maintained at 37 °C in a humid atmosphere. A solution containing 0.05% trypsin and 0.53 mM ethylenediamine tetra acetic acid (EDTA) was used to remove the cells from the flasks. All protocols were performed in a laminar flow cabinet with UV light sterilization. DMEM was added to the suspended cells to neutralize the effect of trypsin**.** The medium and manufacturer are shown in *Table 2*.

Table 2. Cell culture medium used in this study

CELL CULTURE MEDIUM	MANUFACTURER
Dulbecco's Modified Eagle Medium	Sigma Aldrich Chemie, Germany
Fetal Bovine Serum	Biochrom KG, Germany
Penicillin /Streptomycin	Biochrom KG, Germany

Cell Count

The hemocytometer was used to calculate the number of cells in milliliters of the suspension, using the following formula:

Total cell count/ ml = hemocytometer count result x 10^4 x medium amount (ml)

Preparation of Extraction Fluid and Extraction Process

In this study, artificial saliva was preferred as extraction fluid. The content of the saliva solution was; 136.8 mM NaCl (sodium chloride), 3.0 mM KCl (potassium chloride), 2.5 mM $CaCl_2.6H_2O$ (calcium chloride), 1.5 mM $MgCl_2.6H_2O$ (magnesium chloride), 0.5 mM $Na_2SO_4.10H_2O$ (sodium sulfate), 4.2 mM $NaHCO_3$ (sodium bicarbonate), and 1.0 mM $K_2HPO_4.3H_2O$ (dipotassium phosphate).

The prepared test samples were placed in an Eppendorf tube, then 1 ml artificial saliva solution was added. Since the extraction liquid to be obtained after all these extraction procedures will be applied to cell cultures, the following procedures were performed in a laminar flow cabinet with UV light sterilization. The extracts were placed in different Eppendorfs tubes due to the various waiting time (24 h, 48 h and 72 h) of the samples.

xCELLingence System

The xCELLigence system is a method for measuring cell viability in accordance with the manufacturer's instructions. (Roche Diagnostics GmbH, Mannheim, Germany and ACEA Biosciences, Inc., San Diego, CA, USA). The xCELLigence system consists the real time analyser (RTCA), the RTCA single plate (SP) station, the RTCA computer with integrated software and a disposable E-plate 16. While the RTCA SP station fits inside a standard tissue-culture incubator, the measurements are transferred to a software analyser and computer. The E-plate 16 is a disposable device used for performing cell-based assays on the RTCA SP instrument. The single-use E-plate 16 contains gold cell sensor arrays at the bottom which; contributes to the monitoring and testing the cells in each well. The E-plate 16 has a low evaporation lid design: the bottom diameter of each well is 5.0mm ± 0.05mm; with a total volume of 243 ± 5 µL. Approximately 80% of the bottom of each well is covered by circle-on-line-electrodes, which are designed to be used in an environment of +15 to +40 °C, relative humidity 98% maximum without condensation.

Physiological changes of the cells to be examined are determined by the electronic impedance formed in the sensor electrodes. The voltage of the electrodes is about 20 mV (RMS) during the test. In the absence of cells, the main source of electrode impedance is the concentration of ions at both the electrode / solution interface and within the solution. In the presence of cells, which act as insulators, the electrode impedance increases as a result of the change in the local ion environment at the electrode / solution interface. The electrode impedance value changes in proportion to the cell density.

Cytotoxicity Test

In this study, a 16-well E-plate 16 was used; each well had a volume of 250 μL and a base diameter of 5 mm. After 200 μL of the cell suspensions were seeded into the wells (10.000 cells/well) of the E-plate 16 in a laminar flow cabinet with UV light sterilization, and the plates were placed in the incubator. L929 mouse fibroblast cells which were placed on the well of the plates was provided to grow in the appropriate medium and O_2/CO_2 environment, to enter the rapid growth phase (log phase). In addition to the experimental groups, only the cells and medium solution containing no adhesive agents were added to the test plate of the control group. Following this, the E-plate 16 was placed in the incubator. Cytotoxic values of adhesive systems at 24, 48, and 72 h were obtained by applying the extraction liquid at different times (24, 48, and 72 h).

Statistical Analysis

SPSS 22.0 software was used for statistical analysis of the data obtained from this empirical study. The sphericity prerequisite was assessed with Mauchly's test of sphericity. In cases where the sphericity prerequisite was not provided, the difference between repeated measurements was determined by using the Greenhouse-Geise test. ANOVA test was performed for measurements between more than two independent groups. Post-hoc Tukey's test was used for pairwise comparisons in groups with differences. The statistical significance level for all comparisons was $p<0.05$.

