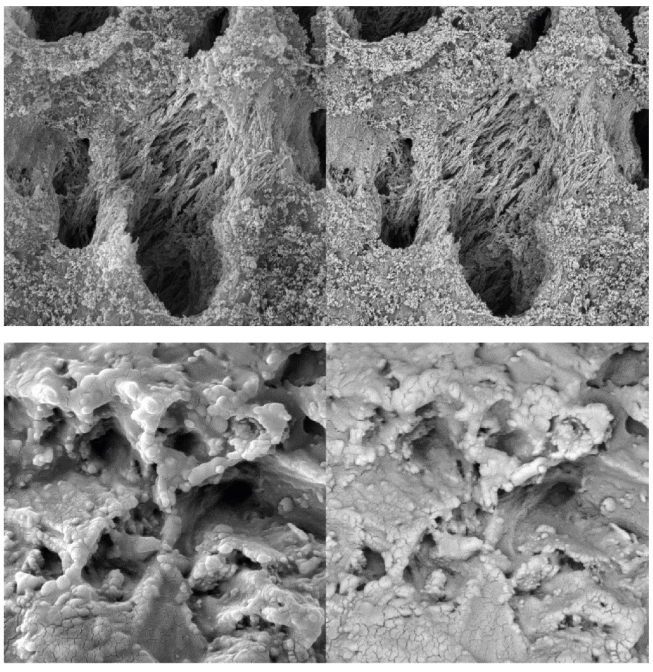
CUMHURIYET DENTAL JOURNAL

The Official Journal of Sivas Cumhuriyet University Faculty of Dentistry



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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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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.

CDJ accepts articles in English. Submitting a paper to CDJ is free of charges. In addition, CDJ has not have article processing charges.

Frequency: Four times a year (March, June, September, and December)

CDJ is published using an open access publication model, meaning that all interested readers are able to freely access the journal online without the need for a subscription. Manuscripts will be reviewed by the editor, and at least two reviewers with expertise within the scope of the article.In addition, CDJ use double- blind review process (every effort is made to prevent the identities of the authors and reviewers from being known to each other)

Review Process

Double-Blind Peer Review Process

CDJ uses double-blind review, which means that both the reviewer and author identities are concealed from the reviewers, and vice versa, throughout the review process. Within this aim, the authors need to ensure that their manuscripts are prepared in a way that does not give away their identity. Editors will email selected Reviewers the title and abstract of the submission, as well as an invitation to log into the journal web site to complete the review. Reviewers enter the journal web site to agree to do the review, to download submissions, submit their comments, and select a recommendation.

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The publication language is English. Authors whose native language is not English should obtain the assistance of an expert in English and scientific writing before submitting their manuscripts. Manuscripts that do not meet basic language standards will be returned pre-review. Authors are requested to submit their original manuscript and figures via the online submission and editorial system for Cumhuriyet Dental Journal. Using this online system, authors may submit manuscripts and track their progress through the system to publication. Reviewers can download manuscripts and submit their opinions to the editor. Editors can manage the whole submission/review/revise/publish process.

Manuscript Format and Style

General

Manuscript length depends on manuscript type. Paper dimensions should be 8.5 × 11 inches with 2.5 cm margins on all sides. Please use normal, plain font (12-point Times New Roman), justified and number all pages consecutively. Indent or space paragraphs.

Manuscript Types Accepted

Original Research Article: Title, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgements, References, Tables and Figure Legends

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.

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

Manuscript Submission Procedure

Submission site

Manuscripts should be submitted online through http://dergipark.gov.tr/cumudj. Full instructions and support are available on the website, and a *user ID* and *password* can be obtained at the first visit. All parts of the manuscript (Main Document, Tables, Figures and Supplemental Information) must be available in an electronic format: Microsoft Word or generic RTF are recommended for text and tables; and TIFF or EPS for graphics (see under Figures).

ELEMENTS OF a MANUSCRIPT

1. Title Page

- ***Title page must be*** **uploaded apart from manuscript and should include;**
- -Title
- -Authors (first name, middle initial, surname) e.g. Burak Buldur, DDS, PhD,[a]
- -Authors' addresses (abbreviated) e.g.
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- Corresponding Author details including name, complete address, phone, fax, and e-mail must be added.

Main Document

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- Should not exceed 300 words and should be presented under the following subheadings:

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3. Keywords

- Up to 5 keywords should be supplied according to **MESH**.

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- This must be presented in a structured format, covering the following subjects, although not under subheadings: succinct statements of the issue in question; the essence of existing knowledge and understanding pertinent to the issue; and the aims and objectives of the research being reported.

5. Materials and methods

- The authors should describe the procedures and analytical techniques and identify names and sources of all commercial products e.g. magnetic attachment (Hyper Slim 5513, Hitachi Metals, Tokyo, Japan)

6. Results

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- The authors should discuss the results of the study also state the agreement with other studies and identify the limitations of the present study and suggest areas for future research.

8. Conclusions

- The authors should concisely list conclusions that may be drawn from the research and do not simply restate the results.

9.Acknowledgements

- If the work was supported by a grant or any other kind of funding, supply the name of the supporting organization and the grant number.

11. Conflicts of Interest statement

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

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

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

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

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

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

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

We invite governments, universities, libraries, journal editors, publishers, foundations, learned societies, professional associations, and individual scholars who share our vision to join us in the task of removing the barriers to open access and building a future in which research and education in every part of the world are that much 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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For the experimental, clinical and drug human studies, approval by ethical committee and statement on the adherence of the study protocol to the international agreements (Helsinki Declaration revised 2008) are required. In experimental animal studies, the authors should indicate that the procedures followed were in accordance with animal rights and they should obtain animal ethic committee approval. The Ethic Committee approval document should be submitted to the Cumhuriyet Dental Journal together with the manuscript.

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.

Cumhuriyet Dental Journal: 2019; 22(3)
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CONTENTS

Cumhuriyet Dental Journal: 2019; 22(3)
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Editorial

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

EVALUATION OF POSTOPERATIVE PAIN AFTER USING DIFFERENT FILE SYSTEMS: A RANDOMIZED CLINICAL STUDY

I am pleased to inform you that I have chosen this article by Ozdemir *et al.*[1] as Editor's Choice for third issue of 2019.

As we all know, clinical trials are essential for the development of new treatments. This study has given very valuable information regarding postoperative pain which is a common complication after root canal treatment. This clinical study aimed to compare the severity of postoperative pain following root canal treatment by using rotational and adaptive techniques. This article revealed that although tested instrumentation techniques caused postoperative pain, the pain scores indicated that both techniques caused limited discomfort associated with slight pain which did not require any additional treatment and medication.

Happy readings in the third issue of 2019!

Assoc. Prof. Burak Buldur

Co-Editor-in-Chief

REFERENCE

1. Özdemir O, Koçak MM, Koçak S, Sağlam BC. Evaluation of Postoperative Pain After Using Different File Systems: A Randomized Clinical Study. Cumhuriyet Dent J 2019;22:3:292-298.

How to Cite: Buldur B. Editorial: The Editor-in-Chief's recommendation of this issue's article to readers. Cumhuriyet Dent J 2019:22:3:247.

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Original research

DEREGULATION OF CANCER-ASSOCIATED GENES IN ODONTOGENIC CYSTS

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ABSTRACT

Objectives: The aim of the present study was to demonstrate the key role of differential expression levels of RB1, TP53, XIAP, BCL2 AIFM3, BAX, CASP3 and CASP9 genes in odontogenic cysts.

Materials and Methods: A total number of 15 patients who diagnosed with odontogenic cyst were enrolled for the present study. For the quantitative gene expression analysis, cyst and adjacent gingival healthy tissues of patients were collected during surgical assessments. Quantitative analysis of gene expression levels RB1, TP53, XIAP, BCL2 AIFM3, BAX, CASP3 and CASP9 were achieved real-time PCR method. For the optimization of gene expression levels GAPDH reference gene was used.

Results: Expression of both RB1 and TP53 genes were markedly diminished in odontogenic cysts tissues as compared to healthy tissues ($p<0.05$). Likewise, levels of CASP3 and CASP9 genes were found to be significantly reduced in odontogenic cysts tissues compared to healthy tissues ($p<0.05$). In contrast, expression levels of XIAP was significantly elevated ($p<0.05$). Although BCL2, AIFM3, and BAX genes were also differentially expressed in odontogenic cysts tissues, these variations were statistically insignificant ($p>0.05$).

Conclusions: The findings of the present study indicates that RB1, TP53, XIAP, CASP3 and CASP9 genes might have chief roles in formation odontogenic cysts and responsible for the increased cell proliferation in these tissues.

Keywords: Gene expression, odontogenic cysts, tumor suppressor genes.

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INTRODUCTION

Cysts are pathological cavities which are surrounded by epithelial connective tissue capsule filled with fluid or semi-fluid material. Cysts are most commonly seen in jaws compared to other parts of the body, as epithelial debris in these regions is more abundant than other parts of the body.[1] Radicular cysts are the most frequently encountered odontogenic cysts and originate from a previously formed apical granuloma in the apical region of a necrotic tooth. These cysts are formed by the proliferation of malessez epithelial residues.[2,3] Dentigerous cysts are cysts that surround the crown of the unerupted or partially erupted teeth. After the formation of the tooth crown, fluid accumulation between tooth enamel epithelium and the tooth crown and additionally proliferation of epithelial islets in the dental follicle wall leads to cyst formation. Approximately 24% of odontogenic cysts are dentigerous cysts and second most common odontogenic cysts seen after radicular cysts.[4,5] Odontogenic keratocysts, which originates from odontogenic epithelial wastes and the basal layer of the oral epithelium, are considered to be the most aggressive odontogenic cyst and more likely to transform into a malignancy due to local aggressive course, high residual rate and invasion to surrounding tissues.[6-9]

Besides, molecular pathogenesis of carcinogenesis involves the accumulation of mutations in oncogenes and tumor suppressor genes and these genetic aberrations are the very early sign of a malignant transformation.[10] In consequence, oncogenes and tumor suppressor genes are the crucial components that drive the formation of cancers.[10,11] Mutations can lead to hyper- or hypo-activity of an oncogene and tumor suppressor gene, respectively. So far, several genes with oncogenic and tumor suppressor functions have been identified to be associated with the development and progression of human cancers.[10,11] Chiefly, deregulation of these genes have been shown to be well-correlated with poor prognosis and clinicopathological characteristics of patients and experimentally associated with the increased proliferation, invasion, migration and metastasis of cancer cells in vitro.[10,11]

Moreover, accumulating body of evidence suggest that several cancer biomarkers were revealed to be deregulated in odontogenic cysts. Particularly, immunohistochemical analysis of Cyclin D1, BCL2, PCNA, P53[12,13], COX-2[14], Ki-67, P63[15,16] HIF-1α and Caspase 3[17] proteins revealed that these proteins markedly involved in pathobiology of odontogenic cysts and associated with the clinicopathological characteristics of patients. Nevertheless, these findings were limited to immunohistochemistry method and a few numbers of markers were investigated. Apart from these studies, we used quantitative PCR approach which is a cost-effective and less time-consuming method enabling rapid quantitative analysis gene expression levels.

Consequently, in this particular study, our aim was to elucidate the roles of differential expression levels of RB1 (Retinoblastoma 1), TP53 (Tumor Protein P53), XIAP (X-linked inhibitor of apoptosis), AIFM3 (Apoptosis inducing factor, mitochondria associated 3), BAX (BCL2 associated X, apoptosis regulator), CASP3 (Caspase 3) and CASP9 (Caspase 3) genes, which have been known to be associated with the formation and progression of several types of cancers, in odontogenic cysts of radicular, dentigerous and odontogenic keratocyst epithelium and their association with local aggressive behavior and osteolytic properties of these cysts. In particular, expression levels of RB1, TP53, XIAP, BCL2 AIFM3, BAX, CASP3 and CASP9 genes were measured by Real-Time Polymerase Chain Reaction (PCR) method.

MATERIALS AND METHODS

Study population and sample collection

Total numbers of 15 patients who admitted to clinic of Department of Oral and Maxillofacial Surgery with the various symptoms (pain, swelling, etc.) and diagnosed with odontogenic cyst were enrolled in the study. Clinical and demographical features of age, gender, anatomic localization of tooth and pathological diagnosis of patients were shown in Table 1. Among these patients, 4 (26.6%) were odontogenic keratocyst,

3 (20%) were dentigerous cyst and 8 of them were 8 (53.4%) radicular cyst (Table 1). Patients included in the study were subjected to routine examination and panoramic radiographs were obtained. Exclusion criteria for the study was presence of systemic disease, infection in the operation area, presence of systemic viral, fungal, or bacterial infection, sensitivity to drugs and anesthetics, cigarette or alcohol addiction, pregnancy or lactation.

Table 1. Demographic and clinicopathological characteristics of patients

Parameters		**n=15 (%)**
Age		34.8± 23.99
Gender	Male	6 (40.0)
	Female	9 (60.0)
Localization of Tooth	Mandibula right anterior	1 (6.67)
	Mandibula left posterior	2 (13.3)
	Maxilla right anterior	3 (20)
	Maxilla left anterior	6 (40)
	Mandibula right posterior	3 (20)
Pathological diagnosis	Odontogenic keratocyst	4 (26.6)
	Dentigerous cyst	3 (20)
	Radicular cyst	8 (53.4)

For the study, tissues and adjacent healthy tissues of patients were collected during surgical operation. Collected tissue samples of patients were stored at -80 °C for expression analysis. Also, every tissues sample were subjected to pathological examination prior to inclusion in the study. A written informed consent was obtained from all individuals participated in the study.

Isolation of total RNA from tissue samples

GeneJET RNA Purification Kit (Thermo Fisher Scientific Inc., Wilmington, USA) was used for the isolation of total RNA from tissue samples. Briefly, 30 mg of tissue samples were homogenized in 300 µl lysis buffer containing β-mercaptoethanol (Sigma-Aldrich, MO, USA) by using TissueLyser LT (QIAGEN Sample & Assay Technologies, Germany) and instructions of the manufacturer were followed to obtain RNA. To ensure any RNase contamination RNase AWAY Decontamination Reagent (Thermo Fisher Scientific Inc., Wilmington, USA) was used. Quantities and concentrations of isolated RNA samples were measured by using NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, USA) and stored -80°C in equal aliquots.

Synthesis of complementary DNA from RNA samples

Complementary DNA was synthesized by using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Wilmington, USA). Briefly, 4 µl of 5X Reaction Buffer, 1 µl of RiboLock RNase Inhibitor, 1 µl of 10 mM dNTP Mix, 1 µl of RevertAid Reverse Transcriptase, and 1 µl of Random Hexamer and variable amounts of ddH2O and RNA samples were mixed and distributed to 0.2 ml PCR tubes. The prepared PCR mix was subjected to following thermal cycling protocol; 5 min at 25°C, 60 min at 42°C and for the termination of reaction 5 min at 70°C. Subsequently, samples were placed on ice block and transferred to -80°C until further application.

Primer Design and Analysis of gene expression by Real-Time PCR

Appropriate expression primers were designed using the "Primer-blast" interface of National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) to analyze the expression of the genes of interest. The primers and their properties used in the analysis of mRNA expressions. For the analysis of the expressions of RB1, TP53, XIAP, BCL2 AIFM3, BAX, CASP3 and CASP9 genes, we used RealQ Plus 2x Master Mix Green without ROX™ (Ampliqon PCR Enzymes & Reagents, Odense M, Denmark). Briefly, for each reaction

12.5 µl of RealQ Plus 2x Master Mix, 0.5 µl of Forward primer, 0.5 µl of Reverse primer, 2 µl of cDNA and 9.5 µl of PCR-grade H2O was used. Real-time PCR reactions were carried out in Rotor-Gene Q instrument (QIAGEN Sample & Assay Technologies, Germany). For the reactions, tree-step cycling PCR program including 15 min at 95°C initial denaturation and 30 seconds at 95°C, 30 seconds at 60°C and 30 seconds at 72°C for 40 cycle was performed. Also, a melting curve analysis between 60 and 95°C immediately was performed after PCR to ensure PCR efficiency. All reactions were studied in triplicate.

Data Analysis

Gene expression levels were calculated by $2^{-\Delta Ct}$ (ΔCt = Target gene − Reference gene) method. GAPDH reference gene was used to normalize resulting expression data. GraphPad Prism (Version 6 for Windows) statistical analysis software was used to analyze statistical changes. For the statistical comparisons, Wilcoxon matched-pairs signed rank test was applied during analysis. All statistics were two-tailed and $p<0.05$ were accepted as statistically significant.

Ethics statement

This present study was reviewed in accordance to the Declaration of Helsinki on medical protocol and ethics. The regional Ethical Review Board of Adıyaman University Ethics Committee approved the study (Approval Number: 2018/1-7).

RESULTS

As a result of the gene expression analysis, we identified significant gene expression variations in odontogenic cysts. In particular, relative gene expression levels of two well-known tumor suppressor genes, RB1 (p=0,0479) and TP53 (p=0.0054) were found to be significantly decreased in tissues of odontogenic cysts as compared to adjacent gingival healthy tissue of patients (Fig 1).

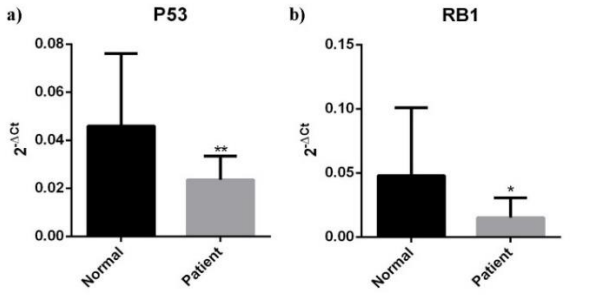

Fig 1. Quantitative expression levels of P53 and RB1 genes in tissues of odontogenic cysts as compared to adjacent gingival healthy tissue of patients.

In addition, gene expression levels of CASP3 (p=0.0085) and CASP9 (p=0.0479), which are involved in the intrinsic pathway of apoptosis, were found to be significantly reduced in tissues of odontogenic cysts as compared to adjacent gingival healthy tissue of patients (Fig 2). Also, gene expression level of XIAP (0.0353), which is an apoptosis inhibitor gene, was found to be significantly advanced in tissues of odontogenic cysts as compared to adjacent gingival healthy tissue of patients. Moreover, gene expression level of AIFM3 (p>0.05), an apoptosis inducing gene, was also found to be diminished but this variation was statistically not significant (Fig 2).

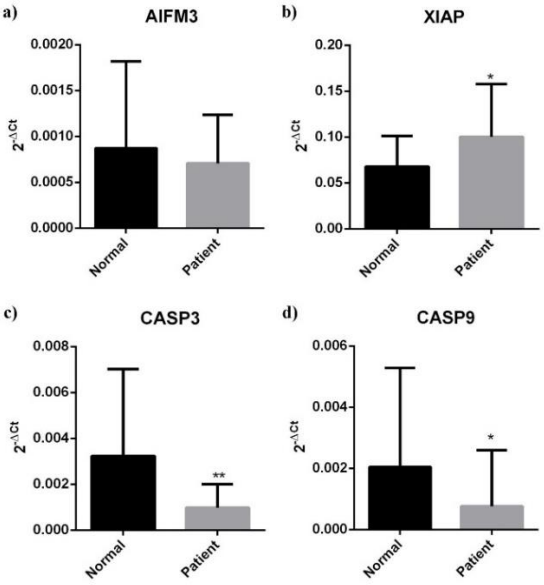

Fig 2. Quantitative expression levels of AIFM3, XIAP, CASP3 and CASP9 genes in tissues of odontogenic cysts as compared to adjacent gingival healthy tissue of patients**.**

Furthermore, gene expression level of pro-apoptotic BAX gene was found to be increased in tissues of odontogenic cysts as compared to adjacent gingival healthy tissue of patients (Fig 3). Also, BCL2 gene expression levels were found to

be reduced in issues of odontogenic cysts as compared to adjacent gingival healthy tissue of patients (Fig 3). However, changes in the expression levels of BAX and BCL2 were statistically insignificant ($p>0.05$).

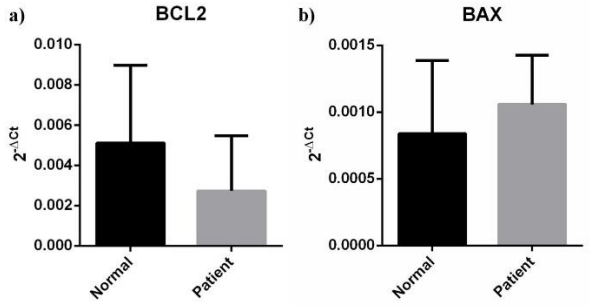

Fig 3. Quantitative expression levels of BCL2 and BAX genes in tissues of odontogenic cysts as compared to adjacent gingival healthy tissue of patients.

DISCUSSION

Increasing mass of evidence indicate that several proteins participated in the formation and progression of tumors are also deregulated in odontogenic cysts. Previous work by Kaczmarzyk *et al.*[14] analysed immunoexpression of PCNA, P53, COX-2, and BCL-2 and reported that these proteins are involved in the physiopathology of odontogenic keratocyst and expression of these proteins were associated with the radiographic evidence of cortical perforation and age of patients. Fatemeh *et al.*[12] also reported significant differential expression of p53 protein in odontogenic cysts. In particular, they established that p53 is highly positive in inflamed odontogenic keratocyst in contrast to non-inflamed odontogenic keratocyst and p53 expression highly decreased in dental follicles.

Furthermore, in a separate study, differential up-regulation of HIF-1α and CASPASE-3 by determined by immunohistochemistry. Particularly, expression of HIF-1α was higher in radicular cyst, dentigerous cyst, and ameloblastoma as compared to dental follicle tissues.[17] These findings strongly suggest the contribution of HIF-1α and CASPASE-3 in the formation of odontogenic cysts and tumors. Moreover, Gadbail *et al*.[15] also demonstrated the role of Ki-67 and p53 in odontogenic keratocyst and ameloblastoma and found that both Ki-67 and p53 expressions are elevated in ameloblastoma as compared to odontogenic keratocyst. Also, both Ki-67 and p53 expressions were advanced in odontogenic keratocyst than dentigerous cyst, suggesting Ki-67 and p53 protein expression might be useful prognostic biomarker in odontogenic lesions. Sreedhar *et al.*[18] also established the expression status of PCNA and BCL-2 proteins in odontogenic keratocyst and dentigerous cyst. Particularly, they showed that while PCNA was markedly increased, Bcl-2 was significantly markedly in odontogenic keratocyst and dentigerous cyst.[18] In a different study comprising p53, Bcl-2 and Bax proteins, while expression of p53 and Bcl-2 was found to be positively correlated, expression of p53 and Bax protein was found to be negatively correlated.[19]

In line with these findings, in tissues of odontogenic cysts, we also identified significant genetic alterations in genes significantly involved in the formation and progression of human cancers. As we all know, loss of tumor suppressive activity is the initial step in formation of tumors and deregulation of these two has been muchly reported in several types of neoplastic growths and cancers.[20] TP53 has been known as "the guardian of genome" because it is activated in harsh conditions, especially in case of DNA damage.[20] Once the TP53 is activated, it temporally arrests cell cycle at the G1 phase to allow DNA repair if the DNA damage is reversible. Alternatively, if the damage is irreversible TP53 forces cells to die by apoptosis.[21] In our study, expression levels of both RB1 and TP53 tumor suppressor genes were found to be significantly reduced in tissues of odontogenic cysts. Our results are also consistent with the previous immunohistochemistry studies. Thus, these findings suggest that these two genes might have important roles in the formation of these cysts and might be reliable biomarkers in characterization of these cysts.

Additionally, deregulation of apoptotic mechanisms is a chief contributor of the development of various types of disease ranging from cancer to neurological disorders and might have significant influence in developing therapeutic strategy.[22] Particularly, growing evidence suggests that tumor cells are extremely

resistant to apoptotic cell death which eliminates damaged cells.[22] Resistant to apoptosis emerges as a result of differential overexpression of anti-apoptotic genes such as Bcl-2 and IAP (inhibitors of apoptosis proteins) family of proteins or reduced expression of pro-apoptotic members such as Bid and Bax.[22,23] Similar to tumors, odontogenic cysts can also acquire resistance programmed cell death by deregulation of pro-apoptotic and anti-apoptotic protein members as we mentioned above. Accordingly, we also evaluated the gene expression levels of pro-apoptotic and anti-apoptotic members of the programmed cell death pathway as well as protein involved in the initiation (caspase-9) and execution (caspase-3) phases of apoptosis. As a result, we recognized that while gene expression levels of anti-apoptotic XIAP was significantly increased in odontogenic cysts, expression levels of apoptosis initiator caspase-9 and executioner caspase-3 was found to be significantly decreased in odontogenic cysts as compared to adjacent gingival healthy tissue of patients. Our findings are also consistent with previous findings about caspase-3 protein expression in immunohistochemistry. These findings supports the notion that apoptosis pathway is markedly deregulated in the formation and progression of odontogenic cysts.

Taken together, odontogenic cysts seems to be acquire molecular changes acquired by tumors by downregulation of tumor suppressor and pro-apoptotic proteins and up-regulation of anti-apoptotic proteins. Here, we identified significant differential expression of genes involved in the formation and progression of tumors.

CONCLUSIONS

In conclusion, the findings of the present study indicates that RB1, TP53, XIAP, CASP3 and CASP9 might have significant impact in formation odontogenic cysts and responsible for the increased cell proliferation in these tissues. On the other hand, there are several limitations of our study. The most important one is the small sample size. In the upcoming studies, it is of great interest to determine the role of these genes in larger study populations. Also, comparing the gene expression results between types of odontogenic cysts is of great interest. Further in vitro studies might also be conducted to demonstrate the functional roles of these genes in the cysts development. Lastly, identifying microRNA and long non-coding RNA regulators of these genes is of great interest to illuminate exact mechanism of pathogenesis.

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None

CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

Odontojenik Kistlerde Kanser İlişkili Genlerin Düzensiz Ekspresyonu

ÖZ

Amaç: *Bu çalışmanın amacı odontojenik kistlerde RB1, TP53, XIAP, BCL2 AIFM3, BAX, CASP3 ve CASP9 genlerinin ayırt edici ekspresyonlarının ortaya konulmasıdır.* ***Gereç ve Yöntemler:*** *Bu çalışmaya odontojenik kist tanısı konulan toplam 15 hasta dahil edilmiştir. Gen ekspresyonu analizi için cerrahi operasyon sırasında hastaların kist ve bitişik gingival sağlıklı dokuları toplanmıştır. RB1, TP53, XIAP, BCL2 AIFM3, BAX, CASP3 ve CASP9 genlerinin kantitatif ekspresyon seviyelerinin belirlenmesi amacıyla real-time PCR yöntemi kullanılmıştır. Gen ekspresyon seviyelerinin optimize edilmesi amacıyla GAPDH referans geni kullanıldı.* ***Bulgular:*** *Hem RB1 hem de TP53 genlerinin ekspresyonu, sağlıklı dokularla karşılaştırıldığında odontojenik kist dokularında belirgin bir şekilde azalmıştır ($p<0,05$). Ayrıca, odontojenik kist dokularında CASP3 ve CASP9 genlerinde sağlıklı dokularla karşılaştırıldığında istatistiksel olarak anlamlı düşüş saptanmıştır ($p<0,05$). Buna karşılık, XIAP ekspresyonunun anlamlı olarak arttığı saptanmıştır ($p<0,05$). Her ne kadar BCL2, AIFM3 ve BAX genleri de odontojenik kist dokularında ayırıcı bir şekilde eksprese edilmiş olsa da, bu değişikliklerin istatistiksel olarak anlamsız olduğu saptanmıştır ($p>0,05$).* ***Sonuçlar:*** *Bu çalışmanın sonuçları, RB1, TP53, XIAP, CASP3 ve CASP9 genlerinin, odontojenik kistlerin oluşumunda önemli rol oynayabileceğini ve bu dokulardaki artan hücre çoğalmasından sorumlu olabileceğini göstermektedir.* ***Anahtar kelimeler:*** *Gen ekspresyonu, odontojenik kistler, tümör baskılayıcı genler.*

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Original research

THE DIAMETER AND LENGTH PROPERTIES OF SINGLE POSTERIOR DENTAL IMPLANTS: A RETROSPECTIVE STUDY

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ABSTRACT

Objectives: The aim of this study was to evaluate the length and diameter properties of single dental implants that posteriorly placed on the mandible and maxilla.

Materials and Methods: Two hundred ninety three posterior single dental implants were evaluated in this retrospective study by same surgical procedure from 2010 to 2016 years. Demographics of patients, anatomic localizations, implant characteristics (length and diameter), satisfaction of the patient and implant loss were recorded. Implants that placed only single in posterior defect site (premolar or molar) with limited by a natural tooth or a prosthetic restored tooth on the either side of edentulous region were included. Visual analogue scale (VAS) was used for the satisfaction of the patients. The descriptive statistical analysis were done.

Results: A total of 275 patients with 293 dental implants (139 male and 136 female), ranging from 18 to 72 years (42.13 mean years) were analyzed. The majority of the dental implants were inserted mandible (156, 53.3%), 137 in maxilla (137, 46.7%). The first molar region was the most implantation area, inserting with 181 (61.9%) implants, of which 115 (39.3%) were in mandible, 66 (22.6%) in maxilla. The most frequent implant diameter placed was the 4 millimeter (mm) (54, 18.4%) and 12 mm (94, 32%) was the most frequent used implant length. Nine implants were failed and all success rate was found to be 97%.

Conclusions: According to these results, single dental implants in the posterior region can be used safely with high success rates.

Keywords: Mandible, maxilla, dental implant, posterior.

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INTRODUCTION

Dental implants have been used to the reconstruct the aesthetic and functional demand of individuals since 1960s. Oral dental implants have been increasingly preferred in replacing single missing teeth according to the traditional fixed dentures.[1] The priority success indicator of implants is osseointegration described as; direct structural and functional connection between living bone and loading implant.[2] Primer stabilization, quality and quantity of jaw bones, implant design, implant surface texture, surgical procedures, aesthetic and function of prosthesis, radiographic bone loss and patient pleasure must be taken into attention as the success criteria of implants success. Also the success and survey of dental implants are related to the clinical and radiographical examinations with description of risk factors before being implantation and follow up after implantation.[3-5]

The survey of dental implants can be evaluated in the two phases as an osseointegration and loading. Failure in the second phase is associated primarily with bone loss around the implant neck. Bone resorption in the neck area is associated with force distribution in that area. There are many factors effecting the distribution of stress including implant position, occlusion, masticatory forces, number of implant, primer stabilization and reasons related to prosthesis.[6]

Implant diameter is the one of the factors effecting the stress distribution because the wider area in the cervical portion of the implant may better distribute the masticatory forces.[7] Increasing the success of posterior implants is related to increased surface area therefore wide-diameter and longer length implants have been suggested in the posterior region.[8] On the contrary, several research in the literature have been advocated that short implants between 6-10 mm with appropriate conditions produce well results.[9,10]

The aim of this study was to evaluate the characteristic features of the implant length/diameter, distribution of the single posterior implants based on anatomic area, implant survival and patient satisfaction.

MATERIAL AND METHODS

Ethical approval was obtained from the Ethics Committee of Sanko University, Gaziantep, Turkey (June 11, 2018; session: 2018/07, decision no: 03).

This retrospective study were conducted on two hundred seventy five patients who had attended to the Faculty of Dentistry, Gaziantep University and rehabilitated by the single dental implants between the 2010 January and 2016 December. Only single implants those had placed in the posterior regions (premolar or molar) of the mandible or maxilla were included. All patients were assessed by age, gender, characteristic of implants (diameter and length), follow-up, failure of implant and patient satisfaction. All of the examined implants had either a natural tooth or a prosthetic restored tooth on either side of the edentulous site.

A total of 293 dental implants were evaluated. Different 11 brand of implants follow as Straumann (Straumann Institute, Waldenburg, Switzerland), Bredent (Bredent medical GmbH & Co.KG, Senden, Germany), Biotech (Biotech Dental, Salon de Provence, France), Zimmer Dental (Carlsbad, CA, USA), Biohorizons (Maestro Dental Implants, Birmingham, AL, USA), Mis® Seven (MIS®, Medical implants System, Israel), NucleOSS, (Şanlılar Tibbi Cihazlar Medikal Kimya San Tic Ltd. Sti, İzmir, Turkey), Implance (AGS Medikal Merkez, Kadıköy, İstanbul), BIOMET 3i, Palm Beach Gardens, FL, USA), Implantium implants (Dentium Co., Seoul, Korea), DIO Implant, Busan, Republic of Korea were performed under local anesthesia with the same surgical procedure.

The patient's satisfaction level were assessed with a 10-point visual analog scale anchored by the verbal descriptors "no satisfaction" (point 0) and "very severe satisfaction" (point 10).

RESULTS

Present study was consisted of 275 patients who had 293 posterior single dental implants placed, of whom 136 (49.4%) were female and 139 (50.6%) were male. The mean age of the patients was 42.13±12.53, ranging from 18 to 72 years. Two hundred seventy five patients had one implant-supported single crown, 16 patients had two single implant and one patient had three single implant (Table 1). The mean VAS value in all patients was 6.94±1.73 to assess the patient satisfaction level.

Table 1. Subject demographics

Subject demographics	Participating patients
Gender, n (%)	139 (50.6)
Male	136 (49.4)
Female	
Age	42.13 ± 12.53
Mean ± SD	(18-72 years)
VAS (0 - 10)	6.94 ± 1.73
Mean ± SD	
Number of implants, n (%)	
1	275 (93.9)
2	16 (5.8)
3	1 (0.3)

According to localization of the jaws, the majority of the dental implants were inserted mandible (156, 53.3%), 137 implants in maxilla (137, 46.7%). The first molar region was the most implantation edentulous area, inserting with 181 (61.9%) implants, of which 115 (39.3%) were in mandible, 66 (22.6%) in maxilla. Eighty seven (29.7%) implants were placed in the premolar region, consisting of maxilla (25, 8.6%) and mandible (62, 21.1%). Twenty-five (8.4%) implants were inserted in the second molar area in both jaw. The distributions of the implants based on anatomic locations were shown in the Table 2.

When evaluating diameters and lengths of the dental implants, the most frequent implant diameter placed was the 4 milimeter (mm) (54, 18.4%), followed by 4.5 mm (42, 14.4%) and 4.8 mm (41, 13.9%). In 293 implants, about 84.4% (247) implants were ranged from 4 to 6 mm in diameter. Other implants had in diameter between 3 and 3.9 mm (Table 3).

