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

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

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

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

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Articles will normally appear in the order in which they were accepted as publication,

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

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

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Evaluation of antioxidant and antimutagenic activities of aluminum chloride

Purpose

Hemostatic agents are used to control hemorrhage and the gingival crevicular fluid for dental applications. In this study; the antimutagenic and antioxidant activities of aluminum chloride ($AlCl_3$), a topical hemostatic agent used especially in the fields of dermatology and dentistry, were determined. To our knowledge, this is the first study that investigates these properties.

Materials and Methods

The antioxidant activity was determined by DPPH free radical scavenging and β -carotene-linoleic acid bleaching assays. The antimutagenic activity was evaluated with the Ames *Salmonella*/microsome mutagenicity test using *Salmonella typhimurium* TA98 and TA100 strains.




Results

The total antioxidant activity of $AlCl_3$, determined by β -carotene bleaching assay was found to be $25.59 \pm 2.55\%$ and the DPPH scavenging activity of $AlCl_3$ was determined as $17.49 \pm 3.07\%$. $AlCl_3$ showed not any mutagenicity at the tested concentrations by the AMES test used *S. typhimurium* TA98 and TA100. This drug demonstrated antimutagenic effects at the test concentrations and the strongest antimutagenic activity was observed on $1.25 \text{ mg}\cdot\text{mL}^{-1}$ /plate concentration of $AlCl_3$.

Conclusion

$AlCl_3$ showed potent antimutagenic and antioxidant activities and these properties are significant for dentistry and dermatology.

Keywords: $AlCl_3$; Ames; oxidative stress; DPPH; hemostatic

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İnci Rana Karaca³ 

Introduction

Mutations and rearrangements in DNA may give rise to development of many degenerative diseases such as atherosclerosis, autoimmune diseases, Alzheimer's disease, some types of diabetes, and aging (1,2). In cancer initiation and other stages of the carcinogenic process, mutation in somatic cells plays a key role (3).

Reactive oxygen species (ROS) made oxidative stress in the organism and these radicals caused oxidation of biomolecules and eventually cellular damage. The damage that ROS brings about on DNA, protein and lipids result in tissue injury (4,5). During oxidative stress lots of ROS are revealed and they are one of the most common reasons of intracellular DNA modifications (6,7). Oxygen radicals react with DNA and this interaction lead to oxidative damage of DNA (8). Following the reactions formed with free radicals, base changes in nucleic acid and chain breaks in DNA take place. If this change is not repaired, mutation and mutagenic DNA forms will be formed (9).

Cancer incidence may be reduced if mutation rates are decreased. The

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best way to decrease mutation rates in human beings is to prevent exposure to mutagenic and carcinogenic agents (10). The natural antimutagens, using control of mutagenicity, can prevent cancer and other diseases caused by genotoxic agents (11,12).

Aluminum chloride is a topical agent used for hemostasis. It is formulated as AlCl_3 and has acidic character. It can be formulated in concentrations of 20-40% in water, alcohol, ether or glycerol as a protein coagulant (13). The 25% buffered aluminum chloride solution is marketed under the brand name Frenna AC Solution (Dharma Research, Inc., US).

Since there is a significant amount of protein in the blood, its protein coagulant property makes this agent a potent hemostatic agent (13). When blood is exposed to AlCl_3 a chemical reaction occurs between blood proteins and hydrochloric acid (HCl) that is believed to be formed by hydrolysis of AlCl_3 (13,14). This causes coagulation of the tissues, vasoconstriction, thrombus and occlusion of small blood vessels, tissue damage and thus activation of the extrinsic coagulation pathway, protein precipitation and coagulation (14,15).

The literature has reported use of this material especially in the fields of dermatology and dentistry (13). In dermatology, AlCl_3 is applied to bleeding areas with a cotton-tipped applicator after the wound is dried as much as possible. The tip is applied on the wound with a slight pressure and a twisting motion perpendicular to the skin. It is known to be used after curettages and shave and punch biopsies (15). It is also used as a successful contrast enhancer to differentiate between normal and cancer cells in Mohs micrographic surgical technique (16). In dentistry, it has been put to use in dental surgeries to obtain hemostasis and also as a medicament solution for gingival retraction cords to obtain proper impressions in the field of prosthetic dentistry (13,17). Furthermore, AlCl_3 is the most popular topical treatment applied to patients with Frey Syndrome who present with complaints about hyperhidrosis (18).