RESULTS

Extracts were obtained from wells at 24 h, 48 h, and 72 h for all experimental groups. There were significant differences between all groups following 24 h applications of universal adhesive systems used in the study ($p<0.05$). The highest cytotoxic effect was observed in the Tokuyama Universal Bond group, and the highest cellular viability was found in the G-Premio Bond group. The extract obtained at 48 h showed significant differences between all groups ($p<0.05$). The highest cytotoxic effect was in the Tokuyama Universal Bond group, while the lowest cytotoxic effect was in the G-Premio Bond group. Similarly, in the other hour measurements, there was a significant difference in the 72nd h cytotoxicity values in all groups ($p<0.05$). In the 72 h findings, the most cellular proliferation was observed in the G-Premio Bond group, while the least cellular proliferation was observed in the Tokuyama Bond Universal adhesive system. When all groups were compared time dependent, it was observed that the mean cell proliferation value (%) decreased from 24 h to 72 h. Cell proliferation (%) was seen in all time periods for all groups (24 h, 48 h, and 72 h) shown in *Table 3* and Figure 1.

Table 3. Mean ± Standard error of cell proliferation values of tested universal adhesive systems

Cell Proliferation (%)	24 h	48 h	72 h
Control	0.23 ± 0.04^{Aa}	0.20 ± 0.04^{Ab}	0.15 ± 0.04^{Ac}
G-Premio Bond	0.19 ± 0.04^{Ba}	0.17 ± 0.05^{Bb}	0.12 ± 0.04^{Bc}
Tokuyama Universal Bond	0.10 ± 0.06^{Ca}	0.08 ± 0.04^{Cb}	0.05 ± 0.03^{Cc}
Universal Bond Quick	0.13 ± 0.05^{Da}	0.11 ± 0.05^{Db}	0.07 ± 0.04^{Dc}
Prime&Bond Universal	0.15 ± 0.06^{Ea}	0.12 ± 0.05^{Eb}	0.08 ± 0.05^{Ec}
Single Bond Universal	0.13 ± 0.05^{Fa}	0.10 ± 0.04^{Fb}	0.06 ± 0.04^{Fc}

**Different letters within columns and lines indicate statistically significant differences. (Uppercases represent columnar differences intergroup, while lowercases represent linear differences intragroup).*

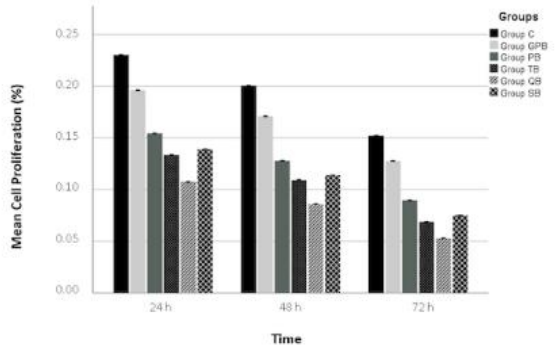

Figure 1. Comparison of mean cell proliferation (%) of all groups at 24, 48 and 72 h

When all groups were compared with the control group, there was a statistically significant difference in all time values in terms of cytotoxicity ($p<0.05$). The lowest cytotoxicity value was observed at 24 h of G-Premio Bond, while the highest cytotoxicity value was found at 72 h of Tokuyama Universal Bond. Cell proliferation (%) seen between control and other groups in all time periods (24 h, 48 h, and 72 h) are shown in Figures 2, 3, 4, 5, 6.

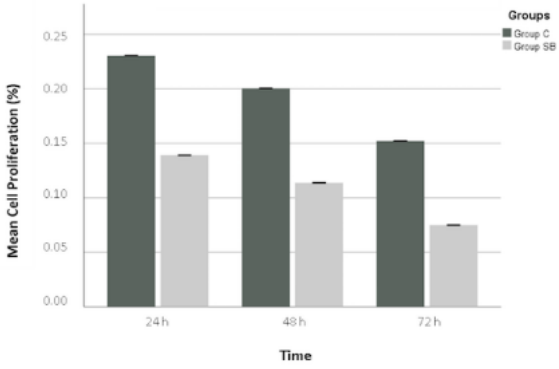

Figure 2. Comparison of mean cell proliferation (%) between control and G-Premio Bond groups at 24, 48 and 72 h