Table 2. Distribution of the dental implants based on anatomic location.

	Premolar region (n/%)	First molar region (n/%)	Second molar region (n/%)	Total
Maxilla	62 (21.1)	66 (22.6)	9 (3)	137 (46.7)
Mandible	25 (8.6)	115 (39.3)	16 (5.4)	156 (53.3)
Total	87 (29.7)	181 (61.9)	25 (8.4)	

Table 3. Distribution of implants according to diameters.

	N	%		N	%
Diameter			**Diameter**		
3 mm	1	0.3	4 mm	54	18.4
3.25 mm	1	0.3	4.1 mm	35	12.2
3.3 mm	11	3.7	4.2 mm	13	4.5
3.4 mm	1	0.3	4.3 mm	2	0.7
3.5 mm	12	4	4.4 mm	3	1
3.6 mm	4	1.4	4.5 mm	42	14.4
3.7 mm	7	2.5	4.6 mm	12	4
3.75 mm	3	1	4.7 mm	18	6.1
3.8 mm	3	1	4.8 mm	41	13.9
3.9 mm	6	2	5 mm	8	2.7
			5.4 mm	2	0.7
			5.5 mm	10	3.6
			5.8 mm	1	0.3
			6 mm	3	1
3-3.9 mm	46	15.6	4-6 mm	247	84.4

The length in 12 mm (94, 32%) was the most frequent used implant size in the implantation, followed by 70 (23.9%) cases were in 14 mm and 56 (19.1%) were in 10 mm. The majority of the cases (220, 75.1%) were in length, ranging from 10,5 and 16 mm (Table 4).

Table 4. Distribution of the implants according to length.

	N	%		N	%
Length			**Length**		
8 mm	16	5.5	10.5mm	4	1.4
9 mm	1	0.3	11.5mm	26	8.9
10 mm	56	19.1	12 mm	94	32
			13 mm	21	7.2
			14 mm	70	23.9
			15 mm	1	0.3
			16 mm	4	1.4
8-10 mm	73	24.9	10.5-16mm	220	75.1

With regards to implant survival, nine (3%) implants were not osseointegrated in all implants. Five of them were located in the mandible and four in the maxilla. All survival rate in 293 dental implants was 97%.

Of these 9 failed implant, the diameter of eight implants were above 4mm and the success rate was 96.8% in implants that placed in diameter between 4 and 6 mm (8/247). Forty six implant were in diameter between 3 and 3.9 mm and only one implant failed. The success rate was found to be 97.9% in this group. The characteristics of failed implant were shown in Table 5.

Table 5. The characteristics of failed implants

Failed implants characteristics	**N**	**(%)**
Mandible	5/156	(3.2)
Maxilla	4/137	(2.9)
Total	9/293	(3)
Diameter		
3 – 3.9 mm	1/46	(2.1)
4 – 6 mm	8/247	(3.2)

DISCUSSION

The dental implant-supported prostheses, which are applied in a single posterior tooth deficiency, have become highly preferred in recent years with high success rates by patients instead of traditional fixed or removable prostheses. In a recent systematic review, Tan *et al.*[11] and Jung *et al.*[12] have reported the success rates of single-tooth implant supported fixed dentures in 10-year follow-up series as 84.4% and 89.1%, respectively. In some studies, it has also been reported that the diameter and length of the implant affect the survival of the implants.[13,14]

The requirement of dental implant following tooth loss is also correlated with age. Villarinho *et al*.[15] and Kim *et al*.[16] reported that the mean age was 52 and 48 years, respectively in their series that inserted posterior single implants. The mean age of the patients included in our study was 42.13 years, ranging from 18 to 72 years. In this study, it was found that the most preferred implant sizes were between 4 and 6 mm (247/293, 84.4%) in diameter and between 10,5 and 16 mm (220/293, 75.1%) in length. Of these implant size, the 4 mm (54/293, 18.4%) diameter and 12 mm (94/293, 32%) length were the most commonly used implant sizes. It has been known that the implant size may be limited by several anatomic factors, particularly the procedures on posterior dental implant placement. In maxilla, the occurrence of sinus pneumatization after tooth loss and resorption in the alveolar crest causes advanced surgical procedures such as sinus floor elevation for bone augmentation or enhance the volume of the alveolar bone with bone substitutes. The limited availability of vertical alveolar bone in the mandible impairs implant treatment without any reconstructive bone surgery.[17]

In the posterior area in partially edentulous subjects, decreased alveolar bone height from the

ridge to the inferior alveolar nerve are effective in determining the length of dental implant. In addition to this, Lee *et al.*[18] reported that there is no linear relationship between the implant length and implant survival. Although the implants less than 10 mm in length are associated with high implant failure after loading[19], it is known that the use of short implants has significant clinical advantages such as minimal overheating during drilling, minimized inferior alveolar nerve injury, maxillary sinus invasion and augmentation procedures for increasing vertical bone.[20]

Although it has been generally known that the mandibular implants have higher success rate than maxilla[21,22], different results were reported by some studies. Mezzomo *et al.*[23] found to be a higher failure rate in the mandible that placed with short implants. But Monje *et al.*[24] reported that there is no difference in failure rates for the arch. In this study, a higher number of implants were inserted in the first molar area in the mandible (115, 39.3%) than maxilla (66, 22.6%). Similar to this result, 53.3% of all implants were placed in the posterior mandible. Of the 9 failed implants, 5 were placed in the mandibular molar region, 2 were placed in the maxillary first molar and 2 were in the maxillary premolar region. Although aforementioned studies have addressed the failure differences between mandible and maxilla in the short dental implants in the posterior, we found to be a higher failure rate in the mandible (5/156, 3.2%) versus maxilla (4/137, 2.9%).

It is known that the implant diameter has important clinical effect on the stress distribution in the cortical plates.[7,25] In recent systematic review by Javed and Romanos[5], they suggested that the critical factors such as surgical protocol, primary stability of implant during surgery or oral hygiene maintenance in the postsurgical period are more important than the implant diameter on the long-term survival of dental implants inserted in the posterior maxilla. When compared the narrow dental implants with wide-diameter implants, wide-diameter implants have better initial stability with increasing the surface area for osteointegration.[26,27] Recent studies demonstrated that the narrow diameter implants are as reliable and predictable as with standard-diameter dental implants.[28,29] But narrow-diameter implants still have a high rate of prosthetic complications.[28] In present study, the majority of implant diameter used in this study was ranged from 4 to 6 mm (247/293, 84.4%). Of these 247 implants, eight implants were failed. The success rate was found to be 96.8% in this group.

Although there are some limitations, it was aimed to present the demographic and characteristics of single dental implants placed in the posterior area in this present study. According to this result, the most common implantation area was the mandibular first molar area. The 4 mm in diameter and 12 mm in length were the most used implant sizes. Single dental implants in the posterior region can be used safely with high success rates.

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None

CONFLICT OF INTEREST STATEMENT

None

Tek Diş Posterior Dental İmplantların Çap ve Boy Özellikleri: Retrospektif Bir Çalışma

ÖZ

Amaç: *Bu çalışmanın amacı, posterior maksilla ve mandibulaya yerleştirilen tek diş implantların çap ve boy özelliklerini değerlendirmektir.* ***Gereç ve Yöntemler:*** *2010 ile 1016 yılları arasında aynı cerrahi yaklaşım ile yapılan 293 posterior tek diş implant değerlendirildi. Hastalara ait demografik bilgiler, implantların boy ve çap özellikleri, implantların yerleştirildiği anatomik lokalizasyonlar ile hasta memnuniyeti kaydedildi. Posterior bölgeye yapılan tek diş implantların her iki yanında doğal veya protetik olarak restore edilen dişlerin olduğu implantlar çalışmaya dahil edildi. Hasta memnuniyeti vizüel analog skala (VAS) ile değerlendirildi. Değerlendirmede tanımlayıcı istatistik uygulandı.* ***Bulgular:*** *Yaşları 18 ile 72 arasında değişen (yaş ortalaması 42,13) 275 hastanın 139'u erkek, 136'sı kadın olup toplamda 293 dental implant çalışmada değerlendirildi. İmplantların çoğunluğu mandibular (156, %53,3) yerleşimliyken 137 (%46,7) implant maksilladaydı. Dişsiz birinci molar (181, %61,9) en fazla implant uygulanan bölge iken bunların 115'i*

(%39,3) mandibulada, 66'sı (%22,6) da maksilladaydı. En fazla uygulanan implant çapı 4 milimetre (mm) (54, %18,4) ve boyu da 12 mm idi. Kayıp 9 implant olup implant başarı oranı %97 olarak bulundu. ***Sonuç:*** *Çalışma sonuçlarına göre, tek diş implantlar yüksek başarı oranları sayesinde posterior bölgede güvenle kullanılabilir.* ***Anahtar Kelimeler:*** *Mandibula, maksilla, dental implant, posterior.*

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Original research

COMPARISON OF ORAL HYGIENE ATTITUDES, AWARENESS AND PERIODONTAL PARAMETERS OF UNDERGRADUATE DENTAL STUDENTS

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ABSTRACT

Objectives: The aim of this study was to compare the differences in self-reported oral health attitudes and clinical measurements between undergraduate dental students who enrolled in or not enrolled in periodontology course in Turkey.

Materials and Methods: A total of 701 students were included in the study. Students asked 26 questions including age, gender, smoking, oral hygiene habits and self-assessment measures for periodontal status. Probing depth (PD) clinical attachment level (CAL), presence of bleeding on probing (BOP), plaque index (PI) and gingival index (GI) measurements performed. The Chi-square test was used for categorical data and one way Anova post hoc Tukey test for ordinal level data.

Results: There were no differences between grades in term of PD and CAL ($p>0.05$). Statistically significant difference was in BOP value between 1st and 5th grade ($p<0.05$). PI values of 1st grade were statistically higher than 3rd, 4th and 5th grades ($p<0.05$). GI of 1st grade was statistically higher than 4th and 5th grades ($p<0.05$). GI of 2nd grade was statistically higher than 3rd, 4th and 5th grades ($p<0.05$). Tooth brushing was not different between grades ($p>0.05$). Interdental care ratios in grades significantly differ from each other ($p<0.05$). According to students, they did not have any kind of periodontal disease with high percentages above 88%. 4th and 5th grades had periodontal treatment comparing the other grades ($p<0.05$).

Conclusions: Starting to take periodontology course in dental faculties from the first year and constituting a periodontal disease prevention program will be beneficial to students in Turkey.

Keywords: Dental education, dental health surveys, periodontal disease, self-report.

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INTRODUCTION

Periodontal diseases are affecting 50-90% of the adult population in the world.[1] Although they are not life threatening, they can affect the quality of life of the patient.[2] The first symptom of periodontal disease is gingival bleeding[3] and the development process of the disease is usually painless unless the root surface is exposed. Clinical-based measurements are standard and also preferred approaches to diagnose periodontal diseases. However, measures derived from responses to self-reported questions included in interview-based surveys of other chronic diseases and conditions such as dietary intake, diabetes, and pain have been successful in producing viable public health data.[4] In 2003, the Center for Disease Control and Prevention launched a Periodontal Disease Surveillance Initiative, in collaboration with the American Academy of Periodontology (AAP), which evaluated many other targets and potential use of personal reporting measures for surveillance of periodontitis.[5]

In some studies comparing periodontal parameters with self-reporting, there was a good agreement between the clinical examination and the opinions of the participants[6,7], but some of them did not show such compatibility.[8] Many studies have assessed self-reported measurements of oral health and periodontal disease, but only a few specific questions were evaluated and the results differ between measurements and populations. There may be differences in the effectiveness of clinic measures, cultural differences, changes in access to dentist/specialist, or differences in periodontal care standards.[4]

Oral health education starts in the family, this predict the actual oral health status, but it can be insufficient in some cases. To control oral diseases, a number of developing countries have recently launched school-based oral health education (OHE) and preventive programs aimed at improving oral health behaviors and the state of the child population. In addition, written and visual media, also dentists, can affect oral health status of people.

Dental students who are dentists of the future take their periodontology course in the 3rd grade. 1st and 2nd grade continues as preclinical. The oral hygiene behaviors of the students up to 3rd grade are independent from the Faculty of Dentistry. The attitudes of dental students towards their oral health affect the oral health habits and have a possible effect on the improvement of the oral health of their patients.[9] The aim of this study was to compare the differences in self-reported oral health attitudes with large number of questions, and clinical measurements between preclinical and clinical dental students in Turkey.

Study population and methodology

Seven hundred one students from all five academic years of Cumhuriyet University and Pamukkale University were included in the study. All dental students who agreed to complete the questionnaire were included in the study. The study was carried out in accordance with the Declaration of Helsinki and approved by Pamukkale University Ethics Committee of Noninvasive Clinical Research (Date: 05/03/2019; No: 05). All participants were signed informed consent. The self-report questionnaire created following the screening of relevant literature and existing self-report measures. Students asked 26 questions including age, gender, also demographic questions about smoking and systemic status, oral hygiene habits and self-assessment measures for periodontal status. The questionnaire form completed by the researcher by asking and explaining the student face to face.

After filling the questionnaire, plaque index (PI)[10] and gingival index (GI)[11] were obtained from all the students. The whole mouth clinical periodontal examination included measurement of probing depth (PD) that measured, clinical attachment level (CAL), presence of bleeding on probing (BOP) performed using a Williams periodontal probe (Hu-Friedy, Chicago, IL) at 6 sites per tooth for whole mouth. Based on clinical diagnostic criteria proposed by 1999 International Workshop for a Classification of Periodontal Diseases and Conditions[12], the students were categorized into periodontal diseases.

Statistical analysis

The SPSS version 21.0 used for performing statistical analyses. The Chi-square test used for categorical data. The normality of data was analyzed using Shapiro-Wilk test and one way Anova post hoc Tukey test for periodontal parameters. The data presented as mean ± standard deviation or percentage. The significance level was taken as p <0.05.

RESULTS

Descriptive data of students represented in Table 1. A total 701 students were included in the study. Age ranges of 1st, 2nd and 3rd grade students were found to be significantly lower than 4th and 5th grade students respectively (p<0.05). There was no difference between grades in terms of gender (p>0.05). There was no difference in smoking ratio between grades (p>0.05).

Table 1. Demographic variables of students

		1st Grade n (%)	2nd Grade n (%)	3rd Grade n (%)	4th Grade n (%)	5th Grade n (%)	P value
Age	***17-24 years***	206(98.6)	188(98.9)	165(100)	64(91.4)	56(83.6)	0.001
	25-30 years	3(1.4)	2(1.1)	0(0)	6(8.6)	11(16.4)	
Gender	***Female***	128(61.2)	120(63.2)	100(60.6)	44(62.9)	45(67.2)	NS
	Male	81(38.8)	70(36.8)	65(39.4)	26(37.1)	22(32.8)	
Smoking Status	***Yes***	45(21.5)	32(16.8)	37(22.4)	18(25.7)	16(23.9)	
	No	158(75.6)	152(80)	118(71.5)	49(70)	49(73.1)	NS
	Quit	6(2.9)	6(3.2)	10(6.1)	3(4.3)	2(3)	

Periodontal parameters

According to periodontal parameters of students there were no differences between grades in term of PD (p>0.05). When comparing CAL values, all grades diagnosed as slight periodontitis but there was no difference between the grades (p>0.05). Although BOP values were similar in all classes, there was a statistically significant difference in BOP value between 1st and 5th grade (p<0.05). PI index values of 1st grade were statistically higher than those of 3rd, 4th and 5th grades (p<0.05) but not 2nd grades (p>0.05) also values of 3rd were statistically higher than 4th and 5th grades (p<0.05). GI of 1st grade was statistically higher than 4th and 5th grades (p<0.05) but not 2nd grades (p>0.05). GI that belongs to 2nd grade was statistically higher than those of 3rd, 4th and 5th grades (p<0.05). There was not a difference between 3rd, 4th and 5th grades in term of GI values (p>0.05). (Table 2)

Table 2. Periodontal parameters of grades

	1st Grade Mean±SD	2nd Grade Mean±SD	3rd Grade Mean±SD	4th Grade Mean±SD	5th Grade Mean±SD	P value
PD	2.045±0.64	2.715±8.65	2.029±0.67	2.080±0.70	2.007±0.63	**0.546**
GI	1.065±0.55ǂ	1.106±0.52#	0.942±0.45	0.754±0.42	0.801±0.43	**0.001**
PI	2.052±0.50*	2.071±0.51**	1.208±0.59***	0.843±0.50	0.816±0.41	**0.001**
BOP	56.08±24.6†	52.23±27.1	51.70±27.7	50.12±27.7	45.08±25.0	**0.039**
CAL	2.051±0.64	2.728±0.64	2.064±0.68	2.180±0.78	2.080±0.72	**0.546**

ǂ p<0.05, 1st vs 4th and 5th grades; # p<0.05, 2nd vs 3rd, 4th and 5th grades; *p<0.05, 1st vs 3rd , 4th and 5th grades; **p<0.05, 2nd vs 3rd, 4th and 5th grades; ***p<0.05, 3rd vs 4th and 5th grades; †p<0.05 1st vs 5th grade

Results of the questions in Table 3

When the answers to the questions asked for the evaluation of the oral hygiene habits of the students, the question answers about tooth brushing were not different between grades (p>0.05). Students in all grades preferred manual toothbrush more to other methods (p<0.05). There was a statistically significant difference between the frequency of tooth brushing in grades (p<0.05). "2-3min brushing" percentages of 4th and 5th grades were higher than the other grades. Interdental care ratios in grades significantly differ from each other (p<0.05). 3rd, 4th and 5th grade students showed high percentages comparing 1st and 2nd grades. 1st, 2nd, 3rd and 5th grades, preferred dental floss in order 48.6%, 73.6%, 64.4%, 80% but 4th grades preferred interdental brush with a 61.4% ratio. Interdental cleaning frequencies were higher in 4th and 5th

grades ($p<0.05$) comparing the other grades. Tongue cleaning was higher in 4th and 5th grades ($p<0.05$) comparing the other grades but mouthwash usage was not different between grades ($p>0.05$). (Table 3)

Table 3. Questions on the determination of oral hygiene habits

	1st Grade n (%)	2nd Grade n (%)	3rd Grade n (%)	4th Grade n (%)	5th Grade n (%)	P value
1-Do you brush your teeth?						
Yes	206(98.6)	188(99.5)	163(98.8)	70(100)	67(100)	**NS**
No	3(1.4)	2(0.5)	2(1.2)	0(0)	0(0)	
2-How many times do you brush your teeth?						
1 time a day	47(22.5)	36(18.9)	27(16.4)	9(12.9)	8(11.9)	
Twice a day	135(64.6)	112(58.9)	109(66.1)	51(72.9)	47(70.1)	
3 times a day	22(10.5)	33(17.4)	24(14.5)	9(12.9)	9(13.4)	**NS**
Seldom	5(2.4)	8(4.2)	5(3)	1(1.4)	3(4.5)	
3-Which product do you prefer for brushing?						
Tootbrush	193(92.3)	175(92.1)	147(89.1)	67(95.7)	63(94)	
Electric toothbrush	14(6.7)	10(5.3)	13(7.9)	3(4.3)	2(3)	
Misvak	1(0.5)	5(2.6)	3(1.8)		2(3)	**NS**
Other	1(0.5)		2(1.2)			
4-Time of tooth brushing						
<1 min	111(53.1)	54(28.6)	75(46.3)	27(38.6)	20(29.9)	
=1 min	90(44)	92(48.7)	60(37.2)	19(27.1)	17(25.4)	
2-3 min	4(2.4)	22(12.7)	19(11.6)	17(24.3)	22(32.8)	**0.001**
>4 min	1(0.5)	19(10.1)	8(4.9)	7(10)	8(11.9)	
5-Do you perform interdental care?						
Yes	70(33.5)	59(31.1)	111(67.3)	51(72.9)	53(79.1)	
No	139(66.5)	131(68.9)	54(32.7)	19(27.1)	14(20.9)	**0.001**
6-Which product do you prefer for interdental cleaning?						
Interdental brush	9(12.5)	11(16.4)	24(20.3)	43(61.4)	6(10.9)	
Dental floss	35(48.6)	49(73.1)	76(64.4)	5(7.1)	44(80)	
Wooden toothpick	28(38.9)	7(10.4)	17(14.4)	5(7.1)	4(7.3)	**0.001**
Water jet			1(0.8)	1(1.4)	1(1.8)	
7-Interdental cleaning frequency						
Everday	10(13.7)	14(20.9)	35(29.7)	21(38.9)	27(49.1)	
Less than a week	13(17.8)	4(6)	16(13.6)	8(14.8)	10(18.2)	
Sometimes		48(71.6)	67(56.8)	25(46.3)	18(32.7)	**0.020**
Seldom	50(68.5)	1(1.5)				
8-Do you clean your tongue?						
Yes	124(59.3)	130(68.4)	101(61.2)	52(74.3)	50(74.6)	
No	85(40.7)	59(31.6)	64(38.8)	18(25.7)	17(25.4)	**0.001**
9-Do you use mouth wash?						
Yes	49(23.4)	63(32.8)	55(32.2)	19(27.1)	17(25.4)	**NS**
No	160(76.6)	127(67.2)	110(67.8)	51(72.9)	50(74.6)	

Results of the questions in Table 4

The answers to questions about oral health and periodontal awareness of students presented in Table 4. Nearly half of the students in all classes defined their gingival health as "good". 3rd, 4th, and 5th grade students with similar percentages (24.8%, 24.3%, 28, and 4%) defined gingival health as "very good". 1st and 2nd grade students defined gingival health as bad with 37.4% and 33.2% respectively. According to students, they did not have any kind of periodontal disease with high percentages above 88% percentages. 4th and 5th grades had periodontal treatment comparing the other grades ($p<0.05$). In terms of both the periodontal pocket question and the oral malodor question, students gave "no" answers with high percentages ($p>0.05$). 1st grade students had pain and swelling on their gums with the highest percentage 31.6%, 5th grade student answered the question the least percentage 6% ($p<0.05$). Less gingival bleeding was reported from 4th and 5th grades comparing the others ($p<0.05$). In addition, less abscess was reported by 3rd, 4th and 5th grades comparing the others ($p<0.05$).

Table 4. Questions about oral health and periodontal awareness of students

	1st Grade n (%)	2nd Grade n (%)	3rd Grade n (%)	4th Grade n (%)	5th Grade n (%)	P value
1-How do you evaluate your gum health?						
Excellent	3(1.4)	12(6.3)	6(3.6)	7(10)	6(9)	
Very Good	28(13.4)	26(13.7)	41(24.8)	17(24.3)	19(28.4)	
Good	95(45.5)	87(45.8)	65(39.4)	35(50)	34(50.7)	
Bad	79(37.4)	63(33.2)	47(28.5)	11(15.7)	8(11.9)	**0.001**
Very Bad	4(1.9)	2(1.1)	6(3.6)	0(0)	0(0)	
2-Do you or did you have periodontitis or any kind of periodontal disease?						
Yes	20(10)	22(11.5)	18(10.7)	7(10.5)	6(11)	**NS**
No	189(90)	166(88.5)	147(89.3)	57(89.5)	50(99)	
3-Has your dentist ever told you that you had periodontitis or periodontal disease?						
Yes	36(17.2)	39(20.5)	36(21.8)	15(21.4)	13(19.4)	
No	173(82.8)	151(79.5)	129(78.2)	55(78.6)	54(80.6)	**NS**
4-Have you ever had periodontal treatment?						
Yes	18(8.6)	21(11.1)	31(18.8)	26(37.1)	24(35.8)	
No	191(91.4)	169(88.9)	134(81.2)	44(62.9)	43(64.2)	**0.001**
5-Has your dentist ever told you that you had pockets or lost bone around your teeth?						
Yes	3(1.5)	5(2.7)	2(1.2)	1(1.4)	2(2.3)	
No	206(98.5)	185(97.3)	163(98.8)	69(98.6)	65(97.7)	**NS**
6-Malodor or bad taste can be caused by certain foods like onions or garlic. Independent of the consumption of such foods, do you have malodor or bad taste?						
Yes	28(13.4)	26(13.7)	21(12.7)	5(7.1)	7(10.4)	**NS**
No	181(86.6)	164(86.3)	144(87.3)	65(92.9)	60(89.6)	
7-Have you had any pain and swelling on your gums?						
Yes	66(31.6)	56(29.5)	34(20.6)	7(10)	4(6)	
No	143(68.4)	134(70.5)	131(79.4)	63(90)	63(94)	**0.001**
8-Have you ever had bleeding in your gums?						
Yes	111(53.1)	101(53.2)	69(41.8)	26(37.1)	24(35.8)	
No	98(46.9)	89(46.8)	96(58.2)	44(62.9)	43(64.2)	**0.008**
9-Have you ever had an abscess in your gums?						
Yes	53(25.4)	50(26.3)	17(10.3)	1(1.4)	3(4.5)	**0.001**
No	156(74.6)	140(73.7)	148(89.7)	69(98.6)	64(95.5)	

Results of the questions in Table 5

The answers of the questions related to periodontology awareness and oral hygiene requirements of the students shown in Table 5. 1st grade students have heard periodontology term in 77% percentage. Most of the students have learned oral hygiene necessity from their parents. 1st and 2nd grade students did not have an information about microbial dental plaque comparing the 3rd, 4th and 5th grade students ($p<0.05$). Also 1st (23.6%) and 2nd (32.1%) grade students thought that "the initial periodontal treatment can harm the teeth" ($p<0.05$). In addition to that, 1st (52.2%) and 2nd (43.7%) grade students thought that dental calculus can be eliminated by natural products with high percentages.

Table 5. Questions related to periodontology awareness and oral hygiene requirements

	1st Grade n (%)	2nd Grade n (%)	3rd Grade n (%)	4th Grade n (%)	5th Grade n (%)	P value
1-Have you heard the "Periodontology" term before?						
Yes	161(77)	171(90)	155(93.3)	70(100)	67(100)	**0.001**
No	48(23)	19(10)	10(6.1)	0(0)	0(0)	
2-Where do you learn the necessity of performing oral hygiene?						
Family	145(69.4)	118(62.1)	105(63.6)	38(54.3)	34(50.7)	
School(Primary, secondary, high)	43(20.6)	35(18.4)	24(14.5)	14(20)	17(25.4)	
Advertisements	7(3.3)	4(2.1)	3(1.8)	0(0)	0(0)	
Friends	5(2.4)	9(4.7)	0(0)	2(2.9)	2(3)	**0.001**
School of Dentistry	9(4.3)	24(12.6)	33(20)	16(22.9)	14(20.9)	
3-Do you have an information about the microbial dental plaque?						
Yes	45(21.5)	87(45.8)	129(78.2)	70(100)	67(100)	**0.001**
No	164(78.5)	103(54.2)	36(21.8)	0(0)	0(0)	
4-Do you think the periodontal treatment will harm the teeth?						
Yes	55(26.3)	61(32.1)	31(18.8)	8(10.2)	2(3.2)	**0.001**
No	154(73.7)	129(67.9)	134(81.2)	62(89.8)	65(96.8)	
5-Do you think the calculus deposits can be eliminated by naturally or natural products?						
Yes	109(52.2)	83(43.7)	38(23)	8(10.2)	3(5)	**0.001**
No	100()47.8	107(56.3)	127(77)	62(89.8)	64(95)	

DISCUSSION

The use of questionnaires has become a more common method for understanding oral health attitudes of people. There are many types of questionnaires used in the literature for this purpose. In this study, we developed questions about both oral hygiene status and periodontal attitude. The aim of this study is to compare the oral hygiene attitudes and knowledge about periodontology field of dental students who take or do not take periodontology course and compare them with their clinical measurements. Theoretical periodontology courses start in the first semester of the third year of five year-education and students meet patients in the second semester of the third year in Turkey. The 4th and 5th grade students actively treat the patients in the periodontology clinic. As the level of education increased, the dental health attitudes of the individuals observed to be more developed[13,14] but some studies reported the absence of an improvement in oral hygiene practices of dental students regardless of having obtained information and education.[15]

Halitosis (fetor oris, bad breath, breath malodour, oral malodour) is the general term that used to describe any unpleasant odour in expired air.[16] Oral halitosis development in younger ages could be due to tongue coating deposition.[17] 1st (13.4%), 2nd (13.7%) and 3rd (12.7%) grade students' percentages belong the oral malodor question higher than 4th (7.1%) and 5th (10.4%) grade students in accordance with their tongue cleaning percentages. These results are very below the results belong the students in Jordan (78%)[13], students in Japan and Finland[18] but higher than Sweden (2.4%). [19] Oral malodor that can occur due to many causes is also an important area of social life but it may originate from oral or non-oral sources. Although a device in this present study did not measure the level or severity of halitosis, the subjective self–reported information evaluated therefore, differences can be observed between survey studies.

Microbial dental plaque is the primary etiologic factor of periodontal disease. Therefore, brushing teeth twice a day recommended as a good procedure for dental care.[20] An adequate plaque control will result in a reduction in PI. In this study the percentage of students brushing twice a day varied between 58.9% and 72.9% and remained below the students in Lithuania (% 92)[21], India (84.6%)[22] and United Arab Emirates (86%)[14] but similar in Turkey (74%).[23] Although interdental cleaning frequency that performed everyday changed between grades, 33.5% -79.1% of students had knowledge regarding interdental aids. The percentage of interdental care in the 3rd (67.3%), 4th (72.9%) and 5th (79.1%) grades almost doubled comparing the 1st (33.5%) and 2nd (31.1%) grades. These findings were similar to India (74%), remained above United Arab Emirates (56%). [14] But these data include all interdental cleaning tools, including toothpicks and all timelines. Rates of regular interdental cleaning results remained low.

Min 13.7% of 1st grade and max 49.1% of 5th grade stated to perform interdental cleaning regularly. In a study 44.6% of clinical and 41.0% of preclinical students stated to use dental floss regularly. [21] In another research conducted in Turkey, 19% of preclinical students indicate the using dental floss regularly, while 31% of clinical students.[23] In the same study, information about microbial dental plaque of preclinical students found to be better than the clinical students.[23] In our study, it increased in direct proportion to the education that student's received and reached 100% in 4th and 5th grade. In line with the results of the questionnaire, the PI values of the 1st, 2nd and 3rd grade students were statistically higher than the 4th and 5th grade students. However, most of these studies did not compare the clinical data that had been collected only declaration of person with clinical measurement. Although, different results may be due to the differences in oral health behavior between countries. Religious and cultural beliefs, also economic factors have effect on oral hygiene behavior. For example, misvak use and toothpicks may not be found in a study in Greece or Italy. But in our study 0.5% of 1st grade, 2.6% of 2nd grade, 1.8% of 3rd grade and 3% of 5th grade students performed their plaque control using misvak.

According to GI values measured in relation to gingival inflammation, 1st and 2nd grade students revealed statistically higher values comparing than those of 4th and 5th grade students. We asked the students if they ever had bleeding, pain or swelling in their gums. 1st grade and 2nd grades had almost the same percent (53.1-53.2%), but this decreased in 3rd (41.8%), 4th (37.1%) and 5th grade (35.8%). Mongolian students had experienced gingival bleeding with 34% [24] similar to our study but Greek students stated the gingival bleeding as %17.9 percent.[25] 3.6% Lithuanian clinical students and 19% preclinical students experienced gingival bleeding.[21] Gingivitis even slight periodontitis diagnosed according to GI and CAL data and questionnaire responses of the students.

Smoking increases the periodontal destruction and reduces the chance of success of periodontal treatments.[26] Also halitosis may be present in the strong smokers' breath, and a history of smoking has been implicated in decreasing olfactory sensitivity. In Saudi Arabia, it has reported that among 13% of male and 2% of female dental students were current smokers.[27] Another study that performed in Saudi Arabia, cigarette smoking has been reported among 27.6% and 2.4 % of male and female dental students, respectively.[28] An international review that evaluated the rate of smoking among dental students and the highest rate was 47% for Greece (1-5 grades) and the lowest rate was 3% for the Canada (all years).[29] In our study, there was no difference between the groups in terms of smoking. The rate of smoking was 16.8% (2nd grade), 21.5% (1st grade), 22.4% (3rd grade), 23.9% (5th grade) and 25.7% (4th grade). Also 43.9% of female students vs 56.1% male students were current smokers as opposed in Japan (33% men versus 7% women)[30], Jordan (31% men vs 4% women)[31], India (15% men vs 2% women)[29] and Saudi Arabia (13% men vs 2% women) [32] but similar to Greece and Serbia.[29] These differences may be caused by the change in the socio-economic situation in which students live, the lack of training on the hazards of smoking and the differences in the stress levels that was experienced. However, smoking rates seem to affect the periodontal status of students. When CAL values considered, periodontitis developed in students of all grades. In addition, the students in our study stated that they did not receive periodontal treatment before with high percentages. This result may be due to inability to reach periodontal treatment or inadequate economic level.

Turkey is a large country linking the Middle East, Asia, and Europe with expansive geography, various ethnic and racial minorities. The cost of dental care with a large and growing adult population and the change in the prevalence of periodontal diseases can lead to significant economic, social and developmental effects.[33] Caries preventive approaches are currently included in the oral health promotion plan. A more comprehensive health plan for peridontal disease prevention has not been established yet. The dental specialization training law was enacted in 2011. Prior to this, a small number of students were given doctoral education in universities and

periodontologists were trained. Therefore, it can be very difficult and costly to have indances reach someone who is educated in the field of periodontology.

CONCLUSIONS

Variations in dental attitudes and behaviors of students depend on clinical training and curriculum. However, dental students can only meet in the 3rd year with the periodontology field that proven relationships with the whole body. Starting to take periodontology course in dental faculties from the first year and addition a periodontal disease prevention program to preventive oral health practice will be beneficial to students on account of their future patients in Turkey.