AlCl_3 is a preferred agent because it is an inexpensive, easy to use, easily stored material that does not require preparation (15). Despite these advantages, it has side effects such as painful paresthesia, burning sensation, tissue irritation and delayed wound healing; thus it must be applied with caution (15,19).

This study is aimed to determine the antimutagenic property of AlCl_3 . The H_0 hypothesis of this study is that there is no difference between the different concentrations of AlCl_3 on the antimutagenic and antioxidant activities. To the best of our knowledge, this property has not yet been studied and this will be the first to be reported in the literature.

Materials and Methods

Frenna AC solution

The sample of AlCl_3 was provided as Frenna AC solution (25% AlCl_3) (Dharma Research Inc., US). The concentrations of 0.025, 0.25, 1.25 and $\text{mg}\cdot\text{mL}^{-1}/\text{plate}$ of AlCl_3 prepared with distilled water were used in the mutagenicity and antimutagenicity tests. For antioxidant activity measurements, 125 $\text{mg}\cdot\text{mL}^{-1}$ concentration prepared with distilled water was used.

Microbial strains

The mutagenicity and antimutagenic activity of AlCl_3 was determined with Ames Salmonella/ microsome mutagenicity assay. In this test the mutant strains *S. typhimurium* TA98 and TA100, histidine dependent *Salmonella* strains were used (20).

Determination of DPPH radical scavenging activity

The radical scavenging activity of AlCl_3 was determined by DPPH free radical method (21). AlCl_3 was used at 125 $\text{mg}\cdot\text{mL}^{-1}$ concentration. Ascorbic acid (5 $\text{mg}\cdot\text{mL}^{-1}$) and α -tocopherol (5 $\text{mg}\cdot\text{mL}^{-1}$) were used as positive controls.

The β -carotene bleaching assay

The antioxidant activity of AlCl_3 was also determined by the β -carotene bleaching assay (22). AlCl_3 was used at 125 $\text{mg}\cdot\text{mL}^{-1}$ concentration; ascorbic acid (5 $\text{mg}\cdot\text{mL}^{-1}$) and α -tocopherol (5 $\text{mg}\cdot\text{mL}^{-1}$) were used as positive controls. Antioxidative activity of the AlCl_3 was compared with the positive controls ascorbic acid and α -tocopherol.

Mutagenic and antimutagenic activity

Mutagenic and antimutagenic activities of AlCl_3 were examined using the plate incorporation method (23) detailed by Sarac and Sen (24). Known mutagens 4-nitro-o-phenylenediamine (4-NPD) (3 $\mu\text{g}/\text{plate}$) and sodium azide (NaN_3) (8 $\mu\text{g}/\text{plate}$) were used as positive controls for *S. typhimurium* TA98 and *S. typhimurium* TA100, respectively. After determining the cytotoxic doses of AlCl_3 , the subcytotoxic doses (0.025, 0.25, and 1.25 $\text{mg}\cdot\text{mL}^{-1}/\text{plate}$ concentrations) were studied for the activity assay. The antimutagenic activity (%) was calculated using the following equation:

$$\text{Inhibition (\%)} = (A-B/A) * 100$$

A: The number of revertants per plate in the positive control,

B: The number of revertants per plate in the presence of mutagen and AlCl_3 .

40% or more inhibition was determined as strong; 25-40% inhibition as moderate, and 25% or less inhibition was determined as low/none antimutagenic activity (11,25).

Statistical analysis

All tests were carried out in triplicates. Data were presented as mean \pm SD. Dose dependent antimutagenic activity of AlCl_3 is also evident from the correlation and regression analyses, the F- and t-tests were used. SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) and Microsoft Excel 2010 were used for the statistical evaluations. All hypotheses were tested at 0.05 significance level.

Results

This study evaluated the antioxidant and antimutagenic properties of AlCl_3 , a buffered hemostatic agent, used in the fields of dermatology and dentistry.