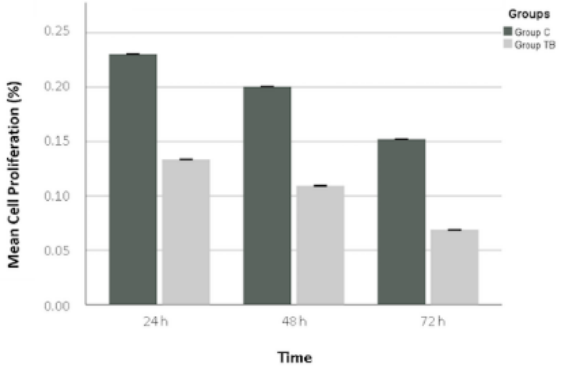

Figure 3. Comparison of mean cell proliferation (%) between control and Prime&Bond Universal groups at 24, 48 and 72 h

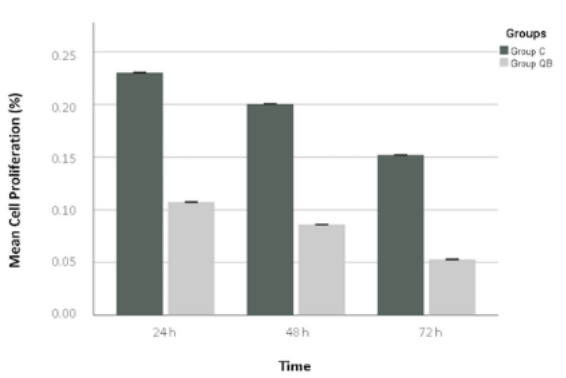

Figure 4. Comparison of mean cell proliferation (%) between control and Single Bond Universal groups at 24, 48 and 72 h

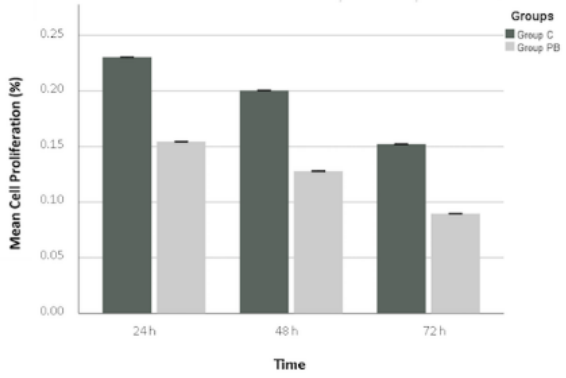

Figure 5. Comparison of mean cell proliferation (%) between control and Tokuyama Universal Bond groups at 24, 48 and 72 h

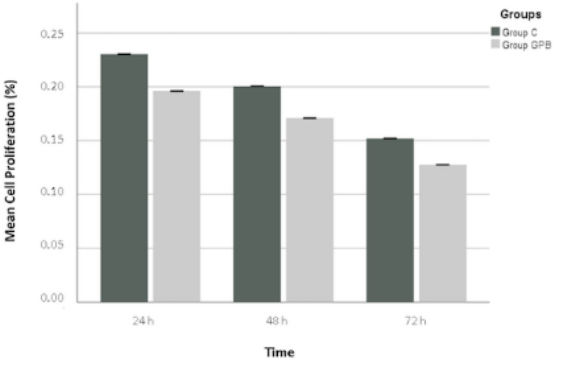

Figure 6. Comparison of mean cell proliferation (%) between control and Universal Bond Quick groups at 24, 48 and 72 h

DISCUSSION

Dental materials which have different contents are offered on the market by the manufacturers in adhesive dentistry with the advancement of technology each passing day. During the evaluation of a newly developed material, its physical, mechanical and biological properties should be taken into consideration.[1,29] The number and variety of tests evaluating the biocompatibility of materials have increased with developed products.[9] The adhesive systems used in restorative dentistry have different cytotoxic properties. Da Silva *et al.*[30] examined the biocompatibility of the

four different generations of adhesive systems and reported that the universal bond systems had the lowest cytotoxic effect on pulpal cells.

Previous research has determined that different universal adhesives have different compositions, such as HEMA, Bis-GMA, UDMA and PENTA, as well as biphenyl dimethacrylate (BPDM), CQ, MDP, polyalkenoic acid, acetone and ethanol, different pH and polymerization methods.[8,9] Several studies have shown that these parameters impact the cytotoxicity of the agent used.[10-12] Therefore, in this experimental study, five different universal adhesive systems with different contents, pH and, different polymerization methods were investigated. Acetone-based G-Premio Bond (pH<2), Tokuyama Universal Bond (pH>2), ethanol-based Prime & Bond Universal (pH>2.5), Universal Bond Quick (pH>2) and Single Bond Universal (pH>2.5), which contain one or more of the resin monomers, were preferred for use in this study.