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CONFLICT OF INTEREST STATEMENT

None

Dişhekimliği Öğrencilerinin Ağız Hijyeni Tutumları, Farkındalıkları ve Periodontal Parametrelerinin Karşılaştırılması

ÖZ

Amaç: *Bu çalışmanın amacı, Türkiye'de periodontoloji dersi alan ve almayan dişhekimliği öğrencileri arasında, kendilerinin bildirdiği ağız sağlığı tutumları ve klinik ölçümler arasındaki farklılıkları karşılaştırmaktır.* ***Gereç ve Yöntemler:*** *Çalışmaya toplam 701 öğrenci dahil edildi. Öğrencilere yaş, cinsiyet, sigara içme, ağız hijyeni alışkanlıkları ve periodontal durum gibi öz değerlendirme soruları dahil toplam 26 soru soruldu. Sondlama derinliği (PD), klinik ataçman seviyesi (CAL), sonlamada kanama varlığı (BOP), plak indeksi (PI) ve gingival indeksi (GI) ölçümleri. Kategorik verilerde Ki-kare testi, gruplar arası karşılaştırmalarda tek yönlü varyans analizi post-hoc Tukey testi kullanıldı.* ***Bulgular:*** *PD ve CAL açısından sınıflar arasında fark bulunmadı (p>0,05). 1. ve 5. sınıf BOP değerleri arasında anlamlı farklılık bulundu (p<0,05). 1. sınıfın PI değerleri 3., 4. ve 5. sınıflarınkinden istatistiksel olarak yüksekti (p<0,05). 1. sınıfların GI değerleri, 4. ve 5. sınıflarda istatistiksel olarak daha yüksekti (p<0,05). 2. sınıfların GI değerleri, 3., 4. ve 5. sınıflarda istatistiksel olarak daha yüksekti (p<0,05). Diş fırçalama açısından sınıflar arasında farklılık bulunmadı (p>0,05). İnterdental bakım oranları sınıflar arasında birbirinden önemli ölçüde farklı bulundu (p<0,05). Öğrencilerin %88'e kendi ağızları periodontal açıdan sağlıklıydı. 4. ve 5. sınıfların periodontal tedavi olma yüzdeleri diğer sınıflarda karşılaştırıldığında daha fazla bulundu.* ***Sonuçlar:*** *Dişhekimliği fakültelerinde 1. sınıftan itibaren periodontoloji dersi verilmeye başlanması ve ülke çapında periodontal hastalıklardan korunma programı oluşturmak Türkiye'deki öğrencilere dolayısıyla onların ilerideki hastalarının yararına olacaktır.* ***Anahtar Kelimeler:*** *Dişhekimliği eğitimi, diş sağlığı anketleri, periodontal hastalık, kendini raporlama.*

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Original research

EVALUATION OF POSTOPERATIVE PAIN AFTER USING DIFFERENT FILE SYSTEMS: A RANDOMIZED CLINICAL STUDY

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ABSTRACT

Objectives: This study evaluated the effect of instrumentation techniques on the postoperative pain after single-visit root canal treatment.

Materials and Methods: Sixty patients having an indication of endodontic treatment were included. Only single rooted teeth were selected The patients were randomly divided into 2 groups. In group 1; the root canals were instrumented using ProTaper Next instruments with rotational motion, in group 2 TF Adaptive instruments with adaptive motion were used during instrumentation. Treatments were completed in a single appointment. Postoperative pain questionnaires were scored by patients using a four-point pain intensity scale for 12, 24, and 48 hours. Mann Whitney-U, Friedman and Wilcoxon tests were used for analyzing the final data.

Results: The comparison of time intervals between groups demonstrated no difference between both groups ($p>0.05$). In both groups, the postoperative pain values of 12h time period were significantly higher than both other periods, and significant difference was found between 24h and 48h time periods ($p<0.05$). The postoperative pain values of 48h time period were significantly lower than the other two time periods ($p<0.05$).

Conclusions: Both instrumentation techniques caused postoperative pain. The pain scores indicated that both techniques caused limited discomfort associated with slight pain which did not require any additional treatment and medication.

Keywords: Pain, root canal preparation, root canal treatment.

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INTRODUCTION

Postoperative pain is a common complication after root canal treatment. The reasons for such pain may include apical debris extrusion, inadequate preparation, preoperative pain, and the presence of periapical pathology.[1-3] Apical debris extrusion affects the postoperative comfort of patients without any significant impact on the outcome of treatment.[4]

Root canal shaping kinematics and file design can affect the amount of extruded debris.[5] Recently, endodontic motors using combined motions, instead of rotational and reciprocal motions solely, were used to reduce the complications that may occur during treatment. However, these new instruments and movements tend to create more debridement.[6]

ProTaper Next (Dentsply Maillefer, Ballaigues, Switzerland) instruments have an asymmetric rectangular cross section and were manufactured from M-WIRE nickel-titanium (NiTi) alloy. The files operate with a rotational movement that makes a snakelike motion during preparation. The rotation of the asymmetric section creates a larger area for the transportation of debris.[7,8] Twisted-File (TF) Adaptive (Kerr Endodontics, Orange, CA, USA) instruments accommodates torsional force in the canal by performing rotational or reciprocal movements depending on the pressure on the file. The adaptive movement provides less compression on file.[9]

Limited information is available comparing the effects of TF Adaptive and ProTaper Next systems on postoperative pain formation. Thus, the aim of this study is to compare the severity of postoperative pain following root canal treatment by using rotational and adaptive techniques. Our hypothesis is that different techniques affect the severity of postoperative pain.

MATERIALS AND METHODS

The plan of the study was registered at www.clinicaltrial.gov (ClinicalTrials.gov Identifier NCT number: NCT03708081). This study was accomplished under the standing orders of the ethics committee (protocol number: 2017-98-20/09). Patients having teeth with asymptomatic irreversible pulpitis or pulp necrosis, who referred to Faculty of Dentistry, Department of Endodontics were included. Only single rooted teeth were selected. The patients with sinus tract, periradiculer abscess or facial cellulitis, who had previously taken any medication, and who had previously undergone root canal treatment with the related tooth were excluded. After the detailed written medical and dental history were obtained, the written consent form was taken from the patients who wanted to participate in the study. The patients having any systemic disease or any medication related to a systemic condition were excluded. Age, gender, and the location of teeth were recorded. Incisors and canine teeth having single root and canal were included. An electric pulp test (Elements pulp vitality tester, SybronEndo, Orange, CA, USA) was applied to determine the vitality of the teeth. An initial pilot study was conducted on overall 30 patients (15 individuals per group). The power analysis, performed for the pilot study, indicated that a minimum of number of 28 patients was required for each group to identificate significant differences. Therefore, 30 patients were included for each group to ensure a significative exemplary.

Overall, 60 patients were included. The ages of participants ranged between 18 and 63 years. For the elimination or limitation of any variables, a single operator completed all treatment procedures. After the teeth were isolated and the access cavities were prepared, the working length was determined by an electronic apex finder (Root ZX mini; J. Morita, Tokyo, Japan).

Patients were randomly divided 2 groups as follows;

In group 1, the root canals were instrumented using ProTaper Next files with a full sequence of X1 to X5 (50/.06), respectively.

In group 2, instrumentation was performed with TF Adaptive ML1, ML2, ML3 (50/.04) instruments. An adaptive motion was expected for each specimen during instrumentation. In case of lacking of any adaptive motion occurrence, the specimen was excluded. A total of 4 specimens was excluded due to this reason.

During the instrumentation, the root canals were irrigated with a total of 10 ml of 2.5% sodium hypochloride. Final irrigation was performed with 5 ml of 2.5% sodium hypochloride, 5 ml of 17% Ethylenediaminetetraacetic acid, 5 ml of 2% chlorhexidine. 5 ml of distilled water was applied after each solution to prevent any reaction that might occur between the solutions.

The root canals were obturated with cold lateral compaction method using matched gutta-percha cones and resin-based canal sealer (Adseal, Meta Biomed, Korea), and the quality of obturation was confirmed with radiographs. Finally, the access cavity was sealed with composite resin (Estelite Σ Quick, Tokuyama Dental Corp. Tokyo, Japan), and the occlusion was checked.

Postoperative pain questionnaires were scored by patients using a four-point pain intensity scale for 12, 24, and 48 hours.[10] The pain scores were as follows;

1- no pain;

2- mild pain (slight discomfort, no treatment required);

3- moderate pain (pain relieved by medication);

4- severe pain (pain and/or swelling not relieved by simple analgesic medication and the requirement of an unscheduled appointment). All scores were processed on the patient's treatment charts and statistical analysis of the results was performed after the planned number of patients had been treated.

The statistical analysis of the data was performed with SPSS 19.0 software. Mann–Whitney U test was used to compare between groups for each time period. Friedman test followed by Wilcoxon signed rank test used for the comparison of time periods in each group. The significance level was set at $p<0.05$.

RESULTS

The mean, standard deviations, minimum and maximum values of two groups, including time periods were presented in Table 1.

Table 1 Mean pain scores, standart deviations, and minimum and maximum values

Groups	Time	Mean	Standart deviation	Minimum	Maximum
PTX	12h	1.63	.890	1	4
	24h	1.43	.728	1	3
	48h	1.20	.484	1	2
TFA	12h	1.50	.731	1	4
	24h	1.23	.430	1	2
	48h	1.10	.305	1	2

Severe pain was only recorded in 12 hours period, whilst the 48 time period did not demonstrate any moderate or severe pain scores for both groups. The comparison of time intervals between groups demonstrated no difference between both groups ($p>0.05$). The total number of patients experiencing pain for both groups in 12, 24, and 48 hours were recorded as 16, 11, and 9, respectively. In both groups, the highest postoperative pain values were found in 12h time periods. In both groups, the postoperative pain values of 12h time period were significantly higher than both other periods, and significant difference was found between 24h and 48h time periods ($p<0.05$). The postoperative pain values of 48h time period were significantly lower than the other two time periods ($p<0.05$). No differences were found between female and male patients, similarly, between maxilla and mandibula ($p>0.05$) (Table 2). The demographic features were presented in Table 3.

Table 2. Mean pain scores according to gender and localization

Gender of patient/ Location of tooth	12h	24h	48h
	Mean (Std Dev.)		
Female (n=24)	1.54 (.884)	1.38 (.711)	1.21 (.509)
Male (n=36)	1.28 (.701)	1.28 (.615)	1.14 (.424)
Mandibular (n=29)	1.41 (.780)	1.31 (.604)	1.17 (.468)
Maxillary (n=31)	1.45 (.798)	1.32 (.702)	1.16 (.454)

Table 3. The demographic features of individuals

Demographic features		ProTaper Next (n= 30)	TF Adaptive (n= 30)
Gender	Female	35%	40%
	Male	65%	60%
Age	Mean	39.65	31.75
	Range	19-63	18-59
Localization	Maxilla	15	16
	Mandibula	15	14

DISCUSSION

The aim of this clinical study was to compare the intensity and duration of postoperative pain after single-visit root canal treatment with rotational and adaptive motions. In general, patients and clinicians prefer single-visit treatments due to their advantages such as low cost, fewer operative procedures, elimination of interappointment leakage and less chair time.[11,12] Therefore, a single visit treatment was selected for all patients. Additionally, final apical file diameter, type and amount of irrigation solution, obturation method were standardized to eliminate any bias in both groups.

Various scales were used to evaluate the severity of postoperative pain after root canal treatment. In this study, a four-point pain intensity scale, which was considered to be an adequate and reliable method, was used.[13] This scale method was also used in various recent studies for measuring the severity of postoperative pain after root canal treatment.[1,13,14] Different time intervals can be selected to evaluate the intensity of pain. The evaluation of postoperative pain was performed for different time periods in recent studies. Comparin *et al.*[15] and Gambarini *et al.*[16] evaluated the first 72 hours, whereas the first 120 hours was selected by Yaylali *et al.*[17] after treatment. In the present study, the postoperative pain was evaluated in three different time intervals. The prevalence and severity of pain after root canal treatment decreases after the first 48 hours.[18] Therefore, 12, 24, and 48 hours were selected similar to previous clinical studies.[1,19,20] The highest postoperative pain values were recorded at 12 h period in both groups. No increase in pain scores was recorded as time progresses, similar to previous clinical studies.[1,20] An unscheduled appointment for emergency treatment of acute apical abscess formation was recorded for only 1 patient. The associated tooth was directed for the extraction due to the patient's request and excluded from the study. Other than this patient, no symptoms such as postoperative swelling or paresthesia were recorded.

Various factors, such as age, gender, pulpal and periapical condition, type of tooth, preoperative pain, and technical characteristics, may affect postoperative pain.[22] In the present study, gender did not affect the incidence of postoperative pain, despite the female patients demonstrated slightly higher scores for all time intervals in accordance with other studies.[13,22] Additionally, the location of the tooth was not found to be a determinant on the pain scores, since no difference was found between mandibular and maxillary teeth. Besides, asymptomatic teeth were included to eliminate a possible preoperative variation. The relationship between age and

postoperative pain demonstrated contradictory results. In the present study, no difference was found in terms of age between groups similar to previous studies.[23,24] However, Azim *et al.*[25] reported higher risk for the patients >50 years in developing flare-ups.

Various instruments and instrumentation techniques are available in the market for clinicians to use in daily endodontic practice. All instruments and techniques may be related to postoperative pain. Several studies compared ProTaper Next system with other instruments and techniques. ProTaper Next demonstrated similar results with various reciprocal instruments in regard to the incidence of postoperative pain.[1,26] However, limited data are available for the TF Adaptive system and adaptive motion in terms of postoperative pain occurrence. Similar to ProTaper Next system, TF Adaptive resulted in significantly less incidence of severe pain symptoms than reciprocal technique.[27] Çiçek *et al.*[1] reported less postoperative pain with modified step-back technique than rotational and reciprocal technique. However, no difference was reported between the rotation and reciprocation techniques. The results of the present clinical trial also corroborate the clinical findings of Relvas *et al.*[28] who reported low and similar occurrence of postoperative pain between the reciprocating and rotary techniques. According to the present results, the null hypothesis was rejected. The ProTaper Next instruments demonstrated higher pain scores compared to the TF Adaptive system without any significance. The occurrence of adaptive motion, including rotational and reciprocal movements may lead to prevent the movement of debris to apical direction. Other possibilities related to this finding could be higher taper of the files which may result in the occurrence of more debris, and the requirement of more files to complete the instrumentation in ProTaper Next system during the root canal shaping procedures.

CONCLUSIONS

Both instrumentation techniques caused postoperative pain. The pain scores indicated that both techniques caused limited discomfort associated with slight pain which did not require any additional treatment and medication.

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None

CONFLICT OF INTEREST STATEMENT

None

Farklı Eğe Sistemlerinin Kullanımı Sonrası Oluşan Postoperatif Ağrının Değerlendirilmesi: Randomize Klinik Çalışma

ÖZ

Amaç: *Bu çalışmada enstrümantasyon tekniklerinin tek seans kanal tedavisi sonrası postoperatif ağrı üzerine etkileri değerlendirilmiştir.* ***Gereç ve Yöntemler:*** *Çalışmaya endodontik tedavi endikasyonu olan 60 hasta dahil edildi. Sadece tek köklü dişler seçildi. Hastalar rastgele iki gruba ayrıldı. Grup 1'de kök kanalları rotasyonal hareket ile ProTaper Next enstrümanları kullanılarak, grup 2'de TF Adaptive enstrümanları adaptif hareket ile kullanılarak genişletildi. Tedaviler tek bir randevuda tamamlandı. İşlem sonrası ağrı, 4 nokta ağrı şiddet skalası ile 12, 24 ve 48. saatler için hastalar tarafından işaretlendi. Verilerin analizi için Mann Whitney-U, Friedman ve Wilcoxon testleri kullanıldı.* ***Bulgular:*** *Zaman aralıklarına göre preparasyon tekniklerinin karşılaştırılmasında fark bulunamadı (p>0,05). Her iki grupta da 12. saatteki ağrı değerleri diğer iki periyottan anlamlı olarak yüksek bulundu ve ayrıca 24 ve 48. saat arasında fark tespit edildi (p<0,05). 48. saat değerleri anlamlı olarak diğer iki saatten daha düşük bulundu (p<0,05).* ***Sonuç:*** *Her iki enstrümantasyon tekniği işlem sonrası ağrıya neden olmuştur. Her iki teknik, ilave bir tedavi veya ilaç uygulaması gerektirmeyen sınırlı rahatsızlık ve hafif ağrı skorları göstermiştir.* ***Anahtar Kelimeler:*** *Ağrı, kök kanalını hazırlama, kök kanal tedavisi.*

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Original research

COMPARATIVE ANALYSIS OF MANUAL, ROTARY, AND RECIPROCAL SYSTEMS ON PRIMARY TEETH ROOT CANALS: AN IN VITRO SCANNING ELECTRON MICROSCOPY STUDY

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ABSTRACT

Objective: To evaluate the residual debris and smear layer formation, and also preparation time of one or multifile manual, rotary, and reciprocal systems on primary teeth.

Materials and methods: A total of 75 primary mandibular molar teeth were randomly divided to five groups (n=15). The distal canals of teeth were shaped with each of the K file, Protaper, Twisted File, OneShape, and Reciproc systems. Preparation time was also recorded. Longitudinal sections groups were prepared and processed for observation under scanning electron microscopy (SEM) at a standard magnification of X1000 for smear layer and X200 for residual debris. The presence of smear layer and residual debris was evaluated by two trained operators. The data of preparation time and also debris and smear scores were analyzed using ANOVA and the Kruskal-Wallis test, respectively.

Results: Reciproc and OneShape systems had significantly less instrumentation time than all other groups ($p<0.001$). The results of statistical analyses were the same for the residual debris and smear layer scores. In the coronal thirds of the canals, the canal preparation with the Protaper system resulted in significantly less debris and smear layer compared with the OneShape system ($p=0.015$). In the middle thirds of the canals, OneShape and Reciproc systems had more residual debris and smear layer than the Protaper system ($p<0.05$). In the apical thirds of the canals, the use of the Protaper instruments resulted in less debris and smear layer than the Reciproc system ($p=0.034$).

Conclusions: Within the limits of this study, the Protaper system, which showed better cleaning efficacy and was also faster than the manual system, can be an effective alternative to other systems in the root canal treatment of primary molars. More in vitro and clinical investigations are needed on root canal treatment of primary teeth.

Keywords: Primary teeth, root canal therapy, smear layer, microscopy, scanning.

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INTRODUCTION

The preservation of primary teeth until they are replaced by their successor is one of the most important missions of pediatric dentistry.[1] Early loss of the primary teeth can cause malocclusion, and masticatory, aesthetic, and speech problems. Therefore, to prevent these complications, root canal treatment of primary teeth has gained importance.[2] On the other hand, root canal treatment of primary teeth has some difficulties when compared with permanent teeth due to the anatomic and morphologic differences between them and the behavioral management problems of children.[3] For these reasons, chair time of pulpectomy in primary teeth should be as short as possible.[4]

Traditionally, stainless steel files have been used in the root canal treatment of primary root canals. However, stainless steel files have some limitations such as increased preparation time and imbalanced preparation of the root canal space.[4] To overcome these disadvantages, engine-driven NiTi instruments were introduced to pediatric dentistry by Barr *et al*.[5] Engine-driven NiTi files have shape memory and high elasticity so they easily follow the original anatomy of the root canal then produce conical-shaped canal preparation, and also reduce the preparation time.[5,6]

To date, many NiTi endodontic file systems have been produced by different manufacturers. Many of them recommend the use of multi-file concepts to shape the root canals. In the present study, the Protaper and Twisted file multi-files systems were used.

ProTaper (Dentsply Maillefer, Ballaigues, Switzerland) has been widely recommended and is one of the frequently used multi-file rotary systems (Mtwo-Twisted file). It has eight instruments; three for shaping (SX, S1 and S2) and the others for finishing. The files have an increasing taper design and a triangular, convex cross-section. This design provides high cutting efficiency.[7]

Twisted File (SybronEndo, Orange, CA, USA), which is another multi-file rotary system, is produced via twisting the metal in combination with R-phase heat treatment to advance super-elasticity.[8] A triangular cross-section and constant tapers as 0.04, 0.06, 0.08, 0.10, and 0.12 are seen in Twisted Files. They also have 5 tip sizes (25-50).[9]

New concepts of the engine-driven NiTi file systems called single-files have simplified the instrumentation protocol.[10] Single-file systems have several advantages including reduced working time, crosscontamination prevention, and improved safety of the shaping procedures.[11,12] The two different motions of single-file systems are continuous rotation and reciprocation.[10]

OneShape (OS; Micro Méga, Besançon, France) was the first single-file continuous rotation endodontic system made of conventional austenite 55-NiTi alloy.[13] Only one instrument is found in the system. It has a constant 6% taper and a noncutting tip of size 25. To prevent a screwing effect, the variable design includes a S-shaped triangular cross-section.[14]

The reciprocating motion has a counterclockwise rotation for cutting the dentin and shorter clockwise rotation to prevent the file from locking into the canal wall.[8] Thus, increased cyclic fatigue life and torsional resistance of NiTi instruments are provided with the reciprocating motion compared with the rotary motion.[15,16] However, less debris accumulation was reported with rotary motion.[17]

Reciproc (VDW GmbH, Munich, Germany) is the single-file endodontic system that has a specialized motor that provides clockwise and counter clockwise motion.[18] Reciprocal motion in a 150° counter-clockwise (cutting direction) and 30° clockwise (release of the instrument) rotation and performs balanced force, which is reliable for curved canals.[19] Reciproc files are made of heat-treated M-wire nickel-titanium alloy that is resistant to fatigue.[20]

Debris and smear layer are produced by root canal instruments during shaping protocols.[21] These formations may create a reservoir area for bacteria and their products may also reduce the adaptation of sealer and gutta-percha.[22] Little information is available about the debris and smear layer in primary teeth, contrary to permanent teeth.[6]

The aim of this study was to compare the formation of debris and smear layer and preparation time of K File, Protaper Universal, Twisted File, OneShape, and Reciproc systems on primary teeth.

MATERIALS AND METHODS

A total of 75 human primary mandibular molar teeth that were extracted for various reasons were collected from the department of pediatric dentistry. Ethical approval was obtained from the Ethics Committee of Karadeniz Technical University, Faculty of Medicine (Protocol # 2016-63). The teeth were virgin without any treatment. The inclusion criteria were absence of external or internal pathologic root resorption, absence of perforation in the internal or external furcation area, moderate root angulation,[23] and two-thirds of an intact root. All the teeth were radiographically evaluated to analyze root canal anatomy. The teeth were cleaned ultrasonically and stored in 1% T-Chloramine solution (Merck, Darmstadt, Germany) until use.

An ideal access cavity was prepared for each tooth to obtain straight-line access to the root canal. The crown of each tooth was removed and the roots of the teeth were divided using a bur. Distal roots, which were standardized to 15-mm length, were selected for shaping. Two longitudinal grooves were prepared on the buccal and lingual surfaces of each root with a diamond bur with the aim of facilitating vertical splitting for SEM evaluation after the canal instrumentation. The roots were divided into 5 groups. Working length determination was done by measuring #10 K-file visible at the apical foramen minus 1 mm. All root canals were shaped by a single operator as follows:

K-file group: Root canals were shaped manually with K-files (Dentsply Maillefer, Ballaigues, Switzerland) up to the ISO file size 25 to the working length.

Protaper group: ProTaper instruments (Dentsply Maillefer, Ballaigues, Switzerland) were used in a modified crown down technique according to the manufacturer's instructions with a gentle in-and-out motion, with attention paid to applying light apical pressure. The shaping of the root canal orifice was performed with SX instruments at two-thirds of the working length. S1 and S2 shaping files were used, followed by finishing files F1 and F2 (25/0.08) with continuous rotation motion.

Twisted file group: Twisted file instruments were used in a modified crown down technique according to the manufacturer's instructions with gentle in-and-out motion, also paying attention to applying light apical pressure. A size 25/0.08 file was used in a passive manner 2 mm short of the working length. Afterwards, a 25/0.06 file was used with continuous rotation motion at the working length.

OneShape group: OneShape instruments (MicroMega, Besancon, France) were used according to the manufacturer's instructions with a gentle in-and-out motion applying light apical pressure. A size 25/0.06 file was used with a continuous rotation motion at the working length.

Reciproc group: Reciprocal instruments were used according to the manufacturer's instructions with a gentle in-and-out motion with light apical pressure. A size 25/0.08 file was used with reciprocating motion at the working length.

The root canals were irrigated between each instrument with 2 mL of 2.5% sodium hypochlorite solution via 27 gauge needles.

The total preparation time for each canal included the time required for active instrumentation, cleaning and changing the instruments, and irrigation was recorded.

After the preparation time recorded standard irrigation regimens with 4 mL of 17% EDTA (Saver, Prime Dental Products, India) for 120 seconds followed by 1 mL of 2.5% sodium hypochlorite for 60 seconds. At the end, 1 mL of ethanol for 30 seconds was used as a final rinse and the canals were dried with calibrated paper points (Absorbent Paper Points, Dentsply-Maillefer, Konstanz, Germany).

Each sample was dipped in liquid nitrogen after the instrumentation to split them into two halves longitudinally using a stainless steel chisel. The sections were then allowed to air-dry overnight at room temperature, sputter-coated with gold

(SC7620 "Mini", Polaron Sputter Coater, Quorum Technologies, Newhaven, England), and prepared for scanning electron microscope (SEM) analysis (JSM-6610; JEOL, Peabody, Massachusetts, USA). One part was chosen for the assessment of residual debris and smear layer.

To standardize the examined area for each sample, the technique previously applied by Gorduysus *et al.*[24] was used. According to this technique, the central beam of the SEM was directed to the center of the specimen under X10 magnification, and the magnification was increased to X200 for debris evaluation, and then to X1000 for smear layer evaluation in coronal, middle, and apical thirds of the roots.

Dentin chips, pulpal remnants, and other particles loosely stuck to the canal wall were accepted as debris as previously defined by Hulsmann[17] and scored with the criteria described by the same author [25] as follows:

Score 1: Clean root canal wall, only a few small debris particles.

Score 2: Few small agglomerations of debris.

Score 3: Many agglomerations of debris covering less than 50% of the root canal wall.

Score 4: More than 50% of the root canal wall covered by debris.

Score 5: Complete or nearly complete root canal wall covered by debris.

A surface film retained on dentin and other surfaces after instrumentation with either rotary instruments or endodontic files was accepted as 'smear layer' like previously defined by American Association of Endodontists[26] and scored with the criteria described by Hulsmann *et al.*[25] as follows;

Score 1: No smear layer, dentinal tubules open.

Score 2: Small amount of smear layer, some open dentinal tubules.

Score 3: Homogenous smear layer covering the root canal wall, only a few open dentinal tubules.

Score 4: Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules.

Score 5: Heavy, homogenous smear layer covering the entire root canal wall.

A total of 450 images (75 samples X 3 region; coronal, middle and apical X2 for debris and smear) were analyzed twice at an interval of 8 weeks by two blinded and experienced observers (Kappa: 0.76). When differences occurred in the scoring of the images, the two observers rescored the images and discussed them until reaching a consensus.

Statistical analysis was performed using the Stata 12.0 software (Stata, College Station, Texas, USA). Descriptive statistics for ordinal data, including the median, minimum and maximum values were calculated for all groups. The preparation time data were analyzed with a parametric test of one-way analysis of variance (ANOVA) and Tamhane was performed for group-wise comparison. Debris and smear scores were analyzed with the non-parametric Kruskal-Wallis test and the Mann-Whitney U test was performed in group-wise comparisons. The significance of all statistical tests was predetermined at $p<0.05$.

RESULTS

Preparation Time

The mean time taken to prepare the canals for the different file systems is shown in Table 1. Reciproc and OneShape systems, which are single-file systems, had significantly less preparation time than all other groups ($p<0.001$). There were no statistically significant differences between these single-file systems ($p=0.85$). The slowest system was the K-File ($p<0.001$), and the Twisted file system had a shorter preparation time than the ProTaper system ($p<0.001$) and longer preparation time than single-file systems ($p<0.001$). The ProTaper system had a shorter preparation time than manual systems and longer preparation times than Twisted File and single-file systems ($p<0.001$).

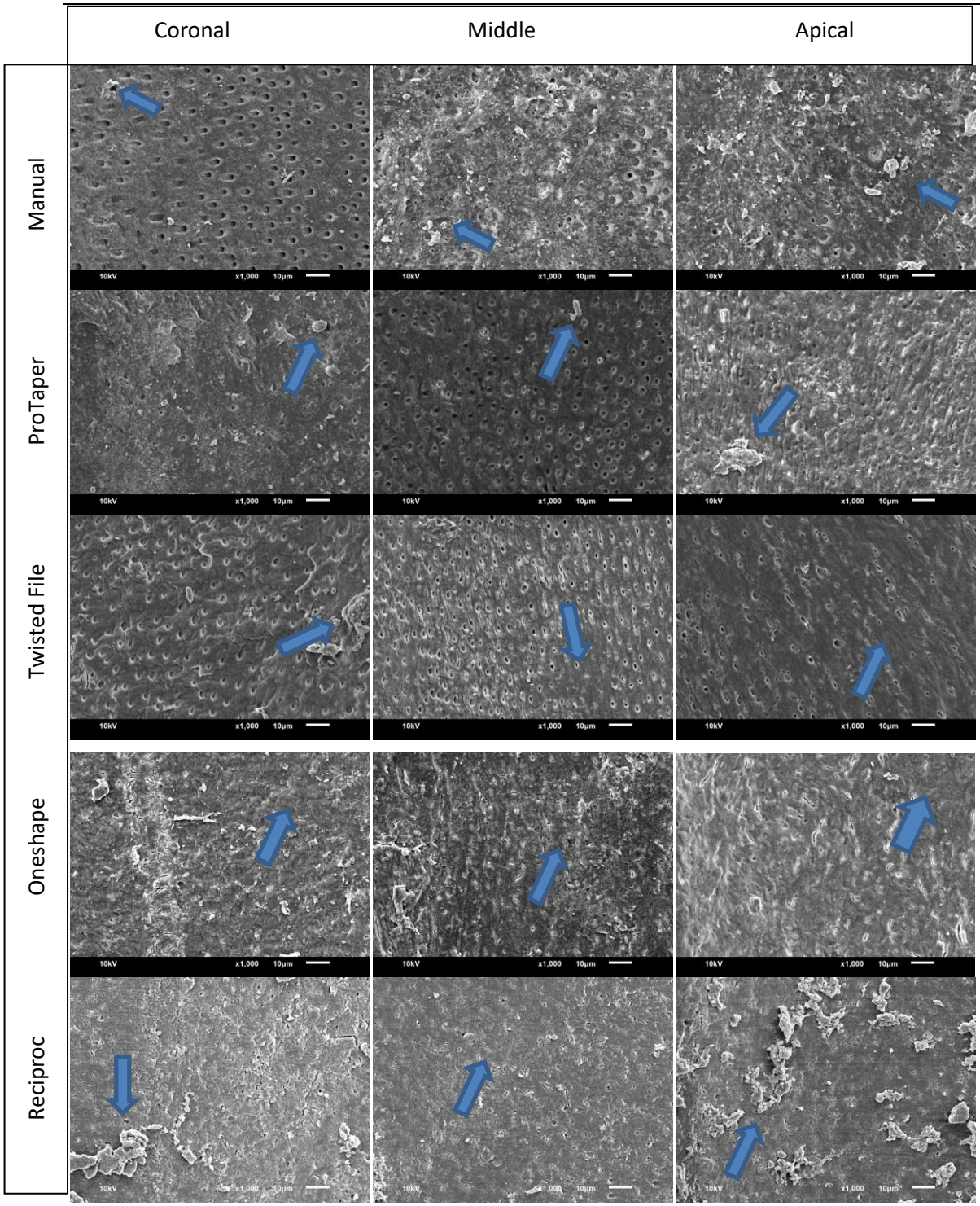

Figure 1. Representative samples of scanning electron micrographs of the root canal dentin surface instrumented with Manual, ProTaper, Twisted file, OneShape, and Reciproc systems at the coronal, middle, and apical third of the root (X1000).

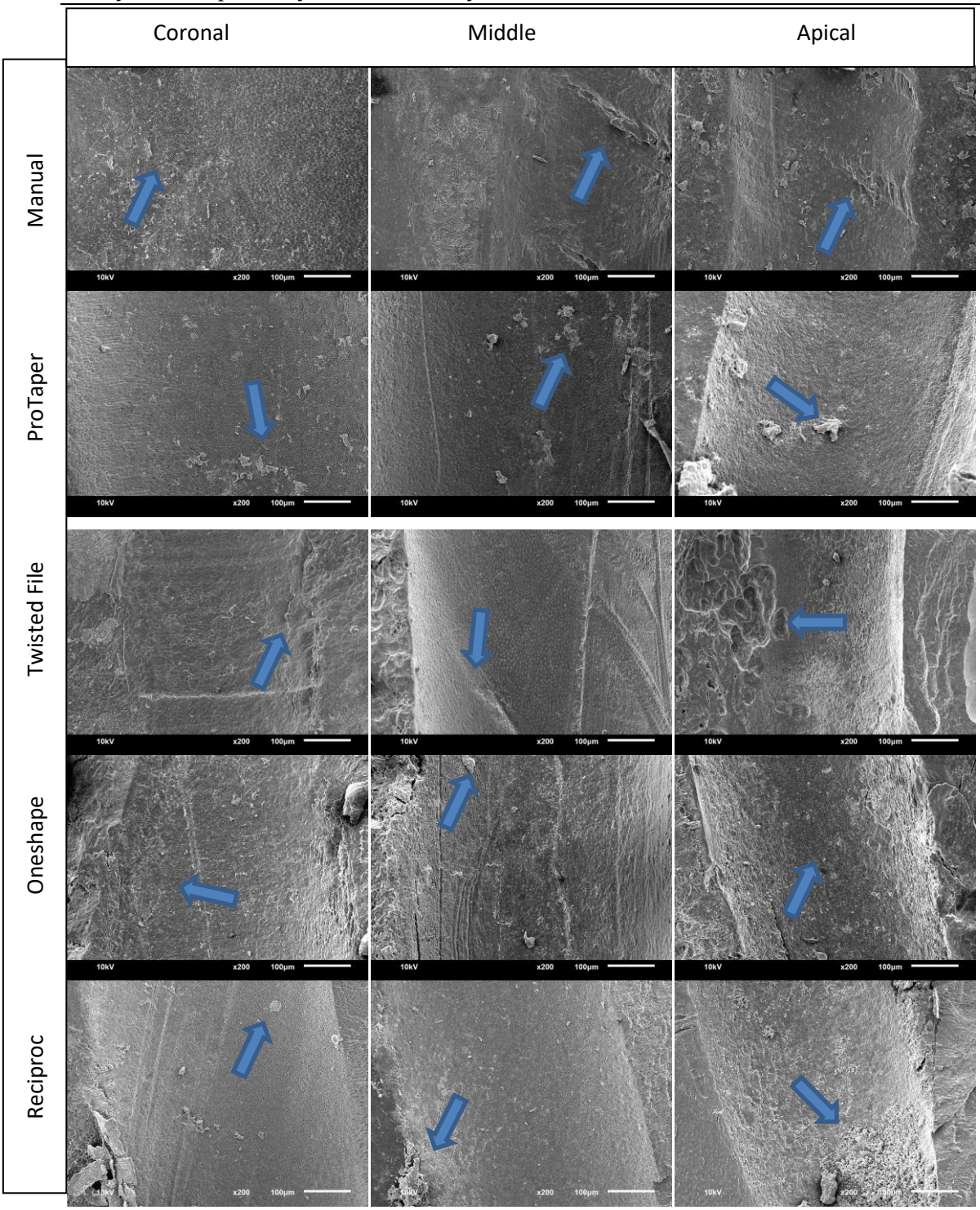

Figure 2. Representative samples of scanning electron micrographs of the root canal dentin surface instrumented with Manual, ProTaper, Twisted file, OneShape, and Reciproc systems at the coronal, middle, and apical third of the root (X200).