H_0 hypothesis was accepted. DPPH assay was used to determine the free-radical-scavenging activity of AlCl_3 (Table 1). The radical scavenging activity of AlCl_3 was less than the

Table 1: Free radical scavenging activity (%) of AlCl₃.

Sample	Activity (%)
AlCl ₃	17.49 ± 3.07
α-tocopherol	92.95 ± 0.54
Ascorbic acid	96.59 ± 0.06

Table 2: Antioxidant activity (%) of AlCl₃ in β-carotene bleaching assay.

Sample	Antioxidant activity (%)
AlCl ₃	25.59 ± 2.55
Ascorbic acid	55.08 ± 2.95
α-tocopherol	91.99 ± 0.61

Table 3: The antimutagenic activity of AlCl₃

Test items	Concentration	Number of revertants			
		TA98		TA100	
		Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)
Negative control		5.66 ± 0.57		48.5 ± 2.12	
4-NPD [†] (μg/plate)	3	378 ± 29.66		-	
NaN ₃ [†] (μg/plate)	8	-		757 ± 33.2	
AlCl ₃ (mg·mL ⁻¹ /plate)	1.25	231.33 ± 24.19	38.8	520 ± 20	31.00
	0.25	300.33 ± 20	20.54	542 ± 20.29	28.40
	0.025	370.66 ± 4.04	1.95	670.66 ± 18.07	11.40

[†]4-NPD and NaN₃ were used as positive controls.

activity of α-tocopherol and ascorbic acid. According to the results, AlCl₃ has moderate scavenging activity (17.49%).

Total antioxidant activity of AlCl₃ was evaluated using the β-carotene bleaching assay (Table 2). The total antioxidant activity of AlCl₃ was found to be lower than that of ascorbic acid and α-tocopherol. The antioxidant activity results showed that AlCl₃ has moderate total antioxidant activity (25.59%).

In the AMES test, firstly, the cytotoxicity of AlCl₃ on *S. typhimurium* TA 98 and TA 100 was evaluated and the minimum cytotoxic dose was determined as 2.5 mg·mL⁻¹/plate concentration. In the mutagenicity and antimutagenicity tests the subcytotoxic doses of AlCl₃ (1.25, 0.25, and 0.025 mg·mL⁻¹/plate concentrations) were used. The AlCl₃ at the tested concentrations showed no mutagenic effects in the Ames test (data not shown).

The antimutagenic activity of AlCl₃ against 4-NPD and NaN₃ was determined with the same strains (Table 3). 0.025, 0.25, and 1.25 mg·mL⁻¹/plate concentrations were used; and AlCl₃ was effective at 1.25 mg·mL⁻¹/plate concentration on TA98, and at 0.25 and 1.25 mg·mL⁻¹/plate concentrations on TA100. The strongest antimutagenic activity was determined at 1.25 mg·mL⁻¹/plate concentration on *S. typhimurium* TA98.

The antimutagenic activity of AlCl₃ increased dose dependently. AlCl₃ has moderate antimutagenic activity in higher test concentrations. The results showed no significant difference between the different doses of AlCl₃ in increasing the number of revertant colonies.

Discussion

The oxidation caused by toxic ROS on cellular structures such as DNA, lipids, proteins, carbohydrates and other biological molecules may result in DNA mutations and/or damage target cells or tissues which frequently leads to cell senescence and death (26,27). Natural antioxidants acquired

from herbs and spices play a role in inhibition or prevention of the destructive consequences of oxidative stress (28).

Ames test is a short-term bacterial reverse mutation test specifically designed to investigate new substances and drugs that can cause damage genetically and lead to gene mutations (20). The *Salmonella* strains used in Ames test have different mutations in the histidine operon and each mutation is designed to respond to mutagens that have various mechanisms of action (20,23).

Genetically transferred metabolic disorders, various human diseases with age related and cancer, are resulted by mutations (29). The best way to decrease mutation rate in human beings is to lessen exposure to mutagenic and carcinogenic agents (10).