The cell type in which the dental materials are in contact with the cytotoxicity is important with the in-vitro studies. Especially in dentistry, using Balb 3T3 or L929 mouse fibroblasts cell cultures to evaluate cytotoxicity is recommended.[31-33] L929 is a continuous cell culture line with a wide range of use as a standard in cytotoxicity testing of dental materials.[34] In a previous study examining cytotoxicity, a dental material showed similar effects on L929 mouse fibroblasts and gingival fibroblasts.[12]

Biological events (cell proliferation, vitality, toxicity, the demonstration of the physical situation of the cells) can be analysed in real time by the xCELLigence system.[35] The greatest advantage of this system is that the respective number of cells in each well of the E-plate 16, the proliferation, attachment and spreading of the cells can be monitored every 15 min.[36] Data from cells in the wells are monitored simultaneously on the connected computer.[37] This property allows for instant changes, such as stopping the experiment and adding a new substance.[36] With the xCELLigence system, it is also possible to observe and evaluate the real-time analysis without the need for staining or marking the cells. In methods such as MTT, XTT and staining methods, it is not possible to use existing cells for different purposes after end-point analysis. Obtaining limited data with three different measurements in a 72-hour experiment using methods sensitive to human error is another disadvantage. In contrast, it is possible to receive cellular data every 15 seconds with real-time analysis using the xCELLigence system. The basic working principle in obtaining data in new systems is based on impedance measurements; therefore the cells used in the xCELLigence system can be evaluated with another test.[38] For example, when a material is evaluated in terms of cytotoxicity, cells are collected from the wells when needed and information about the genotoxicity is obtained by evaluating the oxidative stress level. While only one parameter is evaluated with classical methods, general data (cell proliferation, cell apoptosis, morphological change in cell membrane) can be examined with xCELLigence. The xCELLigence device offers economical, comprehensive and more accurate study to researchers.[37]

Previous studies have reported that different monomers are released from resin-based dental materials during or after polymerization.[39-41] The monomers released from the materials in the different compositions determine the biocompatibility.[13] Although it is possible to fully polymerize the monomer in theoretically, one study that this transformation is at most 70% with conventional light sources.[42] With the increasing power of light sources, the polymerization percentage of monomers is expected to increase. In addition, as heat is generated during the polymerization of dental materials, the dentinal fluid in the tubules moves towards the pulp and the released monomers reach the pulp. These issues increase the damage to the pulp caused by heat. In a study, an 800 mW light device (20 s) and a 650 mW light device (30 s) were used for polymerizing the resin composite, and the number of live lymphocyte cells was examined at the end of the experiment.[43] High cell viability was detected in the group that was applied for a longer time at low power density compared to the group that was applied for a shorter time at high power density.

In the literature, there have been many studies about the release of monomers time-dependent due to insufficient polymerization. Some studies indicate that 1-7 days are required for full-swing, while in some studies it has been stated that 30 days should be waited.[12,44] In addition, there is a positive connection between the contact time of the adhesive systems to the dentine and the cytotoxic effect of the monomers in the contents of the adhesive. Ratanasathien *et al.*[16] found, that the duration of action of monomers released from adhesive agents has a strong effect on the toxicity of adhesive systems. In this study, measurements at 24 h, 48 h and 72 hours were obtained in order to evaluate the short-medium-long-term cytotoxic effects of adhesive systems, and it was observed that the cytotoxicity varied depending on the time of exposure.

Some components of resin-based dental materials are considered to be cytotoxic; furthermore, cytotoxic effects of Bis-GMA, TEGDMA, UDMA and HEMA have been investigated. In previous studies, the cytotoxicity of monomers has been shown to be the following: Bis-GMA> UDMA> TEGDMA> HEMA.[16,36] The cytotoxicity values obtained as a result of this study are achieved by a similar mechanism. G-Premio Bond and Prime & Bond Universal adhesive systems that did not contain any of these resin monomers showed lower cytotoxicity. Also, Tokuyama Universal Bond which contains resin monomers such as Bis-GMA, UDMA and HEMA, had the highest cytotoxic value.

Koulaouzidou *et al.*[45] examined the cytotoxic effects of three different adhesive systems on fibroblast cells and reported that the XP Bond group showed the highest cytotoxic value. This study identified that UDMA and TEGDMA monomers in the content of the XP Bond system are responsible for this result. The same study reported that the molecular weight and components of resin monomers may also affect this result: HEMA, which has a low molecular weight, has a less toxic effect than Bis-GMA, UDMA and TEGDMA. While Bis-GMA and UDMA showed highly toxic effects, HEMA and TEGDMA had moderately toxic effects.[16,42,46] In present study, the Tokuyama Universal Bond group, which includes Bis-GMA, TEGDMA and HEMA monomers had the highest cytotoxicity value, while G-Premio Bond and Prime&Bond Universal adhesives which do not include any of these monomers, had the lowest cytotoxicity value.