Table 1. Mean preparation time in seconds and standard deviation (SD) in different groups.

	Mean	SD
Manual	246,26[a]	72,34
ProTaper	128,53[b]	13,21
Twisted File	71,8[c]	10,28
OneShape	13,2[d]	6,31
Resiproc	22,33[d]	10,61

Values with the different superscript letters showed statistically different groups ($p<0.001$).

Debris and Smear Layer Scores

The debris and smear layer scores are summarized in Table 2 and 3. No completely cleaned root canals were found in any groups. The results of statistical analyses were the same for the scores of residual debris and smear layers. In the coronal thirds of the canals, the canal preparation with ProTaper systems resulted in significantly less debris and smear layer compared with the OneShape instrument ($p<0.05$). In the middle thirds of the canals, OneShape and Reciproc systems showed more residual debris and smear layer than the Protaper system ($p<0.05$). Also, the K-file group showed less debris and smear scores than the OneShape group in the middle thirds. In the apical thirds of the canals, ProTaper instruments resulted in less debris and smear layer than Reciproc systems ($p=0.034$)

Table 2. Median, minimum (MIN), and maximum (MAX) scores of smear layer and residual debris at the coronal, middle and apical area.

		SMEAR LAYER Median (MIN-MAX)			RESIDUAL DEBRIS Median (MIN-MAX)		
Groups	**N**	**Coronal**	**Middle**	**Apical**	**Coronal**	**Middle**	**Apical**
Manual	15	1 (1 - 4)	2 (1 - 4)	3 (2 - 4)	1 (1 - 4)	2 (1 - 4)	3 (2 - 4)
ProTaper	15	1 (1 - 3)	2 (1 - 3)	3 (1 - 4)	1 (1 - 3)	2 (1 - 3)	3 (1 - 4)
Twisted File	15	1(1 - 3)	2 (1 - 3)	3 (2 - 5)	1 (1 - 4)	2 (1 - 4)	3 (1 - 5)
One shape	15	3 (1 - 4)	3 (1 - 4)	3 (1 - 5)	3 (1 - 5)	3(1 - 5)	3 (1 - 5)
Reciproc	15	2 (1 - 4)	3 (1 - 4)	3 (2 - 4)	2 (1 - 4)	3 (1 - 4)	3 (2 - 5)

Table 3. Comparison of the endodontic systems used in the study with respect to the area.

		Groups (p value)				
		Manual	**ProTaper**	**Twisted File**	**OneShape**	**Reciproc**
Area	**Groups**					
Coronal	**Manual**	-	1.00	1.00	0.226	1.00
	ProTaper	1.00	-	1.00	**0.015***	0.689
	Twisted File	1.00	1.00	-	0.258	1.00
	OneShape	0.226	**0.015***	0.258	-	1.00
	Reciproc	1.00	0.689	1.00	1.00	-
Middle	**Manual**	-	1.00	1.00	**0.042***	0.204
	ProTaper	1.00	-	0.581	**0.007***	**0.042***
	Twisted File	1.00	0.581	-	1.00	1.00
	OneShape	**0.042***	**0.007***	1.00	-	1.00
	Reciproc	0.204	**0.042***	1.00	1.00	-
Apical	**Manual**	-	1.00	1.00	1.00	0.204
	ProTaper	1.00	-	1.00	0.284	**0.034***
	Twisted File	1.00	1.00	-	1.00	0.508
	OneShape	1.00	0.284	1.00	-	1.00
	Reciproc	1.00	**0.034***	0.508	1.00	-

*: Statistically significant differences ($p<0.05$).

DISCUSSION

Root canal treatment is a complex procedure comprising removal of pulp tissue, residual necrotic materials, debris, and infected dentin, and also sealing the canals with biocompatible materials.[27] The geometric anatomy of primary tooth pulp is more complicated than in permanent teeth because of the greater numbers of accessory canals, lateral canals, fins, anastomoses between canals and apical delta, and short and thin roots.[28] In addition to anatomic problems, behavioral management of children makes root canal treatment of primary teeth more difficult.[3] For these reasons, completing the root canal procedure in a shorter time and providing good quality treatment are desirable choices for pediatric practitioners.[27]

Engine driven NiTi systems were introduced to pediatric dentistry in order to reduce chair time and produce a more uniform and funnel-shaped preparation through their high elasticity and time-consuming nature.[5,6] Nevertheless, there are limited published data about primary teeth root canal treatment with engine driven NiTi systems.[4,5,27,29] In the present study, manual, rotary, and reciprocal NiTi systems were evaluated for preparation time, residual debris, and smear layers in primary teeth.

Preparation time is an important factor to draw conclusions on the efficacy of endodontic systems and on their clinical suitability.[30] In the present study, the preparation times of K-file–multifile manual systems, ProTaper and Twisted File-multifile rotary systems, OneShape-single file rotary systems, and Reciproc-single file reciprocal systems were investigated. Preparation time included active instrumentation time and the time required for irrigation, changing the file, and cleaning the flutes as in previous studies.[17, 31]

The K-file–multifile manual group was significantly slower than all of the engine-driven groups ($p<0.001$), in agreement with previous studies.[29,32] In the present study, ProTaper, Twisted File, OneShape, and Reciproc groups consisted of 4, 2, 1 and 1 files, to prepare the root canal to a size of 25, respectively. As expected, preparation time increased with increasing file numbers ($p<0.001$). No significant difference was found between the OneShape and Reciproc groups, both of which were single-file systems ($p=0.85$), contrary to the study of Saber *et al.*[33] This status might be due to the extremely curved permanent teeth that were used in the previous study, controversy to the present study. Also, Saber *et al*. declared that from a clinical point of view, the differences between these instruments might be of no importance.[33]

One of the most important objectives of root canal preparation is the removal of vital and/or necrotic pulp tissue, infected dentin, and dentin debris to eliminate most of the microorganisms from the root canal system.[34] In the present study, manual, rotary, and reciprocal systems were evaluated to achieve these objectives by analyzing residual debris and smear layer removal via SEM photomicrographs.

The magnification 200X, which offered a wider view, was used for residual debris evaluation, and the magnification 1000X, which gave detailed information, was used for smear layer evaluations.

The ProTaper system had significantly better results than the OneShape system ($p<0.05$). ProTaper has multi-file system and with the multifile technique canals are irrigated more frequently because irrigation regimen is used with every file change so debris will have less opportunity to accumulate in canals.[35] Other multifile systems also showed better results than OneShape but not statistically different. This result is in accordance with the previous study of Kansal *et al*.[35] Also, the taper sizes of the ProTaper and OneShape are 0.08 and 0.06, respectively. The increase of taper may cause large preparation, which allows a larger volume of irrigants to be in contact with canal walls.[36] The better results of ProTaper may be attributed to these factors.

In this study, the multi-file systems (ProTaper, Twisted File, and K-file) were not statistically different according to the residual debris and smear layer scores ($p>0.50$). This finding is consistent with the previous studies.[37,38] Celik *et al*.[37] compared the cleaning effectiveness of Twisted File, GT series X, Revo-S, RaCe, Mtwo, and

ProTaper Universal rotary files in curved canals. No statistically significant differences were found between the groups in terms of debris scores and smear layer scores, in agreement with the present study. Li *et al*.[39] evaluated the cleaning efficiency of Twisted File and ProTaper rotary instruments. The results of this study are not in agreement with the present study in the coronal area. The probable reasons for the disparity could be the differences between the methods. They used permanent molars, and in the present study, deciduous molars were used.

Reciproc had worse cleaning efficacy results than ProTaper ($p<0.05$). The continuous rotation motion provides an exit for debris up to the flutes of the file. By contrast, with the reciprocal motion, each backward motion of the file pushes debris into the lateral canals and over the apex.[40] Robinson *et al*. [40] also showed that ProTaper had better cleaning efficacy than Waveone, which is a reciprocal system. Although Twisted file and OneShape are continuous rotation systems, they did not show any statistically significant difference ($p>0.05$). This can be because of the taper differences. The taper of Reciproc, Twisted file, and OneShape are 0.08, 0.06, and 0.06, respectively. Despite the reciprocal motion, the greater taper size of Reciproc may have caused the similar results as the Twisted File and OneShape systems.

The limitations of the present study are that SEM images present two-dimensional investigations, whereas three-dimensional methods allow to evaluate root canal anatomy in detail and reflect the clinical conditions more realistically. Also, in vivo studies with more samples are needed.

CONCLUSIONS

Within the limits of this study, all of the engine driven NiTi systems were preferable to manual systems when the chair time was taken into account. These systems may be preferable for the root canal treatments of children who have co-operation problems. However, based on the results of the present study, ProTaper systems, which showed better cleaning efficacy and shorter preparation time than manual systems, can be an effective alternative to other systems on the root canal treatment of primary molars. Nonetheless, more investigations are needed on root canal treatment of primary teeth, which are anatomically and morphologically different from the permanent teeth.

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CONFLICT OF INTEREST STATEMENT

None

Süt Dişi Kök Kanal Şekillendirilmesinde Manuel, Döner ve Resiprokal Sistemlerin Karşılaştırmalı Olarak İncelenmesi; İn Vitro Taramalı Elektron Mikroskobu Çalışması

ÖZ

Amaç: *Manuel, rotary ve resiprokal sistemlerin süt dişi kök kanallarında kullanımının debris ve smear tabakası oluşumuna ayrıca preperasyon süresine etkilerinin incelenmesidir.* ***Gereç ve Yöntemler:*** *Toplamda 75 adet süt mandibular molar diş 5 gruba ayrıldı (n:15). Dişlerin distal kanalları K tipi eğe, Protaper, Twisted File, OneShape and Reciproc sistemlerinden biri ile şekillendirildi. Ayrıca preperasyon zamanı da ölçüldü. Taramalı elektron mikroskopu (SEM) değerlendirilmesi için dişler uzunlamasına ikiye bölündü. Smear tabakasının değerlendirilmesi için 1000, debris için 200 büyütmede inceleme 2 deneyimli hekim tarafından yapıldı. Preparasyon süresi için ANOVA, Smear tabakası ve debris değerlendirilmesi için Krukal Wallis testleri uygulandı.* ***Bulgular:*** *Reciproc ve OneShape sistemleri diğer gruplara göre istatistiksel olarak anlamlı derecede daha kısa preperasyon zamanına sahipti ($p<0,001$). İstatitiksel analiz bulguları debris ve smear tabakası aynıydı. Koronal bölgede Protaper grubu OneShape grubuna göre daha az debris ve smear tabakası gösterdi ($p<0,05$). Orta bölgede OneShape ve Reciproc gruplarında Protaper grubuna göre daha fazla debris ve smear tabakası gözlendi ($p<0,05$). Apikal bölgede Protaper grubu OneShape grubuna göre daha az debris ve smear tabakası sergiledi ($p<0,05$).* ***Sonuçlar:*** *Bu çalışmanın sınırları göz önünde bulundurularak Manuel sisteme göre daha hızlı ve temizleme etkinliği diğer sistemlerden daha yüksek olan Protaper sistemi süt dişi kök kanal şekillendirilmesinde etkin bir alternatif olabilir. Süt dişlerinin kök kanal tedavileri ile ilgili daha fazla in vitro ve klinik çalışmalara ihtiyaç duyulmaktadır.*

Anahtar kelimeler: *Süt dişi, kök kanal tedavisi, smear tabakası, mikroskobi, tarama.*

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Original research

LOWER INCISOR INCLINATION IN RELATION TO HEAD POSTURE, TONGUE AND HYOID POSITIONS

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ABSTRACT

Objectives: To investigate the relationship between head posture, hyoid bone, tongue position and lower incisor inclination.

Materials and Methods: The study sample consisted of 98 subjects (35 males, 63 females), who had a mean age of 17.7 years in the permanent dentition and with a skeletal Class I relationship. Lower incisor inclination, craniovertical, craniocervical and craniohorizontal postural variables, tongue and hyoid position and airway measurements were evaluated on lateral cephalograms taken in natural head position. The study sample was divided into two groups according to lower incisor inclination (48 had IMPA<90 with a mean degree of 88.09 and 50 had IMPA>90 with a mean degree of 102.84) and a Student-t test was performed to compare and describe head posture, tongue and hyoid bone positions.

Results: The subjects with upright incisors had a more posteriorly positioned hyoid bone relative to the mandibular symphysis compared with the subjects with flared incisors ($p<0.05$). Tongue length was greater in the flared incisor group than in the upright incisor group ($p<0.05$). In relation to head posture measurements, the upright incisor group had 3-3.5° larger cranio-cervical angles (NSL-OPT, NSL-CVT, NL-OPT, NL-CVT angles, $p<0.05$) than the flared incisor group. The airway measurements revealed no statistically significant difference between the groups.

Conclusions: The position of the hyoid bone, tongue length and craniocervical head posture showed significant differences between upright and flared lower incisors.

Keywords: Incisor, posture and hyoid bone.

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INTRODUCTION

Increasing attention has been directed to the interaction between form and function in the craniofacial region. Craniocervical, equilibrium and normal oral function has a recorded relationship with the position of the lower incisors within the facial framework.[1,2] The labiolingual inclination of the lower incisors contributes to facial appearance and function. In addition, lower incisor position has an important role in the development of a malocclusion, through the force effects generated by the tongue and lips, habits, occlusal forces, head and cervical posture, and hyoid bone position.

It has been shown that cervical posture is related to craniofacial morphology.[3,4,5] Solow and Tallgren[3] stated that extended craniocervical posture is related to increased anterior facial height, reduced sagittal jaw dimensions and a stepped inclination of the mandible. Whereas a flexed head position related to the cervical column is related to reduce facial height, larger sagittal jaw dimensions and a flattened mandibular plane. Rocabado *et al.*[6] indicated that a strong relationship exists between head posture and malocclusion. Solow and Sonnesen[5] showed that a factor which could affect dental arch crowding was the posture of the head in relation to the cervical column (craniocervical posture). This conclusion was a result of a soft tissue stretching hypothesis associated with an increased dorsally-directed soft tissue pressure which exists in subjects with extended craniocervical posture. Recently, Pachi *et al.*[7] found an association between the extended head posture and lower crowding of 2mm or more. It was also explained that this relation by the stretching of the soft tissue layer that covers the head and neck during extension. A second possible explanation for this association is possibly that the subjects with nasal airway obstruction presented reduced incisor inclinations and crowding.[8,9]

An important feature craniofacial morphology in relation to cervical posture is hyoid bone position. The supra- and infrahyoid muscles connect the hyoid bone to surrounding structures such as the tongue, the mandible, the base of the skull, the sternum, the scapula, the thyroid cartilage and the pharynx. Because of the complex attachments of the hyoid bone to neighboring anatomical structures, changes in the position may influence hyoid bone position.[10] It has been also shown that there is a significant relationship between mandibular position and hyoid bone position, particularly related to head posture changes.[11,12] Based on the assumption that hyoid bone may, therefore, be a dependent structure on many tissues in the craniomandibular system, it is of interest to determine whether lower incisor inclination could be linked to hyoid bone position.

The tongue has the ability to affect the position of teeth and other structures.[13] Peat[14] stated that the tongue has an important role in positioning the dentoalveolar structures. Although it is attached principally to the mandible and hyoid bone[15], the posture, size and the shape of the tongue are the main factors affecting the dentoalveolar form.

Another factor which may affect the lower incisor inclination could be the airway dimensions. A normal airway is accepted as an important factor for the spatial positioning of the craniofacial structures. The relation between airway obstruction and incisor inclination has been described by Linder-Aronson[8] and Woodside *et al.*[9] It has been shown that subjects with nasopharyngeal airway obstruction had greater crowding and reduced incisor inclinations compared with subjects without obstruction. In addition to that, after adenoidectomy, and with the regain of nasal respiration, an increased inclination of the incisors was observed.

The association between lower incisor crowding and head posture[7], skeletal and symphyseal patterns[16] has been discussed, but to the best of our knowledge, no investigation has been performed evaluating the inclination of the lower incisors related to head, tongue and hyoid positions, as well as nasopharyngeal airway dimensions. Therefore, the aim of present study was to evaluate the relationship between head posture, hyoid and tongue position, airway dimensions and lower incisor inclination.

MATERIALS AND METHODS

The material of this retrospective study consisted of pretreatment lateral cephalograms of randomly selected patients treated in the Department of Orthodontics, Baskent University Istanbul Hospital. An informed consent was obtained from all the subjects. A power analysis showed that 98 subjects were sufficient for the study (α=0.05; f (effect size) =0.5; power (1-β err prob=0.80). Ninety-eight subjects were selected age 12-41 years (35 males, 63 females, with a mean age of 17.7 years) on the basis of the following criteria; complete permanent dentition (except the third molars); had good quality lateral cephalometric radiographs, no craniofacial anomalies, systemic muscle or joint disorders; no previous symptoms of upper airway obstruction; no previous orthodontic treatment; and with an ANB angle in the range of 0-4°.

The lateral cephalograms were taken using Sirona Orthopose CD Dental X-ray machine (Siemens, Germany). All lateral radiographs were taken by the same operator with the subjects in the natural head position. The cephalometric landmarks and lines used to assess the changes in lower incisor inclination, tongue, soft palate, hyoid position, and airway dimensions are shown in Table 1 and Figure 1. Nasopharyngeal measurements were performed according to the study by Germeç-Çakan *et al.*[17] The intra-examiner measurement error was calculated by remeasuring randomly selected 20 radiographs after 2 weeks, and their mean differences were used to determine Pearson's correlation coefficient. A high correlation value of 0.86-0.93 was found for all angular and linear measurements.

Table 1. Cephalometric landmarks and lines used to evaluate changes in head posture, hyoid and tongue positions, pharyngeal dimensions, and lower incisor inclination.

Point-line	Definition
S	Centre of the sella turcica of the sphenoid bone
N	Most anterior point of the frontonasal suture in the midsagittal plane
Po	Most superior point of the external auditory meatus
Point A	Most posterior point in the concavity between anterior nasal spine and the dental alveolus
Point B	Most posterior point on the concavity along the anterior surface of the symphysis
Go	The most convex point along the inferior border of the ramus
M	The most inferior point of the symphysis
Rgn	The most posterior point of symphysis
H	The most superior and anterior points on the body of the hyoid bone
TT	Tongue tip
V	Base of epiglottis
U	Tip of soft palate
C3	Antero-inferior limit of the third cervical vertebra
Ans	Tip of the anterior nasal spine
Pns	Tip of the posterior nasal spine
L1	Axial inclination of the mandibular incisor
Cv2tg	Tangent point of OPT line on the odontoid process of the second cevical vertebra
Cv2ip	The most inferior-posterior point on the corpus of the second vertebra
Cv4ip	The most inferior-posterior point on the corpus of the fourth vertebra
Ver line	True vertical line projected on the radiograph
Hor line	True horizontal line projected on the radiograph
NSL	Line extending between sella and nasion
CVT	Posterior tangent to the odontoid process through Cv4ip
OPT	Posterior tangent to the odontoid process through Cv2ip
NL	Line extending between Ans and Pns
MnPl	Line extending between gonion and menton

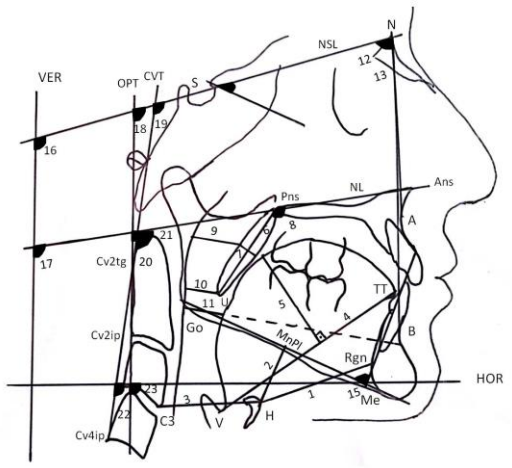

Figure 1. Lateral cephalometric lines and angles. ***Hyoid and tongue positions, pharyngeal dimensions***; 1 Rgn–H, the distance between Rgn and H; 2 H–MnPl, the perpendicular distance from H to the MnPl; 3 C3H, distance between the hyoid bone and C3; 4 TGL, tongue length (V–TT); 5 TGH, tongue height (maximum height of the tongue along a perpendicular line of V–TT line to tongue dorsum); 6 Pns–U, soft palate length, the distance between Pns and U; 7 Mpt, soft palate thickness (maximum thickness of the soft palate measured on a line perpendicular to Pns–U line); 8 Soft palate angle, the angle between soft palate length (Pns–U) and NL; 9 SPAS, superior posterior airway space (width of the airway behind the soft palate along a parallel line to the Go–B line); 10 MAS, middle airway space (width of the airway along a parallel line to the Go–B line through U); 11 IAS, inferior airway space (width of the airway space along the Go–B line); ***dentofacial measurements***; 12 SNA, 13 SNB, 14 ANB, 15 IMPA. ***head posture measurements***; 16 NSL-Ver, downward opening abgle between NSL and true vertical line projected on the radiograph; 17 NL-Ver, downward opening abgle between NSL and true vertical line; 18 NSL-OPT, craniocervical posture, angle formed by NSL line and OPT; 19 NSL-CVT, angle formed by NSL line and CVT, 20 NL-OPT, maxillary base inclination, angle formed by NL line and OPT; 21 NL-CVT, angle formed by NL line and CVT; 22 CVT-Hor, craniohorizontal angle, angle formed by CVT and Hor line; 23 OPT-Hor, angle formed by OPT and Hor line.

The study sample was divided into to two groups. The first group consisted of 48 subjects (34 female, 14 male, mean age of 17.36) with lower incisor inclination angle (IMPA) less than 90°, and identified as flattened incisors. The second group consisted of 50 subjects (29 female, 21 male, mean age of 18.02) with an IMPA angle larger than 90°, and identified as flared incisors. Data analysis was performed using the SPSS software package program (SPSS for Windows, version 16.0, SPSS Inc, Chicago, Ill). Student-t test was performed to compare the inclination of the lower incisors related to head posture, and tongue and hyoid bone positions and airway dimensions. P≤0.05 values were accepted as significant.

RESULTS

The 48 subjects of the flattened incisor group showed a mean IMPA of 88.09°, while 50 subjects with flared incisors had a mean IMPA of 102.84° (p<0.001). Means and standard deviations of the hyoid bone and tongue positions, pharyngeal dimensions and head posture measurements are presented in Table 2.

Table 2. Mean, standard deviation and statistical difference of the variables between flared and flattened lower incisor groups according to Student t test.

	Flattened Lower incisor group (IMPA ≤ 90°, n:48)				**Flared Lower incisor group (IMPA > 90°, n:50)**				
Hyoid and tongue positions, pharyngeal dimensions	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**P**
Rgn-H	24.52	3.32	17	32	23.08	3.71	15	35	0.046*
H-MnPl	10.39	3.72	3	21	9.44	3.59	1	17	0.199
C3H	21.50	2.82	11	28	21.60	2.22	17	28	0.756
TGL	42.08	4.44	26	50	43.79	3.91	32	52	0.046*
TGH	18.95	2.62	14	25	18.78	2.75	15	27	0.744
Pns-U	20.35	2.66	12	25	21.38	2.49	16	28	0.052
MPT	4.79	1.05	3	7	4.99	1.17	3	7	0.379
Soft palate angle	129.35	6.75	120	145	131.62	6.11	120	145	0.085
SPAS	7.83	1.87	5	13	7.82	1.81	4	12	0.972
MAS	5.87	2.00	2	11	5.44	1.76	2	11	0.257
IAS	6.95	1.86	3	10	6.38	2.20	3	12	0.165
Head Posture									
NSL-Ver	99.18	5.27	87	111	98.88	5.71	85	113	0.785
NL-Ver	90.19	5.52	74	101	90.75	5.49	75	102	0.614
NSL-OPT	104.80	6.91	90	128	101.84	7.25	82	115	0.041*
NSL-CVT	109.42	7.59	94	131	105.85	7.49	90	119	0.021*
NL-OPT	96.41	8.07	81	119	93.11	6.36	77	104	0.026*
NL-CVT	102.33	7.64	85	126	99.19	7.52	84	111	0.043*
CVT-Hor	79.24	7.92	58	100	81.89	8.60	62	102	0.116
OPT-Hor	83.89	8.41	59	105	85.94	8.87	67	104	0.243

*p<0.05

Table 2 also shows the differences of various parameters between the two groups. According to hyoid bone and tongue position measurements, the subjects with flattened incisors had a mean of 1.44 mm more posteriorly positioned hyoid bone relative to the mandibular symphysis than the subjects with flared incisors ($p<0.05$). Tongue length was found to be 1.71 mm more in the flared incisor group than flattened group ($p<0.05$). Concerning head posture measurements, the flattened incisor group had 3-3.5° larger cranio-cervical angles (NSL-OPT, NSL-CVT, NL-OPT, NL-CVT angles, $p<0.05$) than the flared incisor group. For the airway measurements, no statistically significant differences were found between the groups.

DISCUSSION

The positions of lower incisor teeth have been considered as a major factor in the development of a malocclusion, facial pattern and orthodontic treatment planning. According to Proffit's equilibrium theory,[18] the dentition is kept in a balanced position by its environment (peri-oral muscles, tongue, periodontal ligaments, etc.). In this theory, altering the oral environment will cause a change in the occlusion and therefore to create a stable occlusion, teeth should be placed in a position balanced by the surrounding forces. Proffit[19] revisited the theory and stated that, while the major primary factors in the dental equilibrium could be the pressures of the tongue and lips, periodontal membrane and eruption forces, the forces from the occlusion and respiratory function also influence head, jaw and tongue postures and thereby altered the "equilibrium".

For the lower incisors, equilibrium is created not only by lip and tongue pressures, but also by the craniocervical mandibular system consisting of the temporomandibular joint, cervical column and by the "hanger system" of the hyoid bone. Other basic parts of this system are the dental occlusion, the swallowing mechanism and, the muscles and ligaments that joined each. From these data the authors evaluated the relationship between the inclination of the lower incisors and the surrounding structures creating craniocervical system equilibrium.

According to the results of the present study, the position of the hyoid bone was significantly closer to the mandibular symphysis, and tongue length was increased as well as craniocervical head posture was more flexed (measured with NSL-CVT, NSL-OPT, NL-CVT, NL-OPT) in the flared incisors group than flattened lower incisors group ($p<0.05$).

The hyoid bone is an important part of the craniofacial complex and its position relates mandibular position adapting to anteroposterior changes in head position by means of the supra- and infrahyoid muscles.[11,20] Adamidis and Spyropoulos[21] also showed more anteriorly position of the hyoid bone in the Class III group correlated with Class I subjects. Amayeri *et al.*[22] argued that the hyoid bone moved backward as the mandible moved forward and; in the vertical plane, the hyoid bone position was lower position in the Class III malocclusion cases than in Class I and Class II malocclusions. Jena and Duggal[23] showed the hyoid bone was placed anteriorly in subjects with a short face. Ferraz *et al.*[24] mentioned no statistical significant differences in the mandible and hyoid bone position related to the respiratory pattern. According to the results of the present study, subjects with flattened lower incisors showed a more posteriorly positioned hyoid bone to the mandibular symphysis than subjects with flared lower incisors. Previously, it was argued[25] that head hyperextension was correlated with a greater distance from the hyoid bone to the mandible and to menton.

The tongue plays a role in dental and skeletal malocclusions, thus the size and position of the tongue must also be taken into consideration when planning orthodontic treatment. A short tongue length has been correlated with upright central incisors, a small overjet, a low ANB angle, impacted maxillary and mandibular teeth, and a steep occlusal plane.[26] Bandy and Hunter[27] stated that the length of the tongue has little influence on the angle between the axial inclination of the lower incisor teeth to the mandibular plane. Consistent with these studies, the results of the

present study showed an increased tongue length in the flared lower incisor group.

According to the results of the present study, the flattened lower incisor group showed a mean of 3° to 4° larger cranio-cervical angles (NSL/CVT, NSL/OPT, NL/CVT, NL/OPT) than subjects with flared incisors ($p<0.05$). Extended head posture caused uprighting of the lower incisors.

Previous studies[3,5] have found that natural head posture is related to respiratory function. Linder-Aronson[8] evaluated the dental measurements of children with mouth breathing, enlarged adenoids and obstructed upper airway, and found that the subjects showed retroclined upper and lower incisors. One year after the adenoidectomy, normalization of dental inclination was observed by the authors. Although the study groups were comprised by the subjects with no previous symptoms of the upper airway obstruction, and results showed the pharyngeal airway dimensions have not any significant differences between groups. Lower incisor inclination was still affected by craniocervical head posture. Similar to the results of the present study, an association between extended the head posture and lower arch dental crowding was shown.[7] It was proposed that another cause of this result may be a "soft tissue stretching mechanism" proposed by Solow and Kreiborg.[28] According to this theory, an extension of the craniocervical posture causes passive stretching of the soft tissue layer (skin, fascia and muscles) covering the head and neck. A dorsally directed force is created by the stretching of this soft tissue layer which affects dentofacial growth in the long term. According to the equilibrium theory, lower incisor inclinations may be retroclined by this backward guiding force, if the tongue muscle activity is not balanced.

However, if the "soft tissue stretching mechanism" is accepted, the magnitude of these forces should be discussed. Hellsing and L'Estrange[29] found a significant difference in upper and lower lip pressures between nose breathing and mouth breathing subjects. It was shown that when the head extends 5° during mouth breathing, a significant increase in upper and lower lip pressures occurred. Archer and Vig[30] studied the lip and tongue pressures in subjects with Class I and Class II dental and skeletal morphologies in the natural head position, head extension and head flexion positions. It was stated that the flexion of the head posture caused a significant decrease in the lingual pressure. In agreement with these researchers, the results of the present study indicate that the influence of head posture should be taken into account when evaluating the facial morphology and planning orthodontic treatment.

In the present study, subjects were a wide range of age of 12-41 years. Thus, there is an inherent limitation of the study as the possible effect of growing on head posture, hyoid bone and tongue position, nasopharyngeal airway dimensions and lower incisor inclination. Araújo *et al.*[31] studied the nasopharyngeal patency change on the respiratory function by means of rhinomanometry in individuals in different age groups. They found that children and adults had similar values in the nasopharyngeal space. Thus, the authors concluded the nasopharynx does not change with age. Abramson *et al.*[32] showed a proportional increase of the nasopharyngeal space to 20 years, and after a stabile period, a reduce of in size until 50 along the craniofacial growth by evaluating 1300 CT scans of healthy individuals aged 6 to 60 years. Considering the change in hyoid bone position, Matsuda *et al.*[33] stated hyoid bone shifted to a posterior, rotated, and lower position with increasing age in their cross sectional study using lateral cephalometric radiographs in 22 to 84 years old patient. Age-lower incisor position relationship was evaluated by Linjawi's study[34] and the author argued that age- and gender-related lower incisor changes significant only in subjects with brachifacial pattern. Present study was focused head posture, hyoid bone, tongue position and lower incisor inclination instead of vertical growth pattern.

CONCLUSIONS

The results of the present study may be summarized as follows:

The hyoid bone is positioned significantly closer to the mandibular symphysis in subjects with flared lower incisors.

Craniocervical head posture showed more flexion and tongue length increased in the subjects with flared incisors than flattened lower incisors.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

Alt Keser İnklünasyonunun Baş Postürü, Hyoid ve Dil Pozisyonu ile İlişkisi

ÖZ

Amaç: *Bu çalışmanın amacı alt keser inklinasyonunun; baş postürü, hyoid kemik ve dil pozisyonu ile olan ilişkinin araştırılmasıdır.* ***Gereçler ve Yöntem:*** *Çalışma grubu ortalama yaşları 17,7 yaş olan daimi dentisyonda, iskeletsel Angle sınıf I ilişkiye sahip 98 bireyden (35 erkek, 63 kız) oluşmaktadır. Alt keser inklinasyonu (IMPA), kraniovertikal, kranioservikal ve kraniohorizontal postüral değişkenler, hyoid ve dil pozisyonları doğal baş pozisyonunda alınan lateral sefalogramlar üzerinde değerlendirilmiştir. Baş postürüyle ilişkili olarak alt keser inklinasyonlarını karşılaştırmak için çalışma grubu ikiye ayrılmış (48 kişide ortalama 88,09º olmak üzere IMPA<90º ve 50 kişide ortalama 102,84º olmak üzere IMPA>90º) ve Student-t testi uygulanmıştır.* ***Bulgular:*** *Dikleşmiş alt keserlere sahip grup, labiale eğimli gruba göre daha arka pozisyonda konumlanmış hyoid kemiğine sahiptir (p<0,05). Bununla birlikte yine dikleşmiş keserlere sahip bireylerin ortalama 3-4° daha geniş kranioservikal açılara (NSL/OPT, NSL/CVT, NL/OPT, NL/CVT) sahip olduğu bulunmuştur (p<0,05). Havayolu ölçümleri iki grup arasında anlamlı farklılık göstermemiştir.* ***Sonuç:*** *Çalışmamızın sonuçlarına göre, hyoid kemiği pozisyonu, dil uzunluğu ve kranioservikal baş pozisyonu; dikleşmiş ve labiale eğimli alt keser gruplarında anlamlı farklılıklar göstermiştir.* ***Anahtar Kelimeler:*** *Kesici diş, postür ve hyoid kemik.*

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Original research

EVALUATION OF THE PREVENTIVE AND INTERCEPTIVE ORTHODONTIC TREATMENT NEED OF AN INNER CITY GROUP OF ANATOLIAN TURKISH CHILDREN

ABSTRACT

Objectives: The aim of this study is to examine the oral and dental health among the primary school children in a city in Turkey and to identify the need for preventive and interceptive practices.