Cancer can be defined as an excessive multiplication of cells, which when followed by a cell invasion in the tissue surrounding it, spreads to other parts of the body. One of the chief characteristics of cancer is consistent cell proliferation, which disrupts the balance of the cell life cycle (30). Usually, cancer occurs when a mutation takes place in a cell and later it undergoes transformation turning into a malignancy of different stages by an acquisition (in a sequence) of further mutations (31).

Oral cancer stands fifth among the most commonly suffered cancer forms around the world; it is a life shattering disease (32). Oral cancer can be described as the cancer of pharynx and mouth, tongue, lips, palate, alveolar mucosa, floor of the mouth, tonsils, salivary glands, buccal mucosa, gingiva, and oropharynx (33).

Cancer potential may be minimised if the mutation rate is decreased. An effective way to control this mutation rate is by avoiding exposure to the ingestion of carcinogens and mutagens (10).

The use of antimutagenic and anticarcinogenic agents in daily life is the most effective method to prevent cancer and genetic diseases (10). The control of cellular mutagens by

natural antimutagenic agents can help prevent the mutations that eventually result in cancer and other diseases caused by genotoxic agents (11,12). Even though the antimutagenic activity of a drug does not definitely indicate that it is anticarcinogenic, it may certainly be considered a sign of anticarcinogenicity (34).

Conclusion

According to analysis performed within the present study, $AlCl_3$ was found to exhibit antimutagenic and antioxidant activity *in vitro*. The results showed that $AlCl_3$ was safe at the tested concentrations, and may represent an easily attainable antioxidant and antimutagenic source for dental applications. Moreover, the antioxidant and antimutagenic activity of $AlCl_3$, used as a hemostatic agent in dermatology and dentistry, has potential characteristics to provide prophylaxis against oxidations and mutations to an extent.

Türkçe Öz: Alüminyum kloritin antioksidan ve antimutajenik aktivitelerinin değerlendirilmesi. Amaç: Hemostatik ajanlar, kanamayı ve dişeti oluşu sıvısını kontrol etmek amacıyla diş hekimliği uygulamalarında kullanılmaktadır. Bu çalışmada; özellikle dermatoloji ve diş hekimliğinde topikal hemostatik ajan olarak kullanılan alüminyum kloritin ($AlCl_3$) antimutajenik ve antioksidan aktiviteleri belirlenmiştir. Edinilen bilgilere göre bu çalışma bu aktivitelerin araştırıldığı ilk çalışma olma özelliğindedir. Gereç ve yöntem: Antioksidan aktivite DPPH serbest radikal giderim ve β -karoten- linoleik asit ağartma deneyi ile belirlenmiştir. Antimutajenik aktivite Ames Salmonella/mikrozomal mutajenite testi ile Salmonella typhimurium TA98 ve TA100 suşları kullanılarak değerlendirilmiştir. Bulgular: $AlCl_3$ 'ün β -karoten- linoleik asit ağartma deneyi ile belirlenen toplam antioksidan aktivitesi % 25.59 ± 2.55 olarak belirlenmiştir. DPPH serbest radikal giderim aktivitesi $17.49 \pm 3.07\%$ olarak tespit edilmiştir. $AlCl_3$ 'ün test konsantrasyonlarında, Salmonella typhimurium TA98 ve TA100 suşları ile yapılan Ames testine göre herhangi bir mutajenik etkiye sahip olmadığı belirlenmiştir. Bu ilaç test konsantrasyonlarında antimutajenik etki göstermiştir ve en güçlü antimutajenik aktivite $1.25 \text{ mg}\cdot\text{mL}^{-1}$ /petri konsantrasyonunda tespit edilmiştir. Sonuç: $AlCl_3$ antimutajenik ve antioksidan aktivite göstermiştir ve bu özellikler diş hekimliği ve dermatoloji için önem arz etmektedir. Anahtar Kelimeler: $AlCl_3$; Ames; oksidatif stres; DPPH; hemostatic

Ethics Committee Approval: Not required.

Informed Consent: Not required.

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Does tooth loss in the mandibular posterior region have an effect on the mental index and panoramic mandibular index?

Purpose

Mental index (MI) and panoramic mandibular index (PMI) are important radiomorphometric indices used for assessing the quality of the mandibular bone. The aim of this study was to investigate the possible effect of mandibular posterior tooth/teeth loss in young adults on the MI and PMI (superior panoramic mandibular index: PMI-s, and inferior panoramic mandibular index: PMI-i).