Several studies reported that camphorquinone, which acts as a photo initiator in adhesive systems, is both cytotoxic and mutagenic.[40,47] In this present study, it is possible that camphorquinone, which is a component of the Prime & Bond Universal adhesive system, may be responsible for the cytotoxic effect of this adhesive agent.

In a study in the literature, the cytotoxicity of composite resin and adhesive systems was examined immediately and following a seven days incubation period, it was reported that all samples were cytotoxicity, but it decreased after seven days.[48] Extraction fluid containing residual monomers released from dental materials was removed to evaluate the samples, and only samples were used. However, in this study, the samples were kept in the extraction liquid for 72 h and cytotoxic effects were determined using the extraction fluid. In the study of Franz *et al.*[48], although the toxic effect of all groups is consistent with this research, it assumed that the reason for the decrease cytotoxic effect at the end of 72 h was performed by removing the extraction fluid of the material.

In the study of Yasuda *et al.*[22], the cytotoxic effect of five different adhesive systems (AQ Bond Plus, Clearfil Tri-S Bond, G-Bond, Adper Prompt and Absolute) in human pulp cells was examined when compared with the polymerized samples and those applied without polymerization, lower cytotoxicity was observed in the polymerized group. Additionally, the cytotoxic effect was significantly higher at 72 h compared to 24 h. This finding is consistent with the present study, in which comparison of 24 h and 72 h groups, revealed significantly more toxic effects present at 72 h.

Schedle *et al.*[12] examined, the cytotoxic effects of composite, adhesive system and / or primer combinations, including experimental

groups for both chemical polymerization and light polymerization of the Optibond adhesive system. On the 2nd and 7th day of the experiment, the number of cells (%) was lower in the chemical polymerization of the Optibond adhesive system. Moreover, in this study, Tokuyama Universal Bond, which is chemically polymerized, was shown to be associated with lower cellular proliferation compared to four other universal adhesive systems polymerized with the LED light device (G-Premio Bond, Prime&Bond Universal, Single Bond Universal and Quick Bond Universal).

In another study[49], the cytotoxicity of Single Bond (pH=4.3), Clearfil SE Bond (primer pH=1.9, bond pH=2.8), Xeno III Bond (pH=1.0), Clearfil Protect Bond (primer pH=1.9, bond pH=2.8) and Adper Prompt Bond (pH=0.8) which have different pH values were investigated by MTT method, and the lowest cytotoxicity was found in the Adper Prompt Bond adhesive system with the lowest pH value. In this study, the G-Premio Bond adhesive system (pH=1.5) had the lowest pH value and demonstrated the lowest cytotoxicity. Similarly, when the Single Bond Universal (pH=2.7) and Universal Bond Quick (pH=2.3) were compared, the Universal Bond Quick adhesive system, which has a lower pH, showed lower cytotoxicity.

According to our results, the null hypothesis that all five different universal adhesive systems with different monomer content, pH level, and polymerization technique will have different cytotoxic effects was accepted.

This experimental study has several limitations, including the inability to fully mimic the in vivo environment of the in vitro study. In addition, cytotoxicity was not followed for time periods longer than 72 h, and mouse fibroblast cells were used. Very few studies to date have evaluated the cytotoxicity of adhesive systems using the xCELLigence system. Ours is the first study to compare the cytotoxic effects of five different universal adhesive systems with the xCELLigence device; however, additional studies are required to clearly elucidate these cytotoxic effects.

CONCLUSIONS

According to the results of this research, all the universal adhesive systems used in were found to have a significant cytotoxic effect on the L929 mouse fibroblast cell line compared to the control group, and it was concluded that this effect increased time-depending (24 h, 48 h and 72 h). It was assumed that the cytotoxic effect of adhesive systems on L929 mouse fibroblast cells is related to the increase in residual monomer concentration released from adhesives.

ACKNOWLEDGMENTS

All procedures performed in this study were in accordance with the ethical standards of the Ethics Committee, University Gaziantep (process no. 2018/374), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The authors would like to thank Prof. Dr. Esra CAN EROĞLU, Prof. Dr. İhsan HUBBEZOĞLU, Assist. Prof. Dr. Ceylan HEPOKUR for their contributions

CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article**.**

FUNDING

This study was supported by Gaziantep University, Scientifics Research Section with DHF.UT.19.03 project number.

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