Materials and Methods: Our study was carried out on total 334 individuals, which include 135 male and 199 female primary school students between 6 and 9 years of age in Sivas, Turkey. These individuals were examined intraorally in the school environment. In order to determine the need for preventive and interceptive orthodontic treatment, pre-prepared data forms were filled in during the examination and existing problems were recorded. Collected data were evaluated through the program of SPSS (Ver:15.0).

Results: The number of the individuals, who needed at least one of the preventive and interceptive orthodontic treatments was 319 (95.5%). The most required one among these practices was found as fissure sealant with a ratio of 52.1%. When the relationship between age and gender data and other parameters was evaluated, a significant relation was observed only between age and the fissure sealant, space maintainer need and posterior cross bite.

Conclusions: The need for preventive and interceptive treatments was quite high and it is a fact that a special attention should be paid to these applications.

Keywords: Preventive orthodontics, interceptive orthodontics, malocclusion.

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INTRODUCTION

Malocclusion is a defect in the craniofacial complex which effects the development of teeth, jaw and facial area and chewing system.[1] Malocclusion is not a public health threat, however it is a prevalent public health problem.[2] While the preventive orthodontic applications are used to allow the development of normal occlusion and prevent the occurrence of malocclusion,[3] the interceptive orthodontic practices are used for the cases of early stage of malocclusion to allow the development of normal occlusion.[3]

These applications are applied with fixed orthodontic appliances during the primary or early mixed dentitions, and used in order to reduce the length and severity of orthodontic treatments.[4] With the help of these practices, the skeletal malocclusions at early ages could be rehabilitated and the possibility of extraction could be lowered despite the need of orthodontic treatment at later ages. Meanwhile, the length of treatment shortens and risk of enamel decalcification and periodontal disease after the treatment decreases. Accordingly, the treatment satisfaction rates of the parents increase.[5-6] Other advantages of these practices are that they are easier and cheaper than the treatments with fixed orthodontic appliances and could be applied by experienced dentists.[7-9]

Various studies were carried out in order to determine the preventive and interceptive treatment need in other countries and populations.[10,11] Onyeaso *et al.*[10] found out that 60.5% of the study group needed at least one of the preventive and interceptive treatments, and Prabhakar *et al.*[11] found out that 63.5% of the study group needed orthodontic treatment. In these studies, the parameters such as cross bite, caries of primary and permanent teeth, deleterious oral habits, molar relationship, need for a habit breaker were examined.

The need for these treatments should be determined in order to popularize the preventive and interceptive practices and establish the skilled employment. Thus, required social policies can be developed accordingly.[12] When the literature was reviewed, it was found out that there has been no study in Turkish society to identify the need for preventive and interceptive treatments, although a few studies were carried out in order to identify the need for orthodontic treatment.[13,14]

The objective of this study is to predetermine the conditions, effecting the primary school children, which can cause malocclusion at later ages, to identify the need for preventive and interceptive applications and to arrange the necessary guidance by raising an awareness among the parents about the benefits of early intervention.

MATERIALS AND METHODS

Ethical Consideration

Before the screening study, the necessary permission was obtained from the directorates and parents. This study was approved also by the ethics committee. (Ethics committee decision no: 2018-12/13, Date: 05.12.2018)

This study was carried out on 350 children at the ages of 6-9 who have participated in oral health screening studies conducted in the village primary schools in Sivas.

Inclusion criteria

Healthy children of the ages 6-9 were included in the study, who received no orthodontic treatment before and diagnosed with no mental or physical disorder.

Exclusion criteria

Children, who are out of the determined age range, who were diagnosed with any physical or mental disturbance, previously received or still receiving orthodontic treatment were excluded from this study. Accordingly, one child who was diagnosed with a mental disorder and 15 children who were out the age range were excluded from the study, and the study was carried out on total 334 individuals.

Examination

Two certified orthodontists, working blind to each other's findings, examined every child. Before the study, two researchers were trained on the examination method and calibrated. Intra-examiner agreement was set at 93%.

The children in upright sitting position on chair were examined by using mouth mirror and sond. Following parameters were evaluated during the examination: age, gender, DMFT, dft scores, oral hygiene, openbite, deepbite, anterior cross bite, posterior cross bite, anterior crowding, space maintainer need, fissure sealant need, fluorine need, impacted teeth, habit breaker appliance need. Previous studies have been used to construct the parameters.

Need for habit breaker, we look at thumbsucking habit and tongue thrusting. Need for fluorine, we look at the scoring of dmft. It was said that the need for fluoride in caries active mouth with high dmft score. A caliper was used to measure crowding. It is thought that there is a need for treatment in patients with a diameter of more than 7mm crowding. The presence of a negative overjet was described as anterior crossbite. The presence of more than 2-4 mm overbite was described as deepbite.

Statistical Method

Data collected from our study were uploaded to the SPSS program (Ver: 15.0, IBM Corp. New York, USA). Average, standard deviation and frequency distributions were studied in data evaluation. Chi square test was applied in the evaluation of the relationship between results and data of age and gender, and differences of $p<0.05$ were accepted as statistically significant. Inter and intra examiner agreement were evaluated by using the weighted kappa statistics.

RESULTS

Gender distribution of the children included into our study was 135 (40.4%) boys and 199 (59.6%) girls. The average age of the children was found as 7.55. Number of the individuals who needed at least one of the preventive and interceptive treatments was 319 (95.5%).

Average DMFT and dft scores and statuses of oral hygiene can be seen respectively in Table 1 and Figure 1.

Table 1. Distribution of DMFT and dft scores of the individuals

	n	Minimum	Maximum	Mean	SD
DMFT	334	0	8.00	0.34	0.84
dft	334	0	13.00	3.32	3.01

SD: Standart deviation

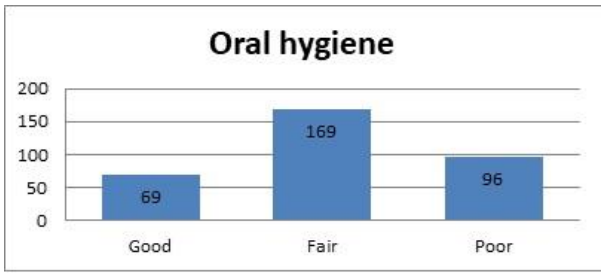

Figure 1. Distribution of participants according to their oral hygiene statuses

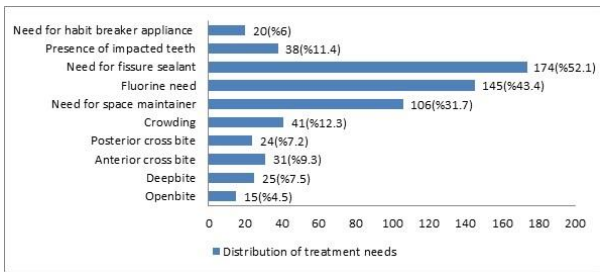

Figure 2. Distribution of participants based on the data obtained from the survey

Distribution of individuals according to the data obtained from the study is shown in Figure 2. Relationships between the results and data of age and gender are shown in Table 2 and Table 3. There is a significant relationship between age and fissure sealant need, space maintainer need and posterior cross bite ($p<0.05$). There is no significant relationship between other parameters and age and gender ($p>0.05$).

Table 2. Distribution of participants' needs for preventive and interceptive treatments by age and gender

Preventive and interceptive applications		**Gender**		**Age**			
Fissure sealant		Female	Male	6	7	8	9
	Presence	104	70	31	37	55	51
	Absence	95	65	46	38	45	31
			p=0.941				p=0.041*
Habit breaker appliance							
	Presence	13	7	2	7	4	7
	Absence	186	128	75	68	96	75
			p=0.648				p=0.194
Fluorine							
Presence		84	61	25	30	49	41
Absence		115	74	52	45	51	41
			p=0.653				p=0.078
Space maintainer							
	Presence	57	49	17	21	46	22
	Absence	142	86	60	54	54	60
			p=0.152				p=0.003*

* Significant at p<0.05

Table 3. Distribution of participants with various malocclusions by age and gender

Malocclusions		**Gender**		**Age**			
Open bite		**Female**	**Male**	**6**	**7**	**8**	**9**
	Presence	10	5	4	4	1	6
	Absence	189	130	73	71	99	76
			p=0.789				p=0.205
Deep bite							
	Presence	11	14	7	4	9	5
	Absence	188	121	70	71	91	77
			p=0.137				p=0.718
Anterior cross bite							
	Presence	20	11	7	10	6	8
	Absence	179	124	70	65	94	74
			p=0.701				p=0.429
Posterior cross bite							
	Presence	16	8	2	2	13	7
	Absence	183	127	75	73	87	85
			p=0.523				p=0.018*
Crowding							
	Presence	25	16	7	10	16	8
	Absence	174	119	70	65	84	74
			p=0.846				p=0.462
Impacted teeth							
	Presence	27	11	13	7	13	5
	Absence	172	124	64	68	87	77
			p=0.160				p=0.161

* Significant at p<0.05

DISCUSSION

Early diagnosis and treatment of malocclusions can prevent the possible future orthodontic disorders. The severity of malocclusions which may occur at later terms can be reduced through early treatments.[15]

Usha Mohandas *et al*.[16] have found that the incidence of any malocclusion and the need for early orthodontic treatment was approximately 63%. The study carried out by Onyeaso *et al*.[17] in Ibadan region of Nigeria has revealed that 27% of the children at the ages of 7-10 needed at least one of the preventive and interceptive treatments. This rate was found in our study as 95.5%. It can be

considered that the reason of this high rate is that the study was carried out in a region of low socioeconomic level.

Burhan *et al*.[18] could not found a significant relationship between the preventive and interceptive treatment need and gender. This finding jibes with the findings of Prabhakar *et al*.[11] and Haider *et al*.[19] However, our study has revealed a significant relationship between age and the needs for fissure sealant and space maintainer.

Presence of untreated caries in primary teeth may result in malocclusion because of the reasons such as shortening of dental arch, deterioration of contact surfaces and early loss of these teeth.[3] Haider's[19] study on 6-year-old children has determined caries or restoration affected at least one surface of primary first molars (37.5%), primary second molars (40.6%), permanent first molars (17.24%) and primary canines (0.0%); these rates in the 9-year-old age group are 61.1%, 66.36%, 21.82% and 0.0%.[19] The scores of DMFT and dft were examined in our study and the average values of 0.347 and 3.326 were found respectively. DMFT score is quite low and dft score is relatively high.

Oral hygiene was classified as good, mean and bad. The objective of oral hygiene examination was to estimate the potential risk of caries. The oral hygiene was determined good for 69 individuals, mean for 169 individuals and bad for 96 individuals. Our study has revealed that the region, where the screening was carried out, needed a training on oral hygiene.

Early loss of primary teeth is considered as the most common local factor of malocclusion. Karaiskos *et al*.[3] carried out a study in Canada in 2005 and determined early primary teeth extraction with a rate of 29.4% in the age group of 9-year-olds and with a rate of 11.9% in the age group of 6-year-olds. Another study carried out in South Africa has revealed a high prevalence for both untreated caries and early extraction of primary teeth.[20] Preservation of the space has a big importance in the presence of early extraction of primary teeth, since the remaining tooth in the posterior of the cavity may use this space. A space maintainer can be used to protect the extraction space. It has been found in our study that 106 individuals (31.7%) needed space maintainer. The reasons for this are that the families do not know the importance of space maintainer and they can not go to the university or hospital to receive health services due to low socioeconomic conditions.

During the evaluation of the need for fissure sealant, we have planned our study on the assumption that fissure sealant application is needed for the individuals with high risk of caries and with colored fissure; and during the evaluation of the need for fluorine application, we have based our study on the fact that the person had a carious-active mouth. In our study, the need for fissure sealant application was found as 52.1% and the need for fluorine application was found as 43.4%.

Oral habits and their potential effects should be determined early.[21] Olatokunbo da Costa *et al*.[12] found that almost half of the population they studied had an oral habit. Büyükbayraktar *et al*.[22] determined oral habits in 62.5% of the girls and 52.1% of the boys. In our study, we have found that 20 individuals (6%) had oral habits and all of them needed habit breaker appliances.

Karaiskos *et al*.[3] determined open bite in 10.0% of the 6-year-old group and 6.7% of the 9-year-old group. In our study, we determined open bite in 15 (4.5%) individuals. Open bite can be observed in the presence of oral habits like thumb-sucking. During the medical history taking in our study, the common reason was determined as thumb-sucking, as well.

Some of the occlusal characteristics (increased overjet, posterior cross-bite, anterior cross-bite, etc.), which occur during the primary dentition and continue during the mixed dentition as well, may cause malocclusion in the early mixed dentition.[23] Posterior cross bite should be treated early, since the presence of posterior cross bite may result in dental malocclusions and skeletal deviations. Likewise, the affected tooth in the presence of anterior cross bite may display periodontal problems such as mobility and

fracture. Grippauda *et al*.[24] found that cross bite was one of the most common malocclusions. Karaiskos *et al*.[3] found a higher prevalence for anterior cross bite than the posterior cross bite. The anterior cross bite was 10.5% in the group of 6-year-olds and 11.9% in the group of 9-year-olds, however the posterior cross bite was 7.8% in the group of 9-year-olds and 3.0% in the group of 6-year-olds. Similarly, Olatokunbo da Costa *et al*.[12] found a prevalence for anterior cross bite higher than the posterior cross bite. In our study, the prevalence for anterior cross bite (9.3%) was higher than the prevalence of posterior cross bite (7.2%) as well.

A study carried out in Lagos University Teaching Hospital has revealed that the most common dental feature was anterior and posterior crowding.[25] In another study, severe crowding (> 7mm) in the upper teeth was highest (30.5%) in Class II division 2 malocclusion group.[26] The most common dental feature determined in our study was crowding with a rate of 41%.

Prabhakar *et al*.[11] found deep bite in 56 and impacted teeth in 15 of 337 individuals. In our study, the incidences of impacted teeth and deep bite were 38 (11.4%) and 25 (41%) respectively.

This study has several limitations. Firstly, X-rays could not be obtained during the examination due to the ethical principles and physical conditions, therefore congenital missing teeth, supernumerary teeth and different dental anomalies could not be determined.

Number of the patients examined is limited and the results concern only one region. In order to generalize the results, more extensive studies should be carried out in larger patient populations.

CONCLUSIONS

This study has revealed a very high prevalence of preventive and interceptive treatment need, therefore not only corrective treatments but also preventive and interceptive treatments should be given more importance. In the light of these findings, we believe that the preventive and interceptive practices should be included in the educational health programs and the families should be informed about the importance of this issue.

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None.

CONFLICT OF INTERESTS STATEMENT

None.

Belli Bir Yöreye Ait Türk Çocuklarında Koruyucu ve Durdurucu Ortodontik Tedavi İhtiyacının Değerlendirilmesi

ÖZ

Amaç: *Bu çalışmanın amacı Sivas ili ilköğretim çocuklarında ağız ve diş sağlığı ile ilgili değerlendirme yapmak ve koruyucu ve durdurucu uygulamalara olan ihtiyacı belirlemektir.* ***Gereç ve Yöntemler:*** *Çalışmamız Sivas ili ilköğretim okullarında eğitim gören, 6 ile 9 yaş arası 135 erkek ve 199 kız toplam 334 birey üzerinde yürütülmüştür. Bireyler okul ortamında ayna ve sond kullanılarak muayene edilmiştir. Koruyucu ve durdurucu ortodontik tedavi ihtiyacını belirleyebilmek için önceden hazırlanmış olan veri formları muayene sırasında doldurularak mevcut problemler kaydedilmiştir. Elde edilen veriler SPSS (Ver:22.0) programında değerlendirilmiştir.* ***Bulgular****: Koruyucu ve durdurucu tedavilerden en az birine ihtiyaç duyan bireylerin sayısı 319 (%95,5) olarak bulunmuştur. Bu uygulamalar içerisinde %52,1 oranıyla en çok fissür örtücüye ihtiyaç duyulmaktadır. Yaş ve cinsiyet verilerinin diğer parametrelerle ilişkisine bakıldığında sadece yaş ile fissür örtücü, yer tutucu ihtiyacı ve posterior cross bite arasında anlamlı ilişki bulunmuştur.* ***Sonuçlar****: Koruyucu ve durdurucu tedavilere duyulan ihtiyacın yüksek olması, bu uygulamalara özel önem verilmesi gerektiğini göstermektedir.* ***Anahtar Kelimeler****: Koruyucu ortodonti, durdurucu ortodonti, maloklüzyon.*

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Original research

DETERMINATION OF THE EFFECTIVE TIME OF DENTURE CLEANSER TABLETS ON THE REMOVAL OF *CANDIDA ALBICANS* ON DENTURE BASE RESINS

Yeliz Hayran[1]

ABSTRACT

Objective: The aim of this study was to determinate of the effective time of different denture cleanser tablets on the removal of *Candida albicans* on various base resins.

Materials and Methods: Conventional heat cured resin, high impact resin, autopolymerized resin, and polyamide resin were used. 160 samples were prepared for each resin type. The biofilm of *C. albicans* was formed on the resins and then exposed to alkaline peroxide tablet and enzymatic tablets for 3, 5, 10, 20, 40, 80, 160 and 200 minutes. Cell viability was assessed by MTT test. 3-way ANOVA was used for statistical analysis.

Results: The effect of resin type, tablet type and application time on cell viability of *C. albicans* were found to be significant (p<0.05). Cell death was at least 65% on the resins even at the minimum time. Furthermore, as the duration of administration of both tablets increased, cell viability in all resins tended to decrease. The administration of both tablets on all resins for 10 min resulted in approximately 80% cell death. Additionally, the most significant antimicrobial activities of the tablets were determined at 20th minutes. The alkaline peroxide tablet on all base resins for application periods of 3 to 200 minutes was generally more effective than the enzymatic tablet.

Conclusions: Alkaline peroxide and enzymatic cleanser tablets showed remarkable anticandidal activity for all resins. The patients with risk of *C. albicans* infection should keep their prostheses in cleanser tablets for at least 20 minutes. Furthermore, the anticandidal effect tended to increase with prolonged exposure time.

Keywords: Denture bases, denture cleansers, candida albicans, cell survival.

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INTRODUCTION

The most commonly used base resin material in dentistry is polymethylmethacrylate (PMMA) resin materials. However, it has some disadvantages such as low flexural and impact strength.[1] Therefore, alternative materials such as polyamide resin and high-impact resins have been developed to obtain a denture base with better mechanical properties. High impact acrylic resin has high resistance to falls[2] and polyamide thermoplastic resin is more elastic than PMMA.[3] In cases where the heat cured PMMA denture base needs to be repaired for reasons such as fracture and tooth replacement, autopolymerized PMMA resins are used.

The oral cavity has a rich and diverse microorganisms.[4] Although the denture base resins appeared to be visually smooth, they were shown to have small pits for the attachment of microorganisms when examined under a microscope.[5] Therefore, denture base resins provide a suitable area for the growth of biofilm.[5-11] Microorganisms hold less on polished surfaces than rough surfaces.[12]

C. albicans is the most common microorganism of denture plaque and causes prosthetic stomatitis in patients with a removable prosthesis.[7,13] The interactions between microorganisms contribute to the development of biofilm communities containing mixed microorganisms.[14] In this way, the developed biofilm is more complex and difficult to remove.[6-8] *Candida* species adhere to the internal or external surfaces of the denture base resin with hydrophobic and electrostatic forces and can be transferred to distinct parts of the oral mucosa after adherence.[15]

In the absence of oral hygiene, microorganisms cause many systemic diseases other than oral diseases.[13] The microbial plaque on the prosthesis must be effectively removed to prevent these diseases. Various methods of denture cleaning have been proposed, including mechanical, chemical, or a combination of mechanical and chemical. Mechanical cleaning includes brushing, while chemical cleaning includes various disinfectants, solutions, and denture cleansing tablets.[16, 17]

Tooth loss generally increase with age[18], and the individuals using removable prostheses are mostly elderly individuals.[19] With increasing age, motor function slows down, and movement limitation occurs. Therefore, mechanically cleaning the dentures is difficult for the elderly, disabled or patients with motor dysfunction.[6] Mechanical cleaning by brush may also cause scratches on the base, which may lead to the attachment of more microorganisms.[6] Therefore, chemical cleaning which can be achieved by using denture cleansers should be considered. Hence, denture cleansing tablets are recommended for the elderly and patients with motor function impairment.[20,21]

Numerous studies have been conducted to determine the effectiveness of cleansing tablets to remove various microorganisms on prostheses.[7,8,22] These studies showed that denture cleanser tablets had significant anticandidal activity, but *C. albicans* biofilm on the resin could not be eliminated entirely. In the studies performed, anticandidal activity was evaluated for random times. However, the application time of the tablets may also be significant in removing microorganisms. No standardization for the duration of application of the tablets exists, and varying periods, from three minutes to overnight usage, are recommended by the manufacturer.

This study aimed to firstly evaluate the effect of two types of heat cured PMMA resins; autopolymerized PMMA and polyamide resin, on *C. albicans* biofilm formations, and secondly to determine the application time effect of two types of denture cleanser tablets for periods ranging from 3 to 200 minutes to remove *C. albicans* biofilm formations. No comprehensive research had previously been conducted to determine the effective duration of tablets used to remove *C. albicans.*

The null hypotheses were that: 1) resin type would affect the amount of microorganism attachment, 2) denture cleanser tablet type would affect the amount of microorganism attachment, and 3) the cell viability of microorganisms would decrease with an increasing application time of tablets.

MATERIAL AND METHODS

Sample preparation

Four different types of resins were used in the study including heat cured conventional PMMA resin (QC-20, Dentsply, Addleston, UK), high impact PMMA resin (Acron-hi, Kemdent, Swindon, UK), autopolymerized PMMA resin (Meliodent Cold; Heraeus Kulzer, Hanau, Germany), and polyamide resin (Deflex, Nuxen SRL, Buenos Ares, AR). The samples were prepared in the form of a disc with a diameter of 10 mm and a thickness of 2 mm following the instructions of the manufacturers (n=160 per resin). The type of resins used in the study are shown in Table 1.

Table 1 Description of tested denture base materials

Trade Name	Material	Processing Method	Manufacturer
Acron-hi	PMMA	Heat cure compression molded	Kemdent, Swindon, UK
Deflex	Polyamide	Injection molded	Nuxen SRL, Buenos Ares, Argentina
Meliodent-cold	PMMA	Cold cure compression molded	Heraeus Kulzer, Hanau, Germany
Qc-20	PMMA	Heat cure compression molded	Dentsply, Addleston, UK

All resin samples were left in the distilled water for 24 hours for residual monomer release after the polymerization. The samples were then smoothen with 600, 800 and 1,000 grit of sandpaper. A profilometer device (Taylor Hobson, Surtronic 25, Leicester, UK) was used to assess the surface roughness (Ra). Measurements were made from three different points of each resin surface, and these readings were averaged. The Ra of all samples were standardized at 0.32 ± 0.02 μm. Before microbial contamination, the sterilization of the samples was performed using an ultrasonic device (Pro-Sonic 600, Sultan Healthcare, Hackensack, NJ) in a distilled water at 50 °C, at 28 kHz for 10 minutes.

Microorganism cultures and growth conditions

C. albicans (ATCC 1023) strain was incubated for 24 h at Sabouraud dextrose agar at 35°C. Twenty-four hours later, the fungal concentration was prepared using RPMI-2% glucose broth and counted using the Neubauer chamber and trypan blue dye to obtain a final concentration of 1-5x10^{6}cell/mL. To produce fungal biofilm on resin samples, the resin and cells were transferred to 24-well polystyrene microtiter plates containing RPMI-2% glucose broth and allowed to grow for 48 hours at 35 °C in a shaker incubator at 150 rpm.

Test tablets

Alkaline peroxide denture cleanser tablet (CoregaTM, GlaxoSmithKline Healthcare, İstanbul, Türkiye) and neutral enzymatic denture cleanser tablet (Polident3minTM, GlaxoSmithKline Healthcare, Moon Township, PA) were prepared following the manufacturer's instructions. The cleansing tablets used in the study are shown in Table 2. For cleaning working solutions, each tablet was dissolved in 150 mL of warm distilled water, and the solution was used immediately.

Table 2. Denture cleanser tablets used in the study

Trade Name	Denture cleanser	Ingredients	Manufacturer
Polident 3 min™	Enzymatic tablet	Sodium perborate, potassium monopersulfate, proteolytic enzyme, detergent and effervescent base	GlaxoSmithKline Healthcare, Moon Township, USA
Corega™	Alkaline peroxide tablet	Potassium monopersulfate, sodium bicarbonate, sodium lauryl sulfoacetate, sodium perborate monohydrate, sodium polyphosphate	GlaxoSmithKline Healthcare, Istanbul, Turkey

Susceptibility testing

The number of living cells was determined by MTT analysis. MTT analysis was performed according to AFST-EUCAST standards. The MTT stock solution (5 mg MTT/ml distilled water) was sterilized by filtration and kept at -20°C until use.

Firstly, the biofilm was grown as previously described. Following incubation of 48 hours, the old medium in the wells was removed, and the cells in the biofilm were exposed to 200 µL of the alkaline peroxide tablet solution and enzymatic tablet solution for 3, 5, 10, 20, 40, 60, 80, 160 and 200 minutes. The cleanser solutions were then replaced with fresh RPMI-2% glucose liquid medium containing MTT (final concentration, 0.5 mg/mL). The mixture was incubated with shaking for 4 hours (150 rpm at 35°C). After the incubation period, 180 µL of the medium was removed. 30 µL of Sorenson buffer and 150 µL of DMSO were added to the well, and the plate was vortexed for 5 minutes. The optical density of the sample and blanks (DMSO with Sorenson's buffer) was measured with a spectrophotometer at 660 nm to 560 nm as the reference range. Percentage of viability was calculated using Microsoft Excel software (Washington, USA).

Statistical analysis

Three-way ANOVA was used to compare the continuous data between/among groups. Analyses were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY).

RESULTS

This study evaluated the antimicrobial activity of two different denture cleansing tablets against *C. albicans* on four different resins and determined the cell viability percentages corresponding to the times of treatment with the tablet solution ranging from 3 to 200 minutes. The mean values and standard deviations of cell viability on acrylic resins for all applied times of cleanser solutions are shown in Table 3. Both tablets were effective against *C. albicans* on all denture base resins at all application times. Cell death occurred in at least 65% of the microorganisms on the resins even at the 3rd minute. The administration of both tablets on all resins for 10 min and over resulted in approximately 80% cell death. As the duration of administration of both tablets increased, a tendency to decrease in cell viability was observed on all resins. The alkaline peroxide tablet on all base resins for application periods of 3 to 200 minutes was generally more effective than the enzymatic tablet. Resin type was found to be effective in *C. albicans* adhesion (p<0.05). Cell viability values for alkaline peroxide tablet and enzymatic tablet for 200 minutes were 8% and 10% for high impact resin, 8% and 12% for polyamide resin, 8% and 11% for autopolymerized resin, 8% and 8% for conventional heat cured resin, respectively. Regardless of time and type of tablet, cell adhesion from high to low was observed in autopolymerized resin, polyamide resin, high impact resin, and heat cured resin, respectively.

Cell viability values of microorganisms cultured on high impact resin

Comparison of times for alkaline peroxide tablets

The cell viability of *C. albicans* on high impact resin exposed to alkaline peroxide tablet was 32% at the 3rd minute and decreased to 30% at the 5th minute, 21% at the 10th minute,10% at the 20th minute (p<0.05) (Table 3) (Figure 1). There was no statistical difference between the cell viability at 20th, 40th, 80th, 160th and 200th minutes (p>0.05). Thus, optimal treatment time for high impact resin to be exposed to alkaline peroxide tablet can be considered as 20 minutes (10%).

Comparison of times for enzymatic tablets

When cell viability results of *C. albicans* on high impact resin exposed to enzymatic tablet were evaluated, the cell viability was 35% at the 3rd minute, decreased to 31% at the 5th minute and 13% at the 10th minute (p <0.05) (Table 3) (Figure 2). The comparisons of 10th vs. 20th, 20th vs. 40th, and 80th vs. 160th, and 200th minutes were found to be insignificant (p>0.05). However, the cell viability at the 200th minute was 10%. Although there is a statistically significant difference between the 40th minute and 200th minute, the difference is not clinically significant. The optimal treatment time for high impact resin can be considered as 40 minutes (12%) for enzymatic tablets.

Table 3. The cell viability of *C. albicans* for all application periods on all resins

Resins	Time (minute)	(Cell viability %) Corega™ (n=10) Mean±SD	Polident 3 min™ (n=10) Mean±SD	Total Mean±SD
High impact resin	3	32.28±0.38(a,x,1)	35.14±0.40(a,y,1)	33.71±1.68 (a,1)
	5	29.73±0.31(a,x,2)	31.13±0.33(a,y,2)	30.43±0.86 (a,2)
	10	21.25±0.29(a,x,3)	13.22±0.36(a,y,3)	17.23±4.65 (a,3)
	20	9.53±0.35(a,x,4)	12.58±0.38(a,y,34)	11.05±1.78 (a,4)
	40	9.15±0.42(a,x,4)	12.01±0.27(a,y,4)	10.58±1.72 (a,45)
	80	8.96±0.25(a,x,4)	10.89±0.32(a,y,5)	9.92±1.15 (a,5)
	160	8.77±0.39(a,x,4)	10.14±0.34(a,y,5)	9.45±0.84 (a,56)
	200	8.39±0.36(a,x,4)	9.76±0.41(a,y,5)	9.07±0.87 (a,6)
	Total	16.01±9.87(a,x)	16.86±9.83(a,y)	16.43±9.7 (a)
Polyamide resin	3	38.93±0.31(b,x,1)	35.41±0.35(a,y,1)	37.17±2.05 (b,1)
	5	31.29±0.36(b,x,2)	34.35±0.38 (b,y,1)	32.82±1.79(b,2)
	10	21.75±0.22(a,x,3)	13.14±0.25(a,y,2)	17.44±4.98(a,3)
	20	8.77±0.27(ab,x,4)	12.77±0.31(a,y,23)	10.77±2.33(a,4)
	40	8.58±0.28(ab,x,4)	12.39±0.34(a,y,234)	10.48±2.22(a,45)
	80	8.39±0.33(ab,x,4)	12.01±0.42(b,y,234)	10.2±2.11(a,45)
	160	8.19±0.37(ac,x,4)	11.64±0.34(b,y,34)	9.92±2.01(a,5)
	200	8±0.36(a,x,4)	11.56±0.36(b,y,4)	9.78±2.08(b,5)
	Total	16.74±11.99(b,x)	17.91±10.14(b,y)	17.32±10.94 (b)
Autopolymerize resin	3	33.2±0.22(a,x,1)	30.79±0.26(b,y,1)	31.99±1.42 (c,1)
	5	24.42±0.32(c,x,2)	28.53±0.36(c,y,2)	26.48±2.39(c,2)
	10	23.66±0.35(b,x,2)	23.28±0.39(b,x,3)	23.47±0.36(b,3)
	20	8±0.25(b,x,3)	21.4±0.27(b,y,4)	14.7±7.74(b,4)
	40	7.97±0.4(b,x,3)	19.52±0.33(b,y,5)	13.74±6.68(b,5)
	80	7.81±0.37(b,x,3)	12.54±0.35(b,y,6)	10.18±2.75(a,6)
	160	7.62±0.26(c,x,3)	11.75±0.29(b,y,67)	9.69±2.4(a,6)
	200	7.62±0.32(a,x,3)	11.26±0.39(b,y,7)	9.44±2.12(ab,6)
	Total	15.04±10.03(c,x)	19.88±7.33(c,y)	17.46±8.98(b)
Conventional heat cured resin	3	35.11±0.3(c,x,1)	34.58±0.35(a,x,1)	34.84±0.42(d,1)
	5	31.29±0.36(b,x,2)	29.14±0.44(c,y,2)	30.21±1.28(a,2)
	10	9.15±0.22(c,x,3)	11.71±0.32(c,y,3)	10.43±1.51(c,3)
	20	8.96±0.46(ab,x,3)	10.89±0.41(c,y,34)	9.92±1.15(c,34)
	40	8.77±0.26(ab,x,3)	10.14±0.34(c,y,45)	9.45±0.84(c,45)
	80	8.58±0.28(ab,x,3)	9.01±0.31(c,x,56)	8.79±0.38(b,56)
	160	8.39±0.46(ac,x,3)	8.63±0.36(c,x,6)	8.51±0.32(b,6)
	200	8.35±0.25(a,x,3)	8.26±0.27(c,x,6)	8.3±0.29(c,6)
	Total	14.82±11.01(c,x)	15.29±10.04(d,y)	15.06±10.37(c)

*a, b, c: Polyamide vs. PMMA, intergroup comparison for interaction and main effects.
x, y: Comparison of denture cleanser tablets for interaction and main effects.
1, 2, 3, 4, 5, 6: Comparison of times for interaction and main effects.
Three-way ANOVA was used, $p<0.05$ was considered significant. Different letters indicate difference with statistical significance.

Cell viability of microorganisms cultured on polyamide resin

Comparison of times for alkaline peroxide tablets

When cell viability results of *C. albicans* on polyamide resin exposed to alkaline peroxide tablet were evaluated, the cell viability of the 3rd minute decreased from 39% to 31% at the 5th minute, to 21% at the 10th minute and 9% at the 20th minute ($p<0.05$) (Table 3) (Figure 1). There was no statistical difference between the cell viability of 40th, 80th, 160th and 200th minutes of cell viability compared to 20th minutes ($p>0.05$). Besides, cell viability at the 200th minute was 8%. The optimal treatment time for polyamide resin for alkaline peroxide tablet can be considered as 20 minutes (9%).

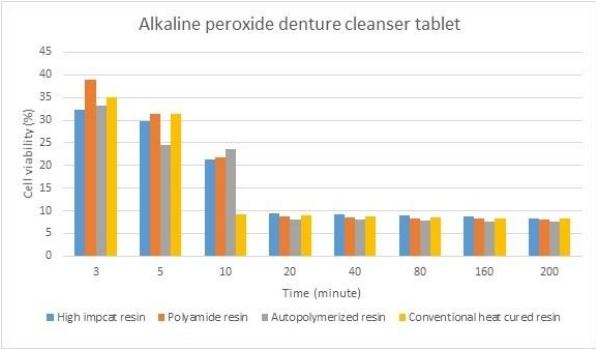

Figure 1: The graphic illustration of the time-dependent efficacy of alkaline peroxide denture cleanser tablets in removal of *Candida albicans* on denture base resins

Comparison of durations for enzymatic tablets

When cell viability results of *C. albicans* on polyamide resin exposed to enzymatic tablet were evaluated, cell viability at 3rd minute decreased from 35% to 13% at the 10th minute ($p<0.05$). No statistical differences were observed in the comparison of 3rd vs. 5th, 10th vs. 20th, 40th, 80th, 20th vs. 40th, 80th, 160th, and 160th vs. 200th ($p>0.05$) (Table 3) (Figure 2). Although there is a statistically significant difference between the 10th minute and 200th minute, this difference does not have clinical importance and can be neglected. Optimal treatment duration for polyamide resin in the enzymatic tablet can be considered as 10 minutes with 13% cell viability.

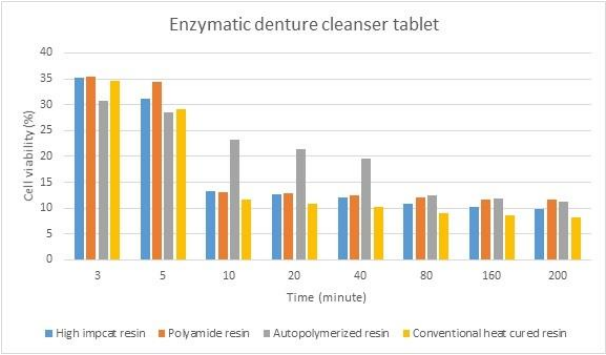

Figure 2: The graphic illustration of the time-dependent efficacy of enzymatic denture cleanser tablets in removal of Candida albicans on denture base resins

Cell viability of microorganisms cultured on autopolymerized resin

Comparison of times for alkaline peroxide tablets

When cell viability results of *C. albicans* on autopolymerized resin exposed to alkaline peroxide tablet were evaluated, the cell viability at the 3rd minute decreased from 33% to 24% at the 5th minute and 8% at the 20th minute ($p<0.05$) (Table 3) (Figure 1). There was no statistically significant difference in cell viability between 5th vs.10th, 20th, 40th, 80th, 160th, and 200th minute ($p>0.05$). The optimal treatment time for autopolymerized resin in alkaline peroxide tablet can be considered as 20 minutes (8%).

Comparison of durations for enzymatic tablets

When cell viability results of *C. albicans* on autopolymerized resin exposed to enzymatic tablet were evaluated, cell viability at the 3rd minute decreased from 30% to 28% in the 5th minute, to 23% at the 10th minute, to 21% at the 20th minute, to 19% at the 40th minute, 12% at 80th minute and to 11% at 200th minute ($p<0.05$) (Table 3) (Figure 2). There was no statistical difference in comparisons of 80th vs. 160th minute and 160th vs. 200th minute ($p>0.05$). Although there is a statistically significant difference between the 80th minute and 200th minute, the difference is not clinically significant. The optimal treatment time for autopolymerized resin can be considered as 80 minutes (12%) for enzymatic tablets.

Cell viability values of microorganisms cultured on heat cured resin

Comparison of times for alkaline peroxide tablets

When cell viability results of *C. albicans* on heat cured resin exposed to alkaline peroxide tablet were evaluated, the cell viability at the 3rd minute decreased from 35% to 31% at the 5th minute, and 9% at the 10th minute ($p<0.05$) (Table 3) (Figure 1). There was no statistically significant difference between cell viability at 10th, 20th, 40th, 80th, 160th and 200th minute ($p>0.05$). Furthermore, cell viability at the 200th minute was 8%. The clinically optimal treatment time for heat cured resin for alkaline peroxide tablet can be considered as 10 minutes (9%).

Comparison of durations for enzymatic tablets

When cell viability results of *C. albicans* on heat cured resin exposed to enzymatic tablet were evaluated, the cell viability at 3rd minute decreased from 35% to 29% at 5th minute, to 12% at 10th minute, 10% at 40th minute, and to 7% at 160th minutes ($p<0.05$). There was no statistical difference in comparisons of 10th vs. 20th minute, 20th vs. 40th minute, 40thvs. 80th, 160th and 200th minutes ($p>0.05$) (Table 3) (Figure 2). Although there is a statistically significant difference

between the 20th minute and 200th minute, this difference is not clinically significant and the clinically optimal treatment time for heat cured resin for enzymatic tablet can be considered as 20 minutes (11%).

Comparison of tablets

Comparison of tablets for high impact resin

The tablets statistically differed for all application times (p<0.05) (Table 3). The anticandidal efficiency of alkaline peroxide tablet was higher than the enzymatic tablet for all the times except for the 10th minute.

Comparison of tablets for polyamide resin

The tablets statistically differed for all application times (p<0.05) (Table 3). The anticandidal efficiency of alkaline peroxide tablet was higher than the enzymatic tablet for all the times except for the 3rd minute.

Comparison of tablets for autopolymerized resin

The anticandidal efficiency of alkaline peroxide tablet was higher than the enzymatic tablet for all the times except for the 3rd minute. However, there was no statistically significant difference between the tablets at the 10th minutes (p>0.05) (Table 3).

Comparison of tablets for heat cured resin

The anticandidal efficiency of alkaline peroxide tablet was higher than the enzymatic tablet for all the times except for the 5th minute. However, there was no statistically significant difference between the tablets at the 3rd, 80th, 160th and 200th minutes (p>0.05) (Table 3).

DISCUSSION

The present study determined the anticandidal efficacy of two different denture cleanser tablets against *C. albicans*. The effective time of different denture cleanser tablets in the removal of *C. albicans* on various base resins was determined on four different types of denture base resins. All hypotheses were accepted. The type of the acrylic resin, type of denture cleanser tablet and duration of the tablet treatment were found to be effective on cell viability of *C. albicans*. Both tablets showed a high anticandidal effect at all the tested durations. Cell death was seen at least 65% on the resins even at the minimum time. The optimal treatment duration for the alkaline peroxide tablet and enzymatic tablets were 20 and 40 minutes for high impact resin, 20 and 10 minutes for polyamide resin, 20 and 80 minutes for autopolymerized resin, and 10 and 20 minutes for heat cured resin. However, the anticandidal effect tended to increase with prolonged exposure time.

Rough surfaces are known to cause increased microbial colonization.[23, 24] Initially, the roughness of the resin surfaces was standardized to 0.32±0.02 μm in order to evaluate the anticandidal effect of alkaline peroxide denture cleanser and neutral enzymatic peroxide denture cleanser on four different denture base resins. Polyamide resin surfaces were generally reported to be rougher than PMMA resins.[8, 25] Although the surface roughness of all the resins were equalized, the cell viability percentages on the resins showed differences according to the resin groups. Regardless of the tablet type and duration, the minimum cell viability was observed in the conventional heat cured resin group. Conventional heat cured resin was followed by high impact resin, polyamide resin and autopolymerized resins respectively.

It has been suggested that the primary factor in the connection of microorganisms to the denture base surfaces is the surface roughness.[24] However, the results of the present study showed that not the only factor that is effective in the amount of *C. albicans* that is formed on the resin is surface roughness and that resin type plays an important role in the attachment of microorganisms. As in this study, it has been shown by other in vitro studies that resin variety is effective in the attachment of microorganisms.[8,10,26] Physical and chemical properties of denture base resins may play a role in the colonization of microorganisms.[27-29] In addition to the physical and chemical properties of the material, characteristic of the microorganism is also effective in the adhesion of microorganisms to the resin surface.[10,30] In this context, Radford et al. suggested that the adherence of *C. albicans* to the prosthetic base resin was due to the hydrophobicity of the organism.[28]

The manufacturers have recommended the minimum time required for antimicrobial activity of their denture cleanser tablets. The recommended

minimum time for Polident 3 min™ enzymatic tablet is 3 minutes, while the recommended minimum time for Corega™ alkaline peroxide tablet is 5 minutes. However, the dentures may be exposed to the tablet solution overnight; but, it has been shown that dentures that have been kept in the denture cleansing agent overnight is damaged.[31, 32] It is essential to determine the optimal duration of administration of these tablets to reduce or eliminate oral microorganisms without damaging the prosthesis. Therefore, in order to determine the most effective duration of tablets in our study, periods ranging from 3 to 200 minutes were studied. In light of the results of the present study, it was determined that the type of cleansing tablet and the application time were also crucial in the reduction or destruction of microorganisms. Tablet solutions significantly reduced the microorganisms on the resins during all their application times. This result also confirms the results from previous studies[6, 8, 26, 33] Both denture cleanser tablets were shown to exhibit high anticandidal activity overall base resins at application times of 10 minutes or more. As the duration of administration increased, cell viability tended to decrease. The cell viability of all resins was approximately 30% after 3 minutes of administration in both tablets, and after 200 minutes of administration, all resins had less than 10% cell viability. However, *C. albicans* could not be removed entirely. Denture cleanser tablets have an antimicrobial effect, but *C. albicans* biofilms show high antifungal resistance.[34, 35] This result confirms that *C. albicans* is a persistent infectious agent. In many studies, as in the present study, denture cleanser tablets have a significant reduction in cell viability of *C. albicans*, but not all microorganisms have been removed.[6-8, 10, 36]

According to the results of the present study, it was found that the type of tablet was as crucial as the application period of the tablets in the reduction of microorganisms. After 3 minutes exposure of the resins to the alkaline peroxide tablet solution, cell viability percentages were determined as high impact resin-autopolymerized resin-conventional heat cured resin-polyamide resin from small to large. After 3 minutes exposure of the resins to the enzymatic tablet solution, cell viability percentages were determined as autopolymerized resin-conventional heat cured resin-high impact resin-polyamide from small to large.

It was determined that the alkaline peroxide tablet on all base resins for application periods of 3 to 200 minutes was generally more effective than the enzymatic tablet. These results showed that the antimicrobial activity of denture cleanser tablets varied according to the resin type. Several studies have been conducted to evaluate the efficacy of denture cleanser tablets in the removal of *C. albicans* on denture base resins.[8-10] As in the present study, Hayran et al. reported that the antimicrobial activity of the alkaline peroxide tablet on high impact resin and polyamide resin was higher than the enzymatic tablet.[10] Fernandes et al. reported that the alkaline peroxide tablet was more effective on the conventional heat cured PMMA based resin and the enzymatic tablet was more effective on the polyamide-based resin.[22] In another study, it was emphasized that enzymatic tablet was more effective on conventional heat cured PMMA based resins than polyamide based resins.[8] However, it is not possible to compare the other studies directly with this study because of the variety of the resins studied, the different types of tablets used, the varying application times and the selection of different methods in the evaluation of cell viability.

This study showed that both alkaline peroxide and neutral enzymatic denture cleanser tablets exhibit potent antimicrobial activity over all the prosthetic base resins studied and for all selected application times. Cell viability tended to decrease as the application time increased from 3 minutes to 200 minutes. However, complete inhibition of *C. albicans* biofilm formed on all resins for both tablets could not be achieved within any time. Although *C. albicans* is a persistent infectious agent, observed death at least 65% of the cells on all resins in a short period such as 3 minutes indicates the antimicrobial efficacy of the tablets. The results also showed that cell viability varies depending on the resin type, tablet type and the duration of administration of the tablets.

CONCLUSIONS

It is an advantage for patients to perform the cleaning procedure of removable prosthesis in a short time without damaging the prosthesis. The results showed the significant anticandidal activity of alkaline peroxide and enzymatic denture cleanser tablets on all resins. As the duration of administration of both tablets increased, a tendency to decrease in cell viability was observed on all resins. Cell death was at least 65% on the resins even at the 3rd minute. The administration of both tablets on all resins for 10 min resulted in approximately 80% cell death. However, the most significant antimicrobial activities of the tablets were determined at 20th minutes. Keeping the prostheses in a tablet solution for more than 20 minutes did not cause significant changes in cell viability. The alkaline peroxide tablet on all base resins for application periods of 3 to 200 minutes was generally more effective than the enzymatic tablet.

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CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

Protez Kaide Rezinleri Üzerindeki Candida Albicans'ın Uzaklaştırılmasında Protez Temizleyici Tabletlerin Etkin Sürelerinin Belirlenmesi

ÖZ

Amaç: *Bu çalışmanın amacı, çeşitli protez kaide rezinleri üzerindeki Candida albicans'ın uzaklaştırılmasında farklı protez temizleyici tabletlerin etkin sürelerinin belirlenmesidir.* ***Gereç ve Yöntemler:*** *Çalışmada geleneksel ısıyla polimerize olan rezin, yüksek çarpma dayanıklı rezin, otopolimerize rezin ve poliamid rezin kullanıldı. Her rezin tipi için 160 numune hazırlandı. C. albicans'ın biyofilmi rezinler üzerinde oluşturuldu ve daha sonra 3-5-10-20-40-80-160-200 dakika boyunca alkalin peroksit tablet ve enzimatik tablete maruz bırakıldı. Hücre canlılığı MTT testi ile değerlendirildi. İstatistiksel analiz için 3 yönlü ANOVA kullanıldı.* ***Bulgular:*** *Rezin tipi, tablet tipi ve uygulama süresinin rezinler üzerindeki C. albicans'ın hücre canlılığı üzerine etkisi anlamlı bulundu (p<0,05). Minimum sürede bile rezinlerde en az %65 hücre ölümü meydana geldi. Bununla birlikte, her iki tabletin uygulama süresi arttıkça, tüm rezinlerdeki hücre canlılığı azalma eğilimi gösterdi. Rezinlerin her iki tablete de 10 dakika maruz bırakılması tüm rezinlerde yaklaşık %80 hücre ölümüne neden oldu. Bununla birlikte, tabletlerin en önemli antimikrobiyal aktiviteleri 20. dakika da görülmüştür. Alkalin peroksit tablet, tüm protez kaide rezinleri üzerinde 3 ila 200 dakikalık uygulama süreleri için genel olarak enzimatik tabletten daha etkili olmuştur.* ***Sonuçlar:*** *Alkalin peroksit ve enzimatik temizleyici tabletler, tüm rezinler için kayda değer bir antikandidal aktivite göstermiştir. C. albicans enfeksiyonu riski taşıyan hastalar protezlerini temizleyici tabletlerde en az 20 dakika tutmalıdır. Bununla birlikte, antikandidal etki tablet süresinin uzaması ile artma eğilimindedir.* ***Anahtar Kelimeler****: Protez kaideleri, protez temizleyicileri, kandida albicans, hücre canlılığı.*

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Original research

COMPARISON OF THE MICROTENSILE BOND STRENGTH OF FOUR DIFFERENT BULK-FILL RESIN COMPOSITES OF CLASS I CAVITIES WITH DENTIN

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ABSTRACT

Objectives: The aim of this study is to compare the microtensile bond strengths of four different bulk-fill composites and a microhybrid composite to the dentin.

Materials and methods: 4 bulk-fill composites and a microhybrid composite were tested. Class I occlusal cavities were prepared on 25 freshly drawn intact human molar teeth, randomly divided into five groups [G-aenial posterior (Control group), Estelite Bulk Fill flow, GrandioSO x-tra, Beautifil Bulk Restorative, Fill-Up] and the cavities were restorated with five composite systems by using their own adhesive systems. Completed restoration teeth bukkolingual and mesiodistal sections were taken, three samples were obtained from each tooth. Microtensile bond strength values of groups were measured by universal test machine. Microscopic changes were examined by SEM. The data were evaluated by one-way ANOVA and Tukey tests.

Results: While the control group showed the highest microtensile bond strength, Fill-Up group showed the lowest. The control group was statistically significant when compared to the four different bulk-fill groups used in the study ($p<0.05$). Although the GrandioSO x-tra group showed the highest bonding strength among the bulk-fill composites, the differences between the Beautifil Bulk Restorative and Estelite Bulk Fill flow were statistically insignificant ($p>0.05$). Fill-Up was found to be statistically significant when compared to the other bulk-fill groups ($p<0.05$). In SEM analyzes a regular gap is seen along the hybrid layer only in Estelite Bulk Fill flow.

Conclusions: No bulk-fill composite group could reach the value of the bond strength of microhybrid composite. Although the Fill-Up group was used with the etch and rinse adhesive system among the bulk-fill composites, it showed the lowest bond strength.

Keywords: Bulk-fill composites, microtensile bond strength, dentin.

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INTRODUCTION

Nowadays, composite resins in dentistry are clinically frequently applied with the development of composite resin and adhesive systems over the years and increased aesthetic expectations of patients.[1] The popularity of light curing composites is due to their aesthetic, biocompatibility and wear resistance properties. However, it is still an unsolved problem to keep the polymerization shrinkage and the clinical performance of restoration in the resin composite restorations. Different clinical methods have been developed to reduce the shrinkage stress during polymerization, such as adjusting the mode and intensity of the light device, applying a flowable resin composite under the material and applying incrementally.[2-4] Hardening of the composite; It is also influenced by factors such as the monomer content of the material, reaction initiators, hue, opacity, wavelength, intensity of light, distance from light device and exposure time. Insufficient light penetration causes the reaction to not complete under the material. For this reason, light curing resin composites have been applied by incremental technique since they are produced.[5] The application of resin composite by incremental technique causes time loss especially in the posterior region, increases the risk of space between the layers and this method adversely affects the success of the restoration while increasing the risk of contamination.[6]

One of the most recently developed restorative materials is bulk-fill resin composites applied as a single layer in the posterior region. Bulk-fill composites placed in a single layer up to a depth of 4 mm took their place in dental markets. The barium and yiterbium particles in the structure increase the radiopacity of the material and allow the effect of the light device to reach deep.[7] Bulk-fill resin composites as well as flowable bulk-fill composites are one of the most up to date materials on the market. Flowability allows these materials to be easily adapted to cavity walls.[8] These materials, which are reported to reduce the possibility of deformation, postoperative sensitivity, microleakage and secondary caries with low polymerization shrinkage, increase the comfort of patient and physician by applying these materials at once.[9,10]

In order to minimize the clinical aesthetics of glass ionomer cements such as poor aesthetic results and moisture related effects, giomers are defined as resin-based restorative material containing fluorine release and PRG fillers.[11,12] In the content of giomers; Bis-GMA, TEGDMA, inorganic glass filler, aluminum oxide, silica, PRG filler and DL-camphorquinone.[13] Composite resins containing S-PRG filler exhibit antibacterial activity by releasing metal ions from the composite.[14] The strontium and fluorine convert the hydroxyapatite into stronsiapatite and fluoroapatite to ensure that the tooth is resistant to acid. In addition, S-PRG fillers change the pH of the surrounding environment when in contact with water or acidic solutions.[15]

It is used in order to obtain preliminary information about bond strength tests, restoration materials activities and clinical success of adhesive systems.[16, 17] The microtensile bond strength test (μTBS) was first proposed by Sano in 1994. With this test method, the bond strength can be measured in an area of approximately 1 mm^2 of dentin, and a large number of samples can be prepared from a single tooth.[18]

The aim of this study is to evaluate the microtensile bond strengths of 2 flowable, 2 condensable bulk-fill composite resins to the dentin compared to a conventional microhybrid composite.

MATERIALS AND METHODS

Selection of Teeth

The study was initiated by the Non-Interventional Clinical Research Ethics Committee of Sivas Cumhuriyet University with the approval of the Ethics Committee dated 26.02.2018 and numbered 2018-01/11. In this study, 25 permanent human molar teeth extracted for orthodontic or periodontal reasons in last 6 months in the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Sivas Cumhuriyet University were used. Immediately after extraction, the teeth were kept in 2.5% sodium hypochlorite (NaOCI) solution for 1 hour and the organic residues on the crown surface were removed. The teeth were then soaked in

distilled water at room temperature until all the teeth were collected and the study started, and the storage fluid was changed every week.

Preparation of Dental Specimens

25 human molar teeth to be subjected to microtensile bond strength test were buried into the L shaped mold using silicon self-curing acrylic (IMICRYL Dental, Konya, Turkey).

Establishment of Working Groups

On each molar, occlusal class I cavities (approximately 3.5 mm wide and 4 mm deep) were prepared using a high speed handpiece and diamond burs (Dia-burs, Mani, Japan, SF-41C, Coarse) with air and water coolant. Tooth samples were randomly divided into five groups (n=5), according to the type of composite used for restoring class I cavities. Five groups were formed according to the composite resin materials used in this study (Table 1). The total number of samples is 75.

Table 1. Materials tested and their composition

Groups	Composites	Composition	Adhesive system
Group 1 (Control group)	**G-aenial posterior** (GC, Japan)	UDMA, dimethacrylatecomonomers (Bis-GMA free), pre-polymerilized silica/lanthanoid fluoride fluoroaluminosilicate/silica	**G-premio bond** (GC, Japan) 10-MDP, 4-META, 10-methacryoyloxydecyl dihydrogen thiophosphate, methacrylate adic ester, distilled water, acetone, photo initiators, silica fine powder.
Group 2	**Estelite Bulk Fill flow** (Tokuyama, Japan)	Bis-GMA, TEGDMA, Bis-MPEPP, mequinol, dibutyl hidroxyl toluene, uv adsorber, silicon oxide, zirconium oxide	**Universal Bond** (Tokuyama, Japan) Primer A: acetone, 3D-SR monomer, MTU-6 (tiourasil monomer),Bis-GMA, TEGDMA, HEMA Primer B: acetone, isopropanol, purified water, borate cataylst, peroksit, silan coupling agent
Group 3	**GrandioSO x-tra** (Voco, Germany)	Bis-GMA, Bis-EMA, organically modified silicic acid, aliphatic dimethacyrlate	**Futurabond U** (Voco, Germany) Organic acids, functionalised methacrylates, organic amine compound, camphorquinone, BHT, ethanol and water
Group 4	**Beautifil Bulk Restorative** (Shofu, Japan)	Bis-GMA, UDMA, Bis-MPEPP, TEGDMA, fluoro-silicate glass (S-PRG filler based on fluoroboroaluminosilicate glass) polymerization initiator, pigments and others	**FL-bond II** (Shofu, Japonya) Primer: water, ethanol, carboxyl acid monomer, phosporic acid monomer and initiator Bond: S-PRG filler based on fluoroboroaluminosilicate glass, UDMA, TEGDMA, 2-HEMA, initiator
Group 5	**Fill-Up** (Coltene, Whaledent, Switzerland)	Dental glass, methacyrlates, amorphous silica, zinc oxide	**ParaBond** (Coltene, Whaledent, Switzerland) Etchant Gel S: 36% phosphoric acid ParaBond A: methacrylates, polyalkenoates, initiators ParaBond B: ethanol, water, initiators

Etching and bonding procedures were done according to the manufacturer's instructions. In group I, horizontal incremental layering technique was used to fill the cavity with G-aenial posterior (GC, Tokyo, Japan) microhybrid composite and light activation was done with LED light curing unit (VALO Cordless, Ultradent, USA, 1000 mW/cm^2) for 20 seconds. In group 2, Estelite Bulk

Fill flow (Tokuyama, Japan) composite resin was placed in a single layer and polymerized with the same light source for 10 seconds. In group 3, GrandioSO x-tra (VOCO, Germany) composite resin was placed in a single layer and polymerized for 10 seconds. In group 4, Beautifil Bulk Restorative (Shofu, Japan) composite was placed in a single layer and poliymerized for 10 seconds. In group 5, Fill-Up composite resin was placed in a single layer and polymerized for 7 seconds. The restorations were then finished and polished (Astropol, Ivoclar Vivadent, USA) under abundant air water spray.

Microtensile Bond Strength Test

Specimens stored in distilled water at 37 ºC for 24 hours were placed on the sectioning device (Isomet 1000, Buehler, USA). Starting from the buccal side parallel to the long axis of the tooth, serial sections of approximately 1 mm width were taken under water cooling with a low speed diamond saw (ATM, Germany) in the buccolingual direction. The depth of the sections was extended to the crown-root junction. Then, serial slices were taken on the same sample starting from the mesial, approximately 1 mm wide. The teeth were cut along the cervical line perpendicular to the long axis of the tooth, resulting in rod-shaped sections of approximately 1 x 1 mm. At least 3 bonded stick shaped specimens were obtained from each tooth. The width of the sections was measured with digital micrometer and the dimensions were recorded. The microtensile bond strength test was performed using the universal test machine LF Plus (LLOYD Instruments, Ametek Inc. England). Each beam was attached to a custom made jig using cyanoacrylate glue (404 Kimya Sanayi ve Tic. A.Ş., Istanbul) and a tensile load was applied at a cross head speed of 0,5 mm/min until the beam fractured. The amount of load required for fracture recorded in newtons was converted to megapascals (MPa).

After the microtensile bond strength test, the fractured specimens of all samples were examined by stereomicroscope (Nikon SMZ800, Tokyo, Japan) under x25 magnification. The failure mode (cohesive, adhesive, mix) was identified for each specimen.

SEM Analysis

After examination of all samples with stereomicroscope, fractured surfaces were evaluated in detail on SEM device (TESCAN MIRA3, Brno, Czech Republic).

Statistical analysis

The data obtained from this study were evaluated with one-way ANOVA and Tukey tests in SPSS 22.0 (SPSS Inc., Chicago, IL, USA) program.

RESULTS

The maximum and minimum microtensile bond strength values, mean and standard deviation of composite resins, the difference between groups are shown in Table 2.

Table 2. The maximum, minimum, mean and standard deviation values of the microtensile bond strength tests of the composite resins used in the study.

Groups	n	Minimum	Maximum	Mean (MPa)	Standard Deviation
Group 1 [a,b,c,d] **G-aenial posterior**	15	23.03	36.90	29.573	3.50
Group 2 [a,e] **Estelite Bulk Fill flow**	15	14.45	21.50	16.952	2.22
Group 3 [b,f] **GrandioSO x-tra**	15	14.44	22.21	17.332	2.53
Group 4 [c,g] **Beautifil Bulk Restorative**	15	14.37	19.95	16.444	1.77
Group 5 [d,e,f,g] **Fill-Up**	15	11.10	15.73	13.426	1.57

F=100.228. P= 0.000. p<0,05.

[a,b,c,d,e,f,g] there is a statistical difference between the groups shown with the same lower case letters.

The control group showed the highest microtensile bond strength, while the Fill-Up group showed the lowest. G-aenial posterior composites showing the highest bond strength were followed by GrandioSO x-tra, Estelite Bulk Fill flow and Beautifil-Bulk Restorative, respectively. When the control group was compared with four different bulk-fill groups, the difference was statistically significant ($p<0.05$). Among the bulk-fill composites, GrandioSO x-tra showed the highest bond strength; The differences between Estelite Bulk Fill flow and Beautifil Bulk Restorative were not significant ($p>0.05$). Fill-Up group showing the lowest bond strength among bulk-fill composites, the difference between the other three bulk-fill groups was statistically significant ($p<0.05$). The most common type of failure is adhesive break across all groups. The least common type of failure is cohesive.

When the fracture surfaces of the G-aenial posterior were examined in SEM, inorganic structures attached to the dense organic matrix were observed on the dentin tubules (Figure 1).

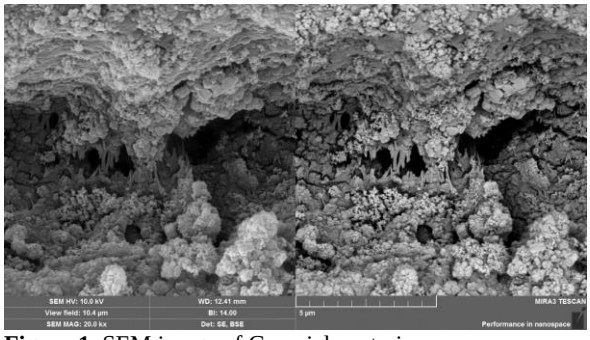

Figure 1. SEM image of G-aenial posterior

Estelite Bulk Fill flow had a distinct 0,5-1 µm gap along the hybrid layer on the fracture surface. (Figure 2).

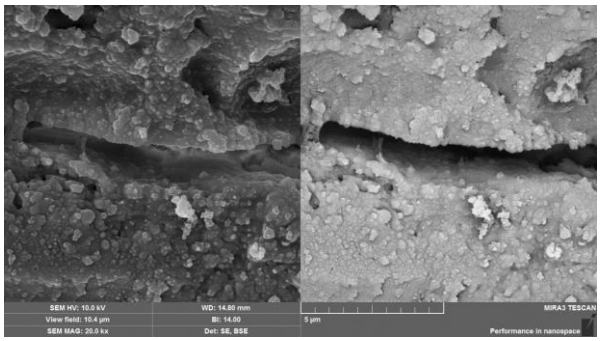

Figure 2. SEM image of Estelite Bulk Fill flow

Macro-resin tags detached from dentin tubules were also found on the fracture surface of GrandioSO x-tra (Figure 3).

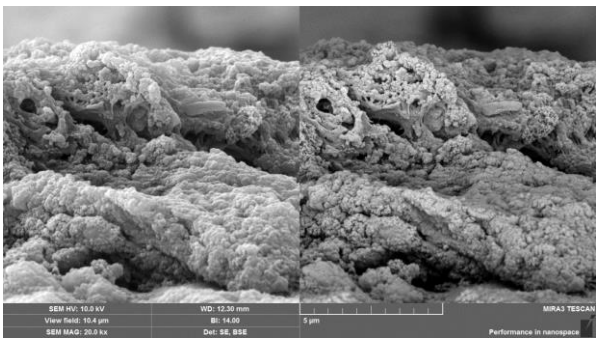

Figure 3: SEM image of GrandioSO x-tra

In the SEM image of the Beautifil Bulk Restorative, a complex plexus-like giomer matrix structure was observed (Figure 4).

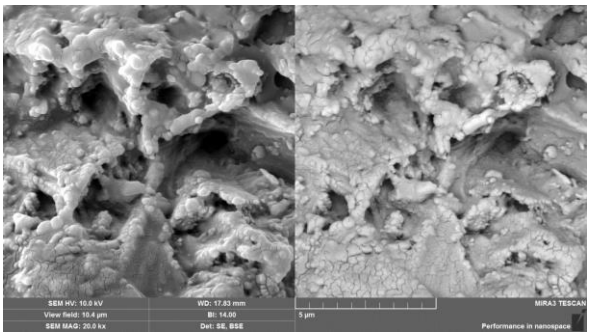

Figure 4: SEM image of Beautifil Bulk Restorative

When the fracture surfaces of Fill-Up were examined, it was seen that there were dilations due to demineralization in peritubular dentin canals (Figure 5).

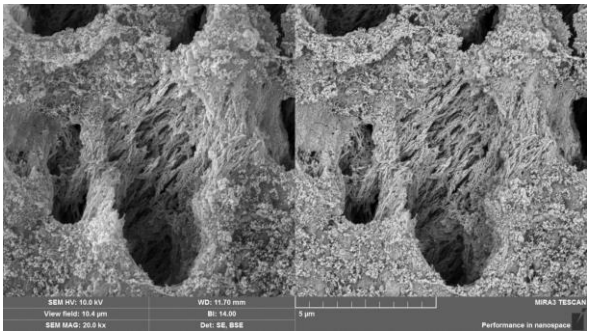

Figure 5: SEM image of Fill-Up

DISCUSSION

In order to spread the use of bulk-fill composite resins, their physical mechanical properties should be known and their bonding to dental tissue should be examined. The microtensile bond strength test applied in most research centers in recent years has many advantages that macro tests (conventional shear and tensile tests) cannot provide. Some of these advantages are; efficient use of teeth by obtaining multiple samples from a single tooth, the remaining dentin thickness to evaluate the effect of

bonding, examination of tooth-induced changes, the assessment of the effect of resin-based composite bonding, more uniform force application than the conventional tensile test, the shape of the break can be easily determined by SEM.[19]

Among the four different bulk-fill and one microhybrid composite (control group) groups used in this study, the highest microtensile bond strength was determined by the control group, the microhybrid composite resin G-aenial posterior (G-premio bond) group. When the fracture surfaces of the G-aenial posterior group were examined in SEM analysis; Inorganic structures attached to dense organic matrix on dentin tubules support the conclusion that there is good bonding between composite and dentin. In the study of Çolak *et al.*[20] evaluated the shear bond strength of 2 bulk-fill (SonicFill Bulk-Fill, Tetric EvoCeram Bulk-Fill) and 2 conventional nanohybrid composites (Herculite XRV Ultra, Tetric EvoCeram) to the middle coronal dentin in premolar teeth. As a result of the study reported that 2 nanohybrid composites exhibited higher values in terms of bond strength than bulk-fill composites. Although different composite types and bonding test methods are used in terms of material method; The study of Çolak *et al*. is similar to this results when compared to this study. Almeida *et al.*[21] studied the microtensile bond strength of 2 bulk-fill (Tetric Bulk Fill, SonicFill) and 1 conventional nanocomposite (Filtek Supreme XTE) in Class I cavities (4x5x4 mm). Before the composite application, they applied etch and rinse adhesive system (Adper Single Bond 2) as a standard to all cavities. SonicFill shows the highest bond strength, followed by traditional composite and Tetric Bulk Fill was the lowest value. Almeida *et al.*[21], although the different types of composites used and the use of a uniform adhesive system differed from this study, other results support this study except SonicFill. The highest microtensile bond strength in SonicFill can be associated with sonic activation technology.

When the fracture surfaces of GrandioSO x-tra are examined in SEM analysis; the absence of open dentin tubules, the observation of irregular polymer structures with crater-shaped recesses on the composite surfaces, and the presence of very few macro resin tags detached from the dentin surfaces support a good bonding between the composite dentin.

Estelite Bulk Fill flow composite is distinguished from other bulk-fill composites by self-cured adhesive system. Although the microtensile bond strength test results of Estelite Bulk Fill flow (+ Universal Bond), which has a flowable structure, followed GrandioSO x-tra, which showed the best bonding among the bulk-fill composites in this study; the difference was not statistically significant ($p>0.05$). When the fracture surfaces of Estelite Bulk Fill flow are examined in SEM analysis; observing a gap as if drawn with a regular pencil along the 0.5-1 μm width hybrid layer may be associated with the application of a self-cured adhesive system. Further research is needed to clarify this situation.

Beautifil Bulk Restorative differs from bulk-fill composites with its giomer structure. According to the results of microtensile bond strength test; Although Beautifil Bulk Restorative (+ FL-bond II) showed lower values than GrandioSO x-tra and Estelite Bulk Fill Flow, the difference between all three bulk-fill composites was statistically insignificant ($p<0.05$). When the rupture surfaces of Beautifil Bulk Restorative were examined in SEM analysis; On the dentin tubules, unlike the other composites, there is a plexus-like complex giomer matrix structure.

Tsujimoto *et al.*[22] evaluated the polymerization depth and volumetric contraction of giomer bulk fillers (Beautifil Bulk Restorative, Beautifil Bulk Flow) and bulk fill composites (SDR, Filtek Bulk Fill Flowable, Tetric EvoCeram Bulk Fill, Filtek Bulk Fill Posterior Restorative). As the polymerization time was increased (20, 30, 40 sec) in all the materials they used, the polymerization depth increased. Beautifil Bulk Flow showed the lowest polymerization depth among low viscosity materials; Among the high viscosity materials, Beautifil Bulk Restorative showed the lowest polymerization depth. Consequently, they report that giomers exhibit less polymerization depth than other bulk-fill composites. In terms of volumetric

shrinkage, they reported that giomers showed more volumetric shrinkage than other bulk-fill composites in both low and high viscosity groups.

The fill-up bulk-fill composite resin differs from the other composites in this study by its dual-cure polymerization and etch and rinse adhesives (+ParaBond). While in this study, Fill-Up bulk-fill composite showed the lowest microtensile bond strength, the differences between other composites were found to be statistically significant ($p<0.05$). When the fracture surfaces of Fill-Up were examined in SEM analysis; clear monitoring of dentin tubules using etch and rinse adhesive system and deep demineralization due to acid roughening of dentin may be related to lack of the simultaneous infiltration of the adhesive.

No literature has been reached to evaluate the bond strength of Fill-Up Composite. Monterubbianesi *et al.*[23] studied the conversion degrees and microhardness values of 5 bulk-fill composites (Fill-Up, SureFil SDR, Filtek, SonicFill, SonicFill2). As a result of the analysis, a significant difference was observed between the lower surface of Fill Up and upper surface conversion degrees; reported no significant difference between the lower-upper surface conversion degrees of other bulk-fill composites. This difference Monterubbianesi *et al.*[23] stated is consistent with the fact that Fill-Up showed low microtensile bond strength in this study.

Gupta *et al.*[24] evaluated microleakage of 3 bulk-fill (Fill-Up, SonicFill, SureFil SDR) and 1 conventional (Filtek) composite in Class I cavities. SonicFill showed the most microleakage as a result of their study; Fill-Up followed this and SureFil SDR showed the lowest value. According to the study of Gupta *et al.*[24]; The excessive microleakage of Fill-Up is compatible with the deep demineralization images of Fill-Up in this SEM images.

CONCLUSIONS

Bulk-fill composites did not show as good bond strength as conventional composite. When bulk-fill composites were used with their own adhesive systems, the bond strengths of other bulk-fill composites except Fill-Up were similar. Further improvement of the bulk-fill composites in terms of bond strength to dentin and follow-up clinical studies are essential for a complete evaluation.

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CONFLICT OF INTEREST STATEMENT

None.

Sınıf I Kavitelerde Dört Farklı Bulk-Fill Rezin Kompozitin Dentine Mikrogerilim Bağlanma Dayanımlarının İncelenmesi

ÖZ

Amaç: *Çalışmamızın amacı, dört farklı bulk-fill kompozitin ve bir mikrohibrit kompozitin dentine mikrogerilme bağlanma dayanımlarının karşılaştırmalı olarak incelenmesidir.* ***Gereç ve Yöntemler:*** *Çalışmamızda 2 akışkan, 2 kondanse edilebilir bulk-fill kompozit ve bir mikrohibrit kompozit (kontrol grubu) kullanıldı. 25 adet yeni çekilmiş sağlam insan molar dişleri üzerine Sınıf I okluzal kaviteler hazırlandı, rastgele 5 gruba ayrılarak [G-aenial posterior + G-premio bond (Kontrol grubu), Estelite Bulk Fill flow + Universal Bond, GrandioSO x-tra + Futurabond U, Beautifil Bulk Restorative + FL-bond II, Fill-Up + ParaBond] her kompozit grubunun kendi adeziv sistemleri kullanılarak restorasyonları yapıldı. Restorasyonu tamamlanan dişler İsomet cihazıyla elmas testere yardımıyla bukkolingual ve meziodistal yönde kesitler alınarak her dişten üçer örnek olmak üzere her gruptan 15 örnek elde edildi. Çalışma gruplarına ait mikrogerilim bağlanma dayanım değerleri universal test cihazında ölçüldü, kuvvet birimi ise "newton" olarak kalibre edildi. Kopma yüzeylerinde meydana gelen mikroskobik değişiklikler Taramalı Elektron Mikroskobunda incelendi. Veriler, istatistiksel yöntem olarak tek yönlü Varyans analizi ve Tukey testleri ile değerlendirildi.* ***Bulgular:*** *En yüksek mikrogerilim bağlanma dayanım değeri kontrol grubunda izlenirken, en düşük bağlanma dayanımını ise Fill-Up grubunda gözlenmiştir. Kontrol grubu, tüm bulk-fill grupları ile karşılaştırıldığında fark istatistiksel olarak anlamlı bulundu ($p<0,05$). Bulk-fill kompozitler arasında en yüksek bağlanma dayanımını GrandioSO x-tra grubu göstermesine rağmen, istatistiksel olarak karşılaştırıldığında; Beautifil Bulk Restorative ve Estelite Bulk Fill flow grupları arasındaki farklar anlamsız*

bulunmuştur (p>0,05). Fill-Up grubu, diğer tüm bulk-fill gruplarıyla karşılaştırıldığında aralarındaki farklar istatistiksel olarak anlamlı bulunmuştur (p<0,05). SEM analizlerinde sadece Estelite Bulk Fill flow'da hibrit tabaka boyunca düzenli bir boşluk görülmektedir. ***Sonuçlar:*** *Bulk-fill kompozitler, geleneksel kompozit kadar iyi bağlanma gösterememiştir. Bulk-fill kompozitler arasında Fill-Up etch and rinse adeziv sistemle kullanılmasına rağmen, en düşük bağlanma dayanımını göstermiştir.* ***Anahtar kelimeler:*** *Bulk-fill kompozit, mikrogerilim bağlanma dayanımı, dentin.*

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Original research

THE EFFECT OF TWO BULK-FILL RESIN COMPOSITES ON FRACTURE RESISTANCE OF ENDODONTICALLY TREATED TEETH

ABSTRACT

Objectives: The aim of this study was to compare the effect of two commercially available bulk-fill restorative materials on the fracture resistance of endodontically treated human molar teeth.

Materials and methods: A total of 44 mandibular third molar were divided into four groups: Group 1; 4 mm thick bulk-fill fluid composite (SDR ™, Dentsply, Konstanz, Germany) was applied to the cavities. The restoration of the proximal walls and the occlusal region was completed with posterior composite (Valux Plus, 3M ESPE, St. Paul, MN, USA). Group 2; 4 mm thick fiber-resin reinforced bulk-fill composite (EverX Posterior; GC Corp., Japan) was placed in the cavities and the procedure in Group 1 was repeated. Group 3 (negative control group); no restoration was applied to the cavities. Group 4 (positive control group); comprised intact molar teeth without any treatment. The root canals were shaped using rotary instruments, irrigated with NaOCl, and obturated. All samples were incubated in distilled water at 37˚C for 1 week and then fracture strength test was performed. The values were recorded in Newton and the data were evaluated using Kruskal Wallis and Mann-Whitney U test.

Results: No statistically significant difference ($p > 0.05$) was observed between group 1, 2, and the positive control group. However, the bulk-fill fluid composite material showed higher fracture resistance than the fiber-resin reinforced composite material ($p<0.05$). The highest fracture resistance was observed in the positive control group. The fracture resistance of the negative control group was statistically lower than the other groups ($p<0.05$).

Conclusions: Both of the bulk-fill restorative materials in endodontically treated teeth showed similar fracture resistance to intact teeth. In addition, the clinical use of the bulk-fill fluid composites may be recommended because of their high fracture resistance and ease of application.

Keywords: Composite resin, fracture resistance, endodontics.

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INTRODUCTION

Long-term success in endodontic treatment can be achieved through a durable and impermeable coronal restoration following an ideal root canal treatment. In these teeth, the loss of healthy tooth structure is usually considerable, and crown fracture following treatment can negatively affect their prognosis due to the secondary caries or unsuccessful restorations. Previous studies have demonstrated that the fragility of endodontically treated teeth was increased due to loss of tooth tissue resulting from the access cavity and root canal preparation rather than the changes in dentin tissue.

For many years, adhesive restorations were quite successful in protecting the remaining hard tissues in the endodontically treated teeth and increasing their fracture resistance.[4,-6] Composite resins could be the material of choice to replace ceramic crowns due to their potential to mimic the dental anatomy, protect the periodontal structure, and provide a cost-effective, durable, and functional restoration of the tooth structure. The most important drawback of composite resin restorations is polymerization shrinkage. Prior to the placement of direct restorative materials; applying flowable composites having a low elastic modulus using a coating technique can reduce the shrinkage stress of composite resin. However, this procedure could be difficult, especially in the posterior teeth because it is time-consuming and isolation is not easy. The recently developed adhesive technologies have aimed at reducing the polymerization shrinkage and increasing the durability of one-stage (bulk-filling) restorations by adding glass or polyethylene fiber to the structure of composite materials.[3-6]

The first bulk-fill composite introduced into the market was the SureFil SDR flow (SDR, Dentsply, Konstanz, Germany). It has advantages such as having a flowing consistency, allowing polymerization of 4-5 mm thick layers at a time, easy and short time of application.[7,8] Another bulk-fill composite material currently used is the fiber-reinforced Ever X Posterior (EXP; GC Europe, Leuven, Belgium), which is a successful product with many proven mechanical properties.[9,10] There has been increasing interest in this material due to the reports of its superior durability, especially against pressure.

The aim of this study was to compare the effect of two commercially available bulk-fill restorative materials on the fracture resistance of endodontically treated human molar teeth. The null hypothesis was that there would be no difference between the materials used regarding the fracture resistance of root-filled teeth.

MATERIALS AND METHODS

In this study, 44 non-carious human third molar teeth extracted due to periodontal reasons, were used. The study was approved by the Akdeniz University Faculty of Medicine Ethics committee No:2012-KAFK-20/238. The extracted teeth were stored in distilled water at +4°C until the study was performed. The specimens were examined under 2.5X magnification with loupes in order to identify the teeth with root caries, broken and cracked teeth, teeth with oblique roots or open apices. The teeth with a mesiodistal width of 11 ± 1 mm and a buccolingual width of 10 ± 1 mm were included in the study. Teeth that meet the criteria of this study were randomly divided into 4 groups with 11 specimens per group. The schematic distribution of the groups is shown in Figure 1.

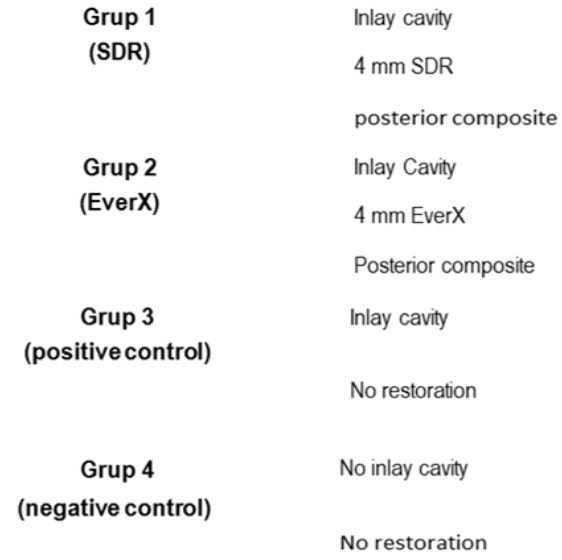

Figure 1. Schematic representation of the groups

Class II mesio-occlusal-distal (MOD) cavities were prepared in all samples except the Group 4. Group 4 was designated as the positive control group and the tooth specimens in this group were

left untreated and stored at +4 C until the fracture resistance test. The buccolingual width of each cavity was measured with a digital caliper (Mitutoyo, Corp., Kawasaki, Japan), and standardized to be half of the intercuspal distance and it included the pulp chamber. For root canal treatment, the access cavities were prepared by using a diamond bur and fissure burs under water cooling. The working length of the root canals was determined with a # 15 K type file (Mani Inc, Tochigi, Japan). The canals were shaped using the Protaper Next (PTN, Dentsply Maillefer, Switzerland) rotary file system. The mesial canals were extended up to X2 and the distal canals up to X3 file. During extension, the canals were irrigated with 2 ml of 2.5% NaOCl between each file. The last irrigation protocol included 2 ml 17% long version (EDTA) and distilled water, respectively. After the root canals were dried with paper points, the root canals were filled using gutta-percha (PTN, Dentsply Maillefer, Switzerland) and resin-based root canal sealer (2Seal, VDW, Munich, Germany) using the single cone method.

In Group 1, Clearfil SE bond (Kuraray Co, Osaka, Japan) was applied to the cavity in accordance with the manufacturers' recommendations and polymerized for 20 light/sec (Valo, Ultradent, USA). The mesial and distal margins of the tooth specimens, shaped using metal matrix bands, were restored with a posterior composite (Valux Plus, 3M ESPE, St. Paul, MN, USA), and a Class I cavity form was created. SDR was placed at a thickness of approximately 4 mm at a time, corresponding to 1.5-2 mm below the occlusal level, and the was polymerized for 40 seconds with dental light curing unit (Valo, Ultradent, South Jordan, USA). The remaining cavity was restored with the posterior composite resin. Following Class I cavity preparations similar to Group 1, EXP was placed in a single phase at a depth of 4 mm to the specimens of Group 2 and polymerized for 40 seconds. The remaining cavity space was restored with posterior composite resin. No restoration was applied on Group 3 specimens. Following these procedures, polishing was performed under water cooling using aluminum oxide coated discs (Soflex, 3M ESPE, MN, USA). The root surfaces of the restored tooth specimens were covered with a thin wax layer up to 1 mm below the cemento-enamel junction in order to mimic the periodontal ligament. The specimens were then placed vertically in a self-hardening acrylic mold (Imicryl, Turkey) in cylinder blocks of 4.5x2.5 cm up to 1 mm lower the level of enamel cement combination.

After being stored in the incubator at 37ºC for a week at 100% humidity, the teeth were inserted to the universal test device (AG-5 kNG, Shimadzu, Tokyo, Japan). At the point corresponding to the central fossa of the teeth, fracture strength test was performed by applying parallel forces to the long axis of the tooth at a speed of 1 mm/min. The forces at the moment of fracture were recorded as Newton (N).

Statistical Analysis

All of the statistical analyses were conducted using SPSS 19 software (SPSS Inc., Chicago, IL USA). The data were assessed by using Kruskal Wallis and Mann-Whitney U tests. The level of significance was determined at $p<0.05$.

RESULTS

No statistically significant difference was found among the Group 1, Group 2 and the positive control group (Group 4) ($p> 0.05$). However, the bulk-fill flowable composite material showed higher fracture resistance than the fiber reinforced composite material. The highest fracture resistance was observed in the positive control group. The fracture resistance of the negative control group was statistically lower than all the other groups ($p<0.05$). The results are shown in Table I.

Table 1. Fracture strenght of the groups (Newton, Mean ± SD)

Group	N	Mean ± SD
Group 1 (SDR)	11	2207.95 ± 431.85 [a]
Group 2 (EverX)	11	2064.13 ± 415.67 [a]
Group 3 (negative control)	11	729.03 ± 161.54 [b]
Group 4 (positive control)	11	2417.11 ± 266.21 [a]

SD: Standart deviation. Same superscript letter indicates statistically similar values ($p > 0.05$)
(Kruskal Wallis and Mann Whitney U test)

DISCUSSION

The most important factor affecting the clinical success of endodontically treated teeth is the remaining coronal tooth tissue. In order to achieve long-term success after treatment, apart from restoring the tooth function, the retention and durability of restoration should also be enhanced. The choice of restorative material and the restoration technique are very important to increase the durability of the teeth against fracture. In many studies it was shown that fiber-reinforced composites have many advantages as better adhesion of composite luting agent to the fiber, physiological stiffness of the denture framework made of fiber, and a better elastic modulus match between fiber-reinforced composite restoration and dentin/enamel.[9,10,11] In this study both bulk fill composite restorations showed fracture resistance values similar to intact teeth.

The preparation of cavity access in posterior teeth having a MOD cavity without marginal support or with significant structural loss may cause loss of resistance.[12] The recently developed bulk-fill composites can replace the layering technique due to the application of a single mass of 4 mm thickness.[13] In our study, when compared to the positive control group, cavity preparation in the negative control group significantly reduced the fracture strength of the dental specimens ($p<0.05$). The findings are consistent with previous studies.[5,14]

In the teeth with considerably decreased fracture resistance due to cavity preparation, completing the canal treatment and coronal restoration with the most suitable material in one session significantly increases the success. EXP, a fiber-reinforced bulk-fill composite, has recently become a preferred material for endodontically treated posterior teeth with large cavities. EXP is designed as a single-layer substructure material consisting 7.2% short fibers by volume.A conventional composite resin restoration is further required. EXP has more advanced fracture strength, bending resistance, and load-bearing capability than conventional composites.[15,16] The reinforcing effect of EXP is based on versatile, discontinuous short E-glass fibers that prevent crack propagation in a semi-intertwined polymer matrix. These fibers can provide an isotropic reinforcing effect, since each fiber acts as a crack stopper and transfers stress from the polymer matrix to stronger fibers.[15]

In this study, there was no statistically significant difference between the positive control group and the EXP group. The fracture strength values were found to be similar to a healthy tooth. The reason for fracture resistance could be because of glass fibers which increase the material stiffness and resistance to bending forces. This result is important because it provides safe clinical use. SDR that is the other composite resin used in our study, was the first bulk-fill composite. It contains a photoactive group in the modified urethane dimethacrylate resin. This structure allows the light, which is required for the polymerization, to reach the deeper layers of the composite. This enables the application of the composite at a thickness of 3-4 mm at a time.[17,18] In this study, no statistically significant difference was observed among the fracture strength values of SDR, EXP, and the positive control group. These findings are consistent with those in previous studies.[7] In another study, EXP showed better results than SDR in terms of compressive and flexural strength

values except hardness. In this study, the higher fracture resistance of SDR than EXP, can be explained by higher nano hardness values.[19]

The chemical solutions, especially NaOCl, used in the root canal treatment may cause mechanical changes in the dentine tissue residues and increase the risk of crown fracture. In a recent study, it has been demonstrated that the irrigation protocol with 5.25% of NaOCl significantly reduces the crown fracture resistance.[20,21] Thus, the choice of materials that are close to the physical and mechanical characteristics of healthy tooth tissue is very important for endodontically treated teeth. In this context, both bulk-fill composite materials used as upper restorations, have demonstrated fracture resistance values close to that of a healthy tooth. As far as the ease of clinical applications is concerned, SDR, through its flowable feature, may provide the clinicians with advantages.

CONCLUSIONS

Within the limits of this study, our findings demonstrate that the bulk-fill composites provide ease of use, decrease the time of application of the upper restoration, and increase the fracture strength of root canal treated teeth. It is important that the findings of this *in vitro* study should be supported with *in vivo* studies.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflicts of interest.

İki Güncel Bulk-Fill Rezin Kompozitin Endodontik Tedavili Dişerin Kırılma Direnci Üzerine Etkisi

ÖZ

Amaç: *İki güncel bulk-fill özellikte restoratif materyalin, endodontik tedavili insan molar dişlerinin kırılma direnci üzerine etkisini karşılaştırmaktır.* ***Materyal ve Metod:*** *Bu çalışmada 44 adet üçüncü molar diş seçilerek rastgele 4 gruba ayrıldı (n=11). Grup 1'de kavitelere 4 mm kalınlığında bulk-fill akışkan kompozit materyal (SDR™, Dentsply) yerleştirildikten sonra proksimal duvarlara rezin kompozit (Valux Plus, 3M ESPE) uygulandı ve kalan okluzal bölgenin de restorasyonu yapılarak işlem tamamlandı. Grup 2'de kavitelere 4 mm kalınlığında fiberle güçlendirilmiş bulk-fill kompozit materyal (EverX Posterior; GC) yerleştirildikten sonra 1. gruptaki işlemlerin aynısı uygulandı. Grup 3'de (negatif kontrol grubu) kavitelere herhangi bir restorasyon uygulanmadı. Grup 4'de (pozitif kontrol grubu) tamamen sağlam dişler kullanıldı. Diş kök kanalları NaOCl ile irrige edilerek döner aletlerle şekillendi ve güta perka ile dolduruldu. Tüm örnekler 37˚de 1 hafta süreyle inkübatörde bekletildikten sonra kırılma dayanımı testi gerçekleştirildi. Newton olarak kaydedilen veriler Kruskal Wallis ve Mann-Whitney U istatistiksel analizi ile değerlendirildi.* ***Bulgular:*** *Grup 1, Grup 2 ve Grup 4 (pozitif kontrol grubu) arasında istatistiksel farklılık gözlenmedi ($p>0.05$). Bununla birlikte bulk-fill akışkan kompozit materyali, fiber-rezinle güçlendirilmiş kompozit materyalinden daha yüksek kırılma direnci göstermiştir ($p<0.05$). En yüksek kırılma dayanımı pozitif kontrol grubunda görülürken, negatif kontrol grubunun kırılma dayanımı diğer gruplardan istatistik olarak anlamlı ölçüde düşük bulunmuştur ($p<0.05$).* ***Sonuç:*** *Her iki güncel bulk-fill restoratif materyali de endodontik tedavili dişlerde benzer kırılma direnci göstermiştir. Bunun yanı sıra bulk-fill akışkan kompozitlerin daha yüksek kırılma direnci sağlaması ve uygulama kolaylığından dolayı klinik kullanımı tavsiye edilebilir.* ***Anahtar Kelimeler:*** *Kompozit rezin, kırılma dayanımı, endodonti.*

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Review

USAGE OF ROTARY INSTRUMENTS IN ROOT CANAL THERAPY OF DECIDUOUS TEETH - REVIEW

ABSTRACT

Maintaining the deciduous teeth in the mouth until the time of physiological exfoliation is of great importance for the health of the permanent dentition. Root canal treatment, also called pulpectomy, performed in cases of inflammation or necrosis of the deciduous teeth pulp, is indicated as a successful method to preserve the tooth functionally. Conventional pulpectomy procedure is performed with stainless steel files, however, the low elasticity of these files may cause undesirable canal shapes and the time-taking treatment period may lead to deterioration of the patient's cooperation, especially in pedodontics. Ni-Ti rotary instrument systems eliminate these disadvantages and provide better quality canal fillings in a shorter duration. The present article reviewed the studies that are comparing the traditional stainless steel files and Ni-Ti rotating instrument for the root canal treatment of deciduous teeth.

Keywords: Tooth, deciduous, dental instruments, root canal therapy, stainless steel.

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INTRODUCTION

The early loss of deciduous teeth in pedodontics is an undesirable condition leading to serious negative outcomes. Deciduous teeth contribute tofunction, phonation, and aesthetics as long as they remain in the mouth. The earlyloss of deciduous teeth in children may result in various harmful habits, an impaired eruption of permanent dentition, lack of space and crowdings.[1-4]

Pulpectomy, which is recommended for pulpitis or necrosis of deciduous tooth, is a more conservative treatment method compared to tooth extraction. As for permanent teeth, the purpose of root canal treatment for deciduous teeth is to provide the disinfection of root canals by eliminating microorganisms, to prevent the reinfection of the tooth with a sealed filling and restoration, and to maintain the functionality of the tooth by preserving the health of periapical tissues.[1,2,4,5] However, unlike permanent teeth, factors such as deciduous teeth's short canal lengths, divergent and curved roots, thinner and more easily abradable dentin walls, canals with more variability and irregularity, physiological root resorption and limited duration for treatment in pediatric patients, render canal treatment more challenging.[2,5-7]

Despite these negative factors, pulpectomy is the recommended and performed method for deciduous teeth with success rates ranging from 68% to 100%.[5,7]

An ideal pulpectomy technique for the root canal treatment of the primary teeth should be completed in a short time period, provide an effective shaping and debridement without weakening teeth structure, not compromise the successor tooth germ, and ensure that the tooth remains in the mouth until its physiological loss with minimal complication.[3,7]

The traditional method for root canal treatment is stainless steel hand instruments. Stainless steel files can cause distruptions in the original shape of root canals and perforations due to their low flexibility. Ni-Ti rotary instrument systems, which were developed for solving the limitations of stainless steel files and used since the 1980s, provide more successful and better conical shaping, better quality debridement and filling in a short time owing to their high flexibility.[2,3,5,7,8]

The studies comparing traditional stainless steel files with Ni-Ti rotary instruments for root canal treatment are mostly focused on permanent teeth. The number of studies evaluating the use of rotary instruments for deciduous teeth is limited.[5] The aim of this article is to present and discuss the advantages and disadvantages of the use of stainless steel files and Ni-Ti rotary instruments in the primary root canal treatments in the light of the previous studies.

Rotary Instruments Used in Root Canal Therapies

Over the years, Ni-Ti rotary systems have been introduced with a wide range of designs and usage techniques.[9-11] ProFile, Hero642 and FlexMaster are the 1st generation rotary systems. ProFile has triple U shaped cross-section, while Hero642 and FlexMaster have triangular cross-section. They create smooth root canal walls and cause low procedural errors, but contain numerous files for root canal preparation.[9,10] Second generation rotary systems include RaCe, ProTaper, K3, Hero Shaper and Mtwo.[9,10] These systems have fewer instruments with active cutting edges. They provide faster preparation and preserve the original shape of even the curved root canals.[9] While designing the 3rd generation rotary systems, the producers focused on to reduce cyclic fatigue and separation risk of the instruments.[9] The reciprocal movement idea inspired the producers to develop the 4th generation rotary systems.[9,12] Reciprocating motion is a back-and-forth motion, in clockwise and anti-clockwise direction. It reduces the risk of instrument separation, due to avoidance of continuous dentinal over engagement.[13] Wave-One, Reciproc and SAF are the examples of 4th generation of rotary systems.[9] The fifth generation rotary files like Revo-S and ProTaper Next, have special designs. Revo-S has only three instruments with asymmetrical cross-section and makes snake-like movement in root canals.[10] ProTaper Next has rectangular off-center cross-section and its offset mass provides greater strength, improved flexibility, and resistance to cyclic fatigue.[10,12]

Efficacy in Cleansing and Shaping

Root canal shaping is considered to be one of the most important steps of canal treatment. It is important to remove the necrotic pulp together with the infected root dentin and to give a shape providing easy debridement in the root cavity and a quality filling.[14] Along with proper shaping and debridement, the removal of organic debris in the canals and reduction of the number of microorganisms are essential for the success of endodontic treatment.[4, 15,16]

Comparing rotary instruments with manual instruments in terms of intracavitary microorganism elimination demonstrated that both systems significantly reduced the number of microorganisms and that there was no statistical difference between them.[17-19]

Conventional stainless steel files were reported to remove more dentin tissue, especially in the coronal region. Although the difference between them and rotary instruments was not significant, stainless steel files were indicated to provide more efficient cleaning.[4,20-22] However, there are also studies comparing ProFile, ProTaper, Hero Shaper rotary instruments and K-type stainless steel files which reported that rotary instruments provided more effective cleaning in a statistically significant manner.[23] Some researchers recommend the hybrid method in which two systems are used in combination for root canal preparation. The hybrid method was reported to clean smears and debris better and to create more successful canal filling with better shaping.[17,24]

Rotary instruments working with reciprocal movements such as Reciproc and WaveOne were shown to provide more successful cleansing compared to rotary instruments working with rotation movement and were recommended to be used for deciduous teeth.[16,25]

In conclusion, the studies comparing the cleansing efficacies of rotary instruments and stainless steel files revealed no statistically significant difference between the two methods.[15,26-28] However, more conical canal shape, and smooth canal walls were obtained with rotary instruments compared to stainless steel files. Thereby, they were reported to provide better quality filling and sealing, thus increasing clinical success.[8,16,23,29]

Filling Volume and Efficacy

The complex morphology of root canals and dissoluble characteristics of the canal filling material cause insufficient filling and failure in apical sealing. This increases the importance of mechanical preparation for the root canal treatment of deciduous teeth.[30]

Although there is a study which found no significant difference in terms of apical sealing quality between ProTaper, Mtwo rotary instruments and K-type files[31], most studies reported that filling density and sealing quality were higher when the tooth preparation was performed using the rotary instruments or the hybrid method.[24,30,32,33]

The Mtwo rotary instrument system was reported to show better sealing and higher filling volume in a study comparing Mtwo and K-type files, although, gaps were observed in the canals of the two groups after filling.[32]

Although apical leakage was observed in all deciduous teeth prepared with Hero642, Race, Mtwo, ProTaper rotary instruments and K-type files, the highest leakage value occurred with K-type files. While Hero642 and Mtwo yielded a significantly lower leakage value, the system providing the most successful apical sealing was stated to be Hero 642.[30]

The preparation is more difficult in the mesial canals of deciduous molar teeth which are generally narrower. In one study, deciduous teeth were prepared with FlexMaster rotary files and K-type files, and completeness of canal filling of molar mesial canals was found to be significantly higher in the rotary group. Preparation with K-type files were noted to result in more incomplete fillings.[33]

More successful filling and better quality apical sealing with rotary instruments are contributed to the conical form obtained by the high taper angle of the rotary instruments. By this way, the flow of the canal paste into the canal is facilitated, the filling density and volume is increased, and apical leakage is decreased.[30,33]

Overflow of Irrigating Solution and Debris from Apical Region

A study on deciduous molar teeth comparing Revo-S, Mtwo, ProTaper Next rotary instrument systems, and K-type stainless steel hand instruments showed that K-type files caused significantly more debris overflow than the rotary instrument systems. The least amount of debris overflow was measured in the ProTaper Next group among the rotary instrument systems used in the study.[34]

Studies have shown that there is not a preparation instrument or a method that does not cause apical debris and irrigating solution overflow, and that hand files do not provide an advantage to rotary systems. Therefore, the researchers reported that the rotary systems could be preferred in the root canal treatment of deciduous teeth.[34-36]

Further studies evaluating the instruments used in the root canal treatment of deciduous teeth in terms of the apical extrusion of irrigant and debris are needed.

Postoperative Pain

Postoperative pain was evaluated at the 6th, 12th, 24th, 48th and 72nd hours and 1 week postoperatively in a study in which deciduous teeth were prepared with stainless steel hand instrument and Revo-S rotary instrument system. The patient group whose root canals were prepared with hand instruments reported more severe postoperative pain, except in the 72nd hour and 1st week measurements. Postoperative pain decreased in time with both methods.[37]

Instrument Failure and Root Perforation

The dentin walls are thin and can be easily eroded in the roots of deciduous teeth. Therefore, aggressive erosive root instruments can cause perforations in the canal walls of deciduous teeth. Also, canal instruments with less flexibility may cause undesirable canal shapes and perforations in the curved deciduous teeth canals. Due to the insufficient flexibility of traditional stainless steel hand instruments, Ni-Ti rotary instrument systems with higher elasticity were developed.[5]

Fractures are encountered more commonly in the Ni-Ti rotary instrument systems as a result of compression within the canal secondary to continuous rotation movement while distortion and deformations occur more frequently due to insufficient elastic properties of stainless steel hand instruments.[8,23]

In a study comparing ProTaper and K-type files, it was reported that there was no significant difference between the two systems in terms of perforation and instrument fracture.[38] Another study evaluating Reciproc rotary instrument system working with reciprocal movement and Mtwo rotary instrument system working with rotation movement revealed no deformation or fracture in the instruments.[16] Since reciprocating instruments move back and forth in the canal, the risk of fracture decreases. Also, they provide less canal transportation compared to the rotary instruments working with rotation movement.[13]

A study comparing K3 rotary instrument system (.02 taper and.04 taper) and K-type files (.02 taper) revealed no instrumental fracture and indicated that the rate of perforation increased as the taper angle increased.[20] The Hero642, ProTaper and K-files were compared in terms of preparation safety and no perforation or instrument fracture were observed in the Hero642 group. This was contributed to the lesser conicity and higher elasticity of Hero642 rotary instrument files. Both perforation and instrument fracture were observed in the K-type file group. Perforation was observed most commonly in the ProTaper group, the reason for this was shown to be the aggressive erosive blades and high taper angle of the ProTaper files. The high taper angle was stated to increase the bending resistance and cause undesirable lateral forces during rotation movement, especially in curved canals such as deciduous teeth have.[5]

A case report suggested that the Kedo-S rotary instrument system designed specifically for pediatric patients reduced the risk of instrument failure, perforation and undesirable canal shaping, and that its use for deciduous teeth was safer than rotating tools designed for permanent teeth. The reason for this was shown to be 16 mm short files facilitating its use in pediatric patients and three files with different taper angles adapting to the thin and curved canals of the deciduous teeth.[39]

In order not to cause iatrogenic errors in the curved, thin and erosive canal walls of the deciduous teeth, flexible files/systems with low taper angle that does not remove excess dentin tissue should be selected.[5,20]

Preparation Time

The duration of preparation in pediatric patients is of great importance in terms of the cooperation of the child and the quality of treatment.[3,40] The duration of preparation in root canal treatments depends on the technique used, physician's experience and the type and number of instruments used.[25]

Many studies were conducted to compare the preparation times of conventional stainless steel files and Ni-Ti rotary instruments and these stated that shorter preparation could be performed with rotary instruments. These studies compared Ni-Ti rotary instrument systems such as ProTaper, Mtwo, Hero642, FlexMaster, K3 and SAF with K-files.[8,15,20,21,23,28,29,31,33,38]

Only a single study compared the H-type stainless steel files and Mtwo rotary instruments and reported shorter preparation time for H-type files. The reason for this was the fact that the time of instrument change was also included in the measured time.[4]

The preparation times of the Hero642, ProTaper rotary instruments and K-files were evaluated and the longest time was observed in K-files, while the lowest preparation time was measured in the Hero642 group. This was contributed to the fact that the Hero642 rotary instrument system contained fewer instruments and that it was easier to apply.[5]

The Kedo-S rotary instrument system, which is including few instruments, was recommended to be used for pediatric patients.[39] The Kedo-S rotary instrument system was noted to provide better quality sealing in the deciduous tooth root canals compared to the K-type and H-type files.[40]

When the rotary instruments were compared among themselves in terms of movement type, rotary instrument system working with reciprocation movement such as Reciproc and Wave-One were reported to allow faster preparation than those working with rotation movement.[13,16,25,35]

Ni-Ti rotary instrument systems shorten the treatment time compared to the traditional method using stainless steel files, thereby increasing patient cooperation and reducing physician's fatigue.[8]

CONCLUSIONS

The root canal treatment of deciduous teeth can be performed with traditional stainless steel files as well as Ni-Ti rotary instrument systems.

The advantages of Ni-Ti rotary instruments are; maintaining the original shape of the canal owing to their flexible structure, reducing iatrogenic errors with the preservation of original anatomy, effective cleansing of irregular canals of deciduous molar teeth, ensuring uniform filling and reducing apical leakage thanks to conical shaped preparation and smooth canal walls, increased patient cooperation with rapid treatment and reduced fatigue of physician.[2,3,13,25]

Factors such as increasing the risk of instrument fracture because of wrong applications since it is a sensitive technique, being expensive and requiring experience are the disadvantages of Ni-Ti rotary instruments.[2,3,25,41]

Patient cooperation is a very important factor determining the success of the treatment, especially in pediatric dentistry. With the use of Ni-Ti rotary instruments for the root canal treatment of deciduous teeth, better quality treatments can be performed in a shorter time period.[2,3,13,25,27,41]

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CONFLICT OF INTEREST STATEMENT

None

Süt Dişlerinin Kanal Tedavilerinde Döner Alet Kullanımı - Derleme

ÖZ

Süt dişlerinin fizyolojik düşme zamanlarına kadar ağızda tutulması daimi dentisyonun sağlığı açısından büyük önem taşımaktadır. Süt dişi pulpasının iltihabı veya nekrozu gibi durumlarda uygulanan kök kanal tedavisi, diğer adıyla pulpektomi, dişin fonksiyonunun devamını sağlayan başarılı bir yöntem olarak gösterilmektedir. Geleneksel pulpektomi uygulaması paslanmaz çelik eğelerle yapılmaktadır, ancak bu eğelerin düşük elastikiyetleri nedeniyle istenmeyen

kanal şekilleri oluşabilmekte, zaman alan tedavi süresi ise özellikle pedodontide hastanın kooperasyonunun bozulmasına sebep olabilmektedir. Ni-Ti döner alet sistemleri bu dezavantajları ortadan kaldırarak daha kısa zamanda daha kaliteli kanal dolumları sağlayabilmektedir. Bu makalede, süt dişi kanal tedavisinde geleneksel paslanmaz çelik eğeler ile Ni-Ti döner aletlerin karşılaştırıldığı çalışmalar derlenmiştir. ***Anahtar Kelimeler:*** *Süt dişi, dental araçlar, kök kanal tedavisi, paslanmaz çelik.*

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Case Report

AGGRESSIVE PERIPHERAL GIANT CELL GRANULOMA IN POSTERIOR MANDIBLE– CASE REPORT IN A CHILD

ABSTRACT

The peripheral giant cell granuloma (PGCG) is a common benign hyperplastic and reactive gingival lesion occurring mostly in adults. We report a case of a 10-year-old boy presenting with a six-month history soft tissue mass of the left mandibular gingiva associated with ulceration area. An excisional biopsy of the lesion followed by histopathologic examination of the biopsy specimen revealed distinctive features of peripheral giant cell granuloma. In these case report after 12 months of follow up spontaenous eruptions of permanent teeth were noticed. Clinical, radiographical and histological characteristics are discussed and recommendations regarding treatment.

Keywords: Giant cell granuloma, pediatric dentistry, children.

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INTRODUCTION

Solitary gingival enlargements in children are relatively common and usually occur in reaction to local irritation or chronic trauma. One of these enlargements is peripheral giant cell granuloma (PGCG), a lesion unique to the oral cavity, occurring only on the gingiva.[1]

Peripheral giant cell granuloma (PGCG) is an oral, non-neoplastic, tumor-like growth that occurs exclusively on the gingiva and the alveolar mucosa. It is never found on non-osseous supported tissues.[2,3] This lesion has been reported to account for 5.1% to 43.6% of reactive gingival overgrowths.[2,4-9] It affects both sexes, with a slight predilection for females especially after puberty. Although peak prevalence is found in the fifth and sixth decades of life, 20 to 30% of cases occur within the first two decades of life. The most common location of the PGCG is the incisor and canine regions with a slight predilection for the mandible.[3,6,7]

The etiology of this lesion is not completely understood. However, local irritating factors such as teeth extraction, ill-fitting prostheses, poor restorations, collections of food remnants and calculus, seem to play an important role in the development of these lesions.[10,11]

PGCG are derived from periosteum and periodontal ligament and occurs frequently in young adults. It occurs in variable sizes, sessile or pedunculated.[2] PGCG appear as a reddish purple or purplish blue lump with smooth shiny or papillomatous surface. It is a well-defined lesion with exophytic growth and rarely exceeds 3 cm in their greater dimension.[12]

It is distinguishable from similar lesion only on the basis of its unique histomorphology, which is essentially identical to that of central giant cell granuloma, intrabony benign neoplasm of the jawbone.[1]

Our study is aimed to report a case of aggressive PGCG in a young child which was misdiagnosed and neglected for more than 6 months.

CASE REPORT

A healthy 10-year-old boy was referred for evaluation of a gingival mass on the left mandibular canine-premolar region that was first observed six months prior to examination. A clinical examination was performed after obtaining the required consent form from the patient's parent. The lesion was interfering with normal permanent premolars eruption. The medical history did not reveal any unusual findings. The patient reported that the lesion first appeared 6 months ago, after primary tooth caries and fractures. Clinical examination revealed that the lower permanent left canine and premolars were absent, and presence of retained primary second molar tooth residues with a reddish-purple, sessile nodule, of approximately 7 mm in diameter. The surface was smooth, except for local areas of ulceration (Figure 1).

Fig 1. Intraoral preoperative view of lesion

The lesion was painless and with no associated spontaneous bleeding except for occasional interference of the swelling with mastication. A panoramic radiograph of the site demonstrates destruction of the alveolar bone and migration of permanent canine and premolar germs. (Figure 2). No other oral or cutaneous lesions were noted in this healthy child.

Fig 2. Panoromic radiograph showing destruction of the alveolar bone and migration of permanent toooth

TREATMENT

Oral hygiene instructions were given to the patient, and oral prophylaxis was done. After one week, under local anaesthesia, the mass was excised with retained tooth residues and submitted for histological examination with a preoperative diagnosis of reactive hyperplasia. Patient was motivated and educated to maintain his oral hygiene.

Histopathological examination of the specimen revealed an ulcerated giant cell granuloma characterized by the proliferation of elongated fibroblastic cells with ovoid nuclei lacking atypia or mitosis and by the diffuse infiltration of osteoclast-like multinucleated giant cells in a fibrous connective tissue. The connective tissue exhibited multinucleated giant cells dispersed throughout a fibrovascular stroma. Histologically, the specimen was suggestive of PGCG. Based on the clinical and histological findings, the lesion was diagnosed as PGCG.

After surgical procedure, healing was uneventful, and no recurrence was observed after 12 months follow-up. During this period the patient did not report any complaints. Tooth eruption was observed in clinical and radiograph examinations, and no other treatment was needed (Figures 3 and 4).

Fig 3. Clinical follow-up 12 months after surgical excision showing the permanent erupted tooth and no recurrence of the lesion

Fig 4. Follow-up 12 months after surgical excision

DISCUSSION

In children, single gingival enlargements are relatively a common finding and are usually the result of a reactive response to local irritation. These lesions may grow rapidly and reach a significant size within several months of its initial diagnosis.[4,7,13,14] PGCG is more common in fifth and sixth decades of life than in children and the pyogenic granuloma is the most common reactive lesion in children.[3,7,13] In a review of 720 cases, 33% were seen in patients younger than 20 years of age, which concurs with the findings of another study in which 33 of 97 cases (34%) occurred in individuals between 5 and 15 years of age.[2,15] The most common location for the PGCG is the incisor and canine regions, with a slight predilection for the mandible.[3] In this paper we reported a case of PGCG located on mandible premolar region in an 10-year-old boy.

Peripheral giant cell granuloma can behave very aggressively, especially in children. The criteria used to define aggressiveness of a PGCG are its size, the extension of the lesion in neighbouring tissues, its ability to relapse, the associated bone resorption, bone displacements, interfere with eruption of adjacent teeth, minor to moderate tooth movement, and have multiple recurrences.[16,17]

The aetiology of PGCG is unknown but local irritation factors such as poor dental restorations, unstable dental prosthesis, dental extractions, plaque and calculus accumulation, food retention seem to play an important role in the development of PGCG.[3,4,7] In the case we describe, the identifiable trigger is neglected decayed primary tooth and accordingly retained tooth residues.

Clinically, PGCG presents as a firm, soft, bright pedunculated or sessile nodule with various sizes that range from small papules to enlarged masses, though they are generally less than 20 mm in diameter with the color ranging from dark red to purple or blue commonly with ulcerated surface.[3,5] Pain is not a common characteristic, and in most cases lesion growth is induced by repeated trauma.[1] In this case, we present a reddish-purple, sessile nodule, of approximately 7 mm in diameter and the lesion was painless and with no associated spontaneous bleeding.

Microscopically, PGCG is often ulcerated or eroded. The underlying connective tissue consists of fibroblast and multinucleated osteoclast-like giant cells. Extravasated red cells and deposits of haemosiderin, which are responsible for the classic brown-reddish colour of the PGCG are often present. Inflammatory cells and bone (woven and/or metaplastic) are found in about one-third of the cases.[18] The stroma may contain osteoblasts, myofibroblasts, macrophages, and Langerhans cells. Histopathologically, the differential diagnosis of the PGCG must be made primarily with the Central Giant Cell Granuloma (CGCG).[19-21]

In differential diagnosis in the cases of gingival enlargements in children we consider gingival lesions that mimic PGCG, like; peripheral ossifying fibroma, peripheral odontogenic fibroma, parulis, and hemangioma. The peripheral ossifying fibroma is a reactive gingival growth that shares similar clinical features as the PGCG. In radiological evaluation, the identification of small calcification spots in the lesion helps to diagnose peripheral ossifying fibroma.[16] The pyogenic granuloma may be difficult to differentiate from the PGCG based on clinical features alone. In general the pyogenic granuloma presents as a soft, friable nodule that bleeds freely with minimal manipulation. Unlike the PGCG, displacement of teeth and resorption of alveolar bone are not observed.[16] The PGCG is distinguishable from pyogenic granuloma and peripheral ossifying fibroma only on the basis of its unique histomorphology, which is the same as central giant cell granuloma.[2,15] Another erythematous nodule of the gingiva is the parulis, which is associated with an stucked foreign body, a gingival pocket and/or a nonvital tooth. Pain and the expression of a purulent exudate with fluctuation in lesion size help to differentiate this inflammatory disease from the PGCG.[16] The other consideration based on the red or blue discoloration of the soft tissue nodule is a hemangioma. Although many hemangiomas are congenital lesions, some vascular malformations increase in size during childhood. Brisk bleeding, increased warmth of the tissue and blanching upon palpation are characteristic of this vascular entity.[16] Clinically, peripheral odontogenic fibroma must be considered in the differential diagnosis of dome-shaped or nodular, nonulcerated, growths on the gingiva like PGCG. Peripheral odontogenic fibroma is characterized by a fibrous or fibromyxomatous stroma containing varying numbers of islands and strands of odontogenic epithelium which is clearly distinguishable from PGCG histopathology.[6]

Management of this gingival lesion includes surgical excision and elimination of any local provocative factors.[14,21] Recurrences of the PGCG have been reported in 5% to 70.6% of cases. This great variation is probably attributable to the surgical technique used, since recurrences re-excised up to the periosteum have not recurred thereafter.[3,9]

Although, multiple recurrences with eventual loss of the adjacent teeth are a potential complication.[16] Early diagnosis based on clinical and radiological findings, confirmed by pathological analysis especially in children, allows for conservative management with less risk of destruction for the adjacent teeth and tissues.[22]

In rare cases, PGCG may be the sole expression of a hyperparathyroidism. Bergdhal[23] showed that 1.9–6% of the patients with a gingival lesion with multinucleated cells also have hyperparathyroidism. A case of recurrent PCGC has also been described by Stratakis *et al.*[24] in a 9-year-old boy affected by X-linked hypophosphatemic rickets, a condition associated with subclinical hyperparathyroidism. In a patient with typical lesions suggesting PGCG, especially when multiple or reoccurring after

treatment, hyperparathyroidism should hence be excluded.[19,25-27]

CONCLUSIONS

Peripheral giant cell granuloma is a disease encountered often in the adult population. It is very rarely found in children and represents only a very small proportion of the hyperplasic gingival lesions. PGCG, as our case illustrates it, may well occur after a dental traumatism. Because of the potential local aggressive behaviour of this type of lesion, an early diagnosis and effective surgical management is warranted especially in children. Early diagnosis based on clinical and radiological findings and confirmed by pathological analysis allows for conservative management with less risk of destruction for the adjacent teeth and tissues.

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CONFLICTS OF INTEREST STATEMENT

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

Posterior Mandibular Bölgede Agresif Periferal Dev Hücreli Granüloma - Bir Olgu Sunumu

ÖZ

Periferik dev hücreli granüloma (PGCG), çoğunlukla yetişkinlerde görülen yaygın, benign, hiperplastik ve reaktif dişeti lezyonudur. Bu çalışmada, ülserasyon bölgesi ile ilişkili sol mandibular dişetinde altı ayldır bulunan yumuşak doku kitlesi ile başvuran 10 yaşında bir erkek hasta bildirildi. Lezyonun eksizyonel biyopsisi, ardından biyopsi örneğinin histopatolojik incelemesi, periferik dev hücreli granülomun ayırt edici özelliklerini ortaya çıkardı. Bu olgu sunumunda 12 aylık takip sonrasında daimi dişlerde spontane erüpsiyonlar gözlendi. Bu vaka sunumunda, klinik, radyografik ve histolojik özellikler tartışılmakta ve tedaviyle ilgili önerilerde bulunulmaktadır. ***Anahtar Kelimeler:*** *Granülom, periferal dev hücre, çocuk diş hekimliği.*

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Case Report

PYOGENIC GRANULOMA OF THE INCISIVE PAPILLA: A RARE CASE REPORT

ABSTRACT

Pyogenic granuloma is a non-neoplastic reactive growth commonly found in the oral cavity and skin. It is benign in origin and may arise due to factors like trauma, local minor irritation and an imbalance in the levels of hormones. Oral pyogenic granuloma occurs commonly in young females in second decade of their life possibly due to hormonal influences leading to changes in the vascular system. Oral pyogenic granuloma presents itself as a smooth or lobulated growth, mostly pedunculated but occasionally with a sessile growth. The colour of pyogenic granuloma may vary from pink, red and purple and this variation in colour is related to the age of the lesion. Clinically the most common site for oral pyogenic granuloma is gingiva, lips, tongue and buccal mucosa. This report presents a unique location for oral pyogenic granuloma at incisive papilla. Palatal pyogenic granuloma is rarely reported.

Key words: Oral, gingiva, pyogenic granuloma, pregnancy, incisive papilla.

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INTRODUCTION

Several soft tissue enlargements can occur in the oral cavity and often present a diagnostic challenge. Such enlargements in the oral cavity may indicate a developmental anomaly, adaptation of a normal structure, cysts, inflammation and neoplasms. One of the most common among oral soft tissue enlargements is pyogenic granuloma, a reactive hyperplasia that appears secondary to either a hormonal change, trauma, a minor local irritation or sometimes due to certain drugs. The term pyogenic granuloma is a double misnomer for the lesion as it neither contains pus nor represents a granuloma in true sense. The first case of pyogenic granuloma was reported in 1884 by Hullihen and although several names have been proposed, its current term i.e., pyogenic granuloma was coined by Hartzell in 1904.[1]

Oral Pyogenic granuloma can appear at any age and most commonly results due to irritation from calculus, overhanging restoration or a foreign object in gingival crevice.[2] It also occurs commonly in mucosa of females who have elevated steroid hormones, and these hormones are believed to play a vital role in pathogenesis of oral pyogenic granuloma.[3] Gingiva accounts for nearly 75% of all oral pyogenic granulomas. The other common locations being lips, tongue and buccal mucosa and on rare occasions the palate.[4] The treatment of choice for oral pyogenic granuloma is surgical excision. The recurrence rate of 16% is attributed to incomplete excision, persistent etiological factors or continued trauma[5], therefore, to avoid recurrences any potential irritants should be completely removed at the time of surgical intervention.

CASE REPORT

A female patient aged 32 years reported to the department of oral medicine and radiology with a chief complaint of growth behind the upper front teeth for 2 years. The growth was initially small and gradually increased to the present size. The patient also gave history of difficulty during speech. The patient gave history of occasional bleeding from the area especially while brushing. No relevant dental and medical history was reported by the patient. On general examination no abnormalities were detected. On intra oral examination, well-defined mass reddish pink in colour was noticed in the anterior palatal region posterior to 11 and 21, measuring approximately 3x2 cm in size (Fig 1).

Figure 1: Clinical photograph of the patient showing a growth on the anterior palate

On reflection with a periodontal probe, the mass appeared to be pedunculated and attached to the incisive papilla (Fig 2).

Figure 2: Clinical photograph of the patient showing growth arising from the incisive papilla

The mass was firm in consistency and non-tender. No other abnormalities were detected intra orally. Based on these findings a provisional diagnosis of pyogenic granuloma was made. The differential diagnosis of fibroma, peripheral ossifying fibroma and peripheral giant cell granuloma were considered.

An intra-oral periapical radiograph was made of 11, 21 region which showed generalised horizontal bone loss interdentally along with rarefaction in between 11 and 21 suggestive of localised periodontitis (Fig 3).

Figure 3: Intra oral periapical radiograph showing bone loss

The mass was surgically excised (Fig 4) with sutures in place under local anaesthesia followed by histopathology investigation.

Figure 4: Clinical photograph of the excised lesion

The Haematoxylin & Eosin stained section showed epithelium and connective tissue. The epithelium was stratified squamous parakeratinized type showing surface ulceration at few areas and hyperkeratinisation. The connective tissue was composed of numerous blood vessels and proliferating blood capillaries lined by endothelium in a background of dense bundles of collagen fibres, fibroblasts and dense inflammatory infiltrate consisting mainly of lymphocytes, neutrophils, plasma cells and few mast cells. Few extravasated RBCs were also seen (Fig 5a and b).

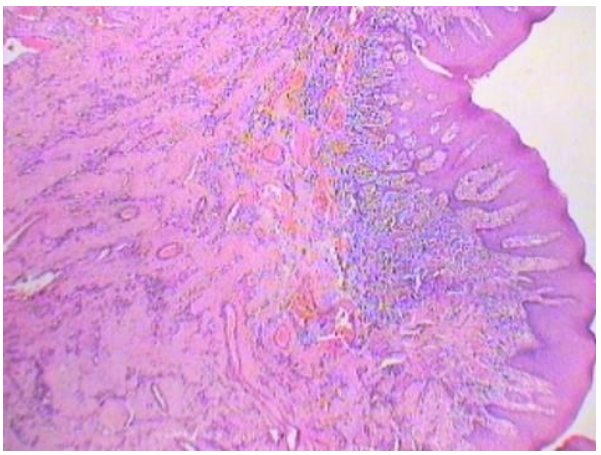

Figure 5a: H&E Staining (40x) Shows stratified squamous parakeratinized epithelium along numerous with proliferating blood capillaries in the underlying connective tissue.

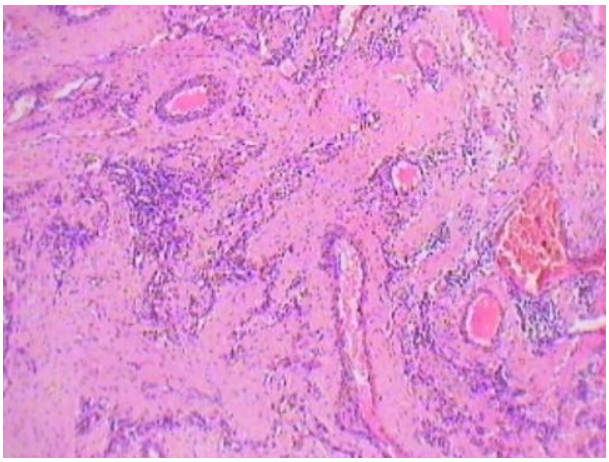

Figure 5b: H&E Staining (100x) shows proliferating and budding blood capillaries lined by endothelium.

A confirmed diagnosis compatible with the clinical diagnosis of pyogenic granuloma was made. The patient was followed up for 2 months and no recurrence was noted.

DISCUSSION

Pyogenic granuloma, a benign vascular lesion is also called as lobular capillary hemangioma and occurs due to inflammatory hyperplasia of skin or mucosa.[6] Oral pyogenic granuloma is either pedunculated or sessile and can present with an appearance of smooth or lobulated surface. The lesion can occur at any age but is frequently seen in the third decade of life, with a predilection of 1.5:1 in favour of the females.[7] The oral pyogenic granuloma occurs more in the maxilla than the mandible with most of the cases seen in the anterior region, involving buccal side more than the lingual.[5] Oral pyogenic granuloma usually has a painless and slow growth but on occasions may grow fast till its full size and thereby remains stable. Initially the lesions are more vascular,

bleed easily and are often elevated and ulcerated and later become collagenized and turn pinkish.[2] The size of this benign vascular lesion, which presents either as a papule or nodule, is usually in millimetres but sizes more than 1 centimetre have also been witnessed in the reported literature.[8] Oral pyogenic granuloma mostly occurs on gingiva, making around 75% of this variant. It usually manifests as a red and purple nodule originating from the interdental gingiva, occasionally with a fibrinopurulent layer covering an ulcer. Among the most common extra gingival locations, namely lips, tongue, palate, buccal mucosa and floor of the mouth, its palatal location is only rarely seen. The incisive papilla location for an oral pyogenic granuloma is even rarer among common extra gingival locations and hence makes the present case report a unique case. Although the precise etiology is still not clear, local trauma is an underlying factor in up to 50% of cases, the other frequently reported factors include infection, poor oral hygiene and hormonal imbalance.[9] Estrogen receptors seen on epithelial mucosa of women may predispose them to the development of oral pyogenic granuloma.[10] Few drugs (e.g., Isotretinoin, oral contraceptives), bacteria, viruses, primary dentition injury, aberrant tooth development and even eruption of teeth could lead to oral pyogenic granuloma.[5]

Oral pyogenic granuloma has two histopathological variations, Lobular Capillary Hemangioma (LCH) and non-LCH. The LCH type shows lobular collections of proliferating blood vessels, although superficially there is no specific change in the lesion including capillary dilation, edema or inflammatory granulation reaction in the tissues.[11] The non-LCH type has abundant vascular proliferation resembling granulation tissue.[12] Oral pyogenic granuloma should be differentially diagnosed with peripheral ossifying fibroma, peripheral giant-cell granuloma, conventional granulation tissue, hemangioma, and hyperplastic gingival inflammation.[9] On occasions differential diagnosis may include malignant lesions, like melanotic melanoma, metastatic carcinoma, or non-Hodgkin's lymphoma.[13] Oral pyogenic granuloma being a benign lesion is successfully treated with surgical excision. The lesion can be treated non-surgically by suture ligation, cryotherapy, pulsed dye laser or CO_2 laser, sclerotherapy, cryotherapy or electrocauterization.[14] Steroid injections have also been used (e.g., Triamcinolone acetonide), and is advocated particularly for recurrent cases after failed surgical methods.[15] We managed our case with conventional surgical technique depending upon enough access and least number of recurrences reported with the surgical approach. The patient in this case report was followed for 2 months with no evidence of any relapse.

CONCLUSIONS

Oral pyogenic granuloma occurs on gingiva in about 75% of cases. Among extra-gingival sites, the palate is a unique location for this lesion. Any potential source of irritation should be dealt with while designing the treatment strategy for successful treatment of this lesion.

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CONFLICTS OF INTEREST STATEMENT

NIL

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Case Report

SPINDLE CELL LIPOMA OF THE BUCCAL MUCOSA- A CASE REPORT

ABSTRACT

Spindle cell lipomas are a distinct variant of lipomas that occur very rarely in the oral cavity. The most common site for occurrence of this entity in the oral cavity is the tongue. The presence of spindle cells in the lesion calls for critical histopathological evaluation of the lesion. Here we present a new case of spindle cell lipoma that occurred on the buccal mucosa of a 45-year-old female patient. We provide an insight to the pathogenesis and the pathology of the entity.

Key words: Lipoma, adipocytes, mouth mucosa, CD34, mast cells.

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INTRODUCTION

Spindle cell Lipoma (SCL) is a rare, histologically distinct variant of lipoma that was first described by Enzinger and Harvey in 1975.[1] They seldom occur in the oral cavity and account for 0-9.8% of lipomas of the oral cavity.[2] Currently, in literature 44 cases of Oral Spindle cell lipomas has been reported.[3]

Oral Spindle cell lipomas usually occur in patients of an age range of 31 years to 78 years.[4] While some authors report a balanced distribution between males and females others report a male predominance for this lesion.[4,5] Oral spindle cell lipomas commonly present as a painless, well-circumscribed submucosal slow growing mass of 1 cm in diameter. They are soft in consistency and yellow in color and usually located on the lateral border of the dorsal anterior 2/3rd of the tongue.[4,6] The tongue is the most common site for Spindle cell Lipomas followed by the floor of the mouth and the buccal mucosa.[4,7.8]

Histologically, Spindle cell Lipomas are characterized by the presence of mature adipocytes surrounded by a thin fibrous capsule that originates in mature fat cells. Bland mitotically inactive spindle cells arranged parallel to the adipocytes, bundles of thick rope-like collagen, a myxoid stroma, and scattered mast cells are also components of spindle cell lipomas.[9,10,11]

The presence of spindle cells in the lesion warrants special attention and needs to be differentiated from other benign and malignant spindle cell lesions. Here we present a new case of spindle cell lipoma that occurred on the buccal mucosa and provide an insight to the pathogenesis and the pathology of the entity.

CASE REPORT

A 45-year-old female patient presented with a 1.5 cm X 1.0 cm X 0.6 cm growth on the buccal mucosa. Informed patient consent was obtained and the mass was excised and sent for histopathological examination. The gross specimen showed focal yellow and grey-white areas. Histopathological examination showed a parakeratotic stratified squamous epithelium overlying a tumor composed of mature adipocytes with bland spindle cells with wispy eosinophilic cytoplasm, mast cells, few congested vessels and collagen fragments (Fig 1, Fig 2 and Fig 3). No mitotic figures were seen. The spindle cells were positive for CD34 (Fig 4). Based on the histopathological appearance and the immunohistochemical positivity for CD34 the lesion was diagnosed as a Spindle cell Lipoma.

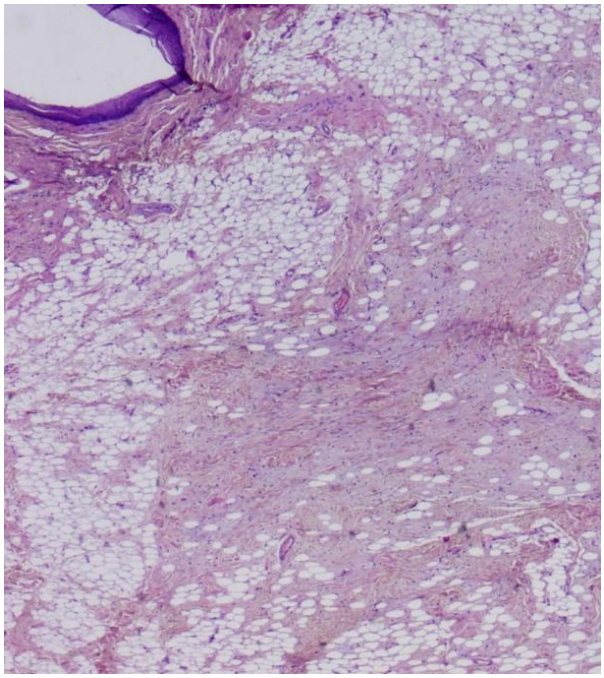

Fig 1: Photomicrograph showing mature adipocytes in cellular stroma comprising of spindle cells (H&E-10X)

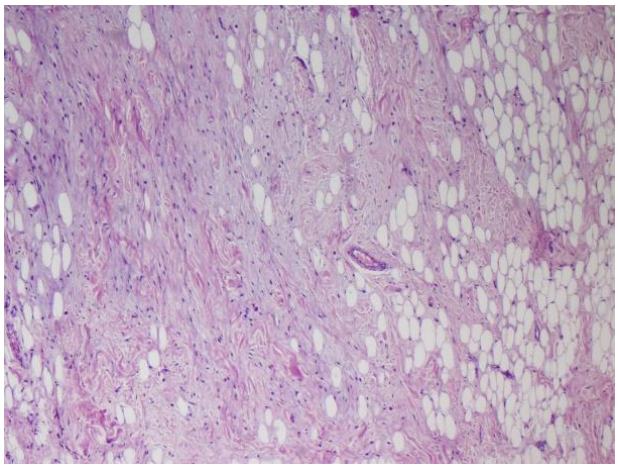

Fig 2: Photomicrograph showing mature adipocytes in a myxomatous stroma (H&E 10X)

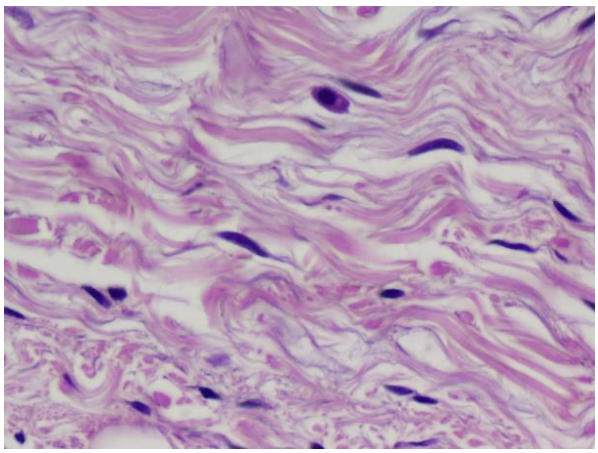

Fig 3: Photomicrograph showing bundles of rope- like collagen (H&E- 40X)

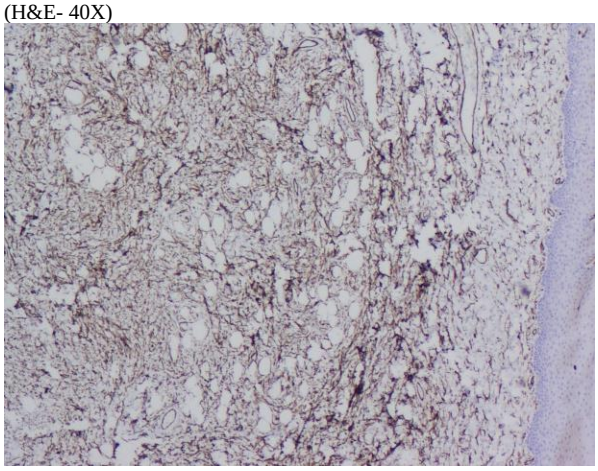

Fig 4: Photomicrograph showing the Spindle cells positive for CD34 (IHC -40X)

DISCUSSION

Oral spindle cell lipomas are rare lesions Among the 44 cases of SCL reported so far, twelve cases occurred on the buccal mucosa (Table 1). Among the 12 cases of spindle cell lipomas that occurred on the buccal mucosa, 10 of them occurred in males and 2 occurred in females. In this report the patient is a 45-year-old female. The size of the lipomas occurring in the buccal mucosa ranges from 10 mm to 50 mm. It has been noted that many patients who present with spindle cell lipomas are diabetic and hypertensive patients as well.

Table 1. Twelve reported cases of Spindle cell lipoma of the buccal mucosa[18]

Author /Year	Age (Y)	Gender	Location	Size (mm)
Tosios et al 1995	55	M	Buccal Mucosa	40
Khoo and Lian, 1995	23	M	Buccal Mucosa	50
Piatelli et al 1999	75	M	Buccal Mucosa	20
Piatelli et al 2000	63	M	Buccal Mucosa	25
Agoff et al, 2001	61	F	Buccal Vestibule	30
Kawasaki et al 2006	42	F	Buccal Mucosa	50
Billings et al 2006	88	M	Buccal Mucosa	10
Vecchio et al2009	52	M	Buccal Mucosa	25
Calderia et al 2011	38	M	Buccal Mucosa	50
Chandrashekar et al 2012	58	M	Buccal Mucosa	10
Manor et al 2013	43	M	Buccal Mucosa	25
Miloro et al 2015	**71**	**M**	**Buccal Mucosa**	**35**

This lipomatous lesion comprises spindle cells, adipocytes with collagenous and occasionally a mucinous matrix in a spectrum of variation among these components.[1] Although the cellular nature of the tumor is similar to several benign mesenchymal tumors the ropey- collagen bundles are an unique feature of SCL.[12]

The spindle cells in the tumors arise from fibroblasts or are similar to the stellate

mesenchymal cells of the primitive fat lobules.[1,13,14] Yet, other investigators have suggested that spindle cells are actually immature mesenchymal cells that remain in position during the transformation to mature lipocytes and are capable of synthesizing only collagen at an early stage.[15]

The main differential diagnosis for Spindle cell lipoma is a well-differentiated Liposarcoma (WDL). While the spindle cells in SCL have an orderly appearance without pleomorphism and scarce mitotic activity, the multivacuolated pleomorphic adipocytes are the components of WDL. In addition the WDL exhibits a prominent plexiform capillary pattern.[15,16,17]

Cytogenetic analysis of the cells in spindle cell lipoma has shown a characteristic karyotypic aberration, notably loss of material from the long arm of chromosomes 13 and 16. These changes are also seen in pleomorphic lipoma.[18]

The treatment of choice for SPL is surgical excision. The prognosis for oral spindle cell lipomas is good. Recurrences are rare and are encountered only when the lesion is infiltrating and invading the surrounding muscle.

CONCLUSIONS

Spindle cell lipoma is benign slow growing soft tissue neoplasm that rarely occurs in the oral cavity. Although a benign lesion, spindle cell lipomas can grow to a large size. Histologically the lesion typically comprises mature adipocytes and spindle cells. Immunopositivity for CD34 by the spindle cells in the lesion helps in confirming the diagnosis.

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CONFLICTS OF INTEREST STATEMENT

None

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