Materials and Methods

Digital panoramic radiographs belonging to 253 patients aged between 18-35 years old were examined. According to the inclusion criteria of the study, a patient and control group was formed. The patient group (n=46) included individuals having at least one extracted tooth in the posterior region of the mandible, and the control group (n=45) included full dentate patients. The MI and PMI (PMI-i and PMI-s) were calculated bilaterally for all of the individuals.

Results

No significant difference was found between the MI, PMI-i and PMI-s indices of the patient group and the control group among both genders ($p>0.05$).

Conclusion

The mandibular premolar and/or molar teeth loss in young adults did not lead to any change in the MI, PMI-i and PMI-s indices among both genders.



Keywords: Mandible; mental index; panoramic mandibular index; panoramic radiography; tooth loss

Introduction

Bone metabolism, skeletal mineral status, the extraction of teeth, surgical procedures, occlusal forces transmitted by prosthesis, physical and muscular activity, presence of teeth, different types of denture support, thickness of the mandibular bone, body mass index, and drug intake are factors reported to influence bone mineral density (1).

Panoramic radiographs are commonly used in dental practice for radiographic assessment. Studies have reported that these radiographs could be used to predict local bone loss in patients. Such an evaluation could be performed with specially designed radiomorphometric indices.

The mental index (MI) and the panoramic mandibular index (PMI) are among these radiomorphometric indices (2-4). The MI defines the cortical width in the mental foramen region. It is calculated as the distance of the mandibular cortex on the line perpendicular to the inferior border of the mandible in the middle of the mental foramen (2). The PMI evaluates the cortical thickness of the mandible normalized for the mandibular size. The PMI is assessed as superior (PMI-s) and inferior (PMI-i) panoramic mandibular indices. This index is calculated by dividing the cortex thickness to the distance of the superior and inferior

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margin of the mental foramen to the inferior border of the mandible separately (3).

The jaws undergo continuous alveolar ridge atrophy after the extraction of teeth. This process happens more severely in the mandible than the maxilla (5). Some studies have investigated the effect of the age, gender, edentulism, dental status, and residual alveolar ridge resorption on the radiomorphometric indices in adults and the elderly (6-9). In this study, we tested the null hypothesis that teeth loss in the mandibular posterior region does not have an effect on the MI and PMI in young adults.

Materials and Methods

Population characteristics

The present study protocol was approved by the ethical committee of Gazi University (2017-368). The digital panoramic radiographs present in the archive of the Oral and Dentomaxillofacial Radiology department in the time period of January-May 2017 belonging to patients aged 18-35 years old were evaluated in this study. Informed consent was routinely obtained from all the patients prior to the radiographic examinations. The anamnesis data recorded in the system was assessed and non-medicated individuals free from systemic diseases or trauma were chosen. According to this, a total of 253 digital panoramic radiographs were present in the archive. Out of these 253 digital panoramic radiographs, individuals having at least one extracted tooth in the posterior region of the mandible; excluding the third molars, were accepted into the patient group. Subsequently, the patient group was divided into three sub-groups: 1-Patients having missing teeth on the right side of the posterior mandible 2-Patients having missing teeth on the left side of the posterior mandible, and 3-Patients having missing teeth both on the right and left side of the posterior of the mandible. After this, the individuals having no missing teeth in the mandible and maxilla; excluding the third molars in both jaws, were accepted into the control group. Ninety-one panoramic radiographs out of 253, provided these criteria. Forty-six of these belonged to the patient group (25 males, 21 females), and 45 (22 males, 23 females) belonged to the control group. The four radiographs belonging to the patient group and the four radiographs belonging to the control group were excluded from the study due to undefined mental foramen and /or inferior mandibular cortex borders.

Imaging protocols

The digital panoramic radiographs were obtained with a machine (Sirona Dental Systems, Bensheim, Germany), operating at 66 kVp, 8 mA, with a 0.5 mm focal spot and an exposure time of 14 seconds with standard positioning according to the manufacturer's recommendation. The magnification factor of the machine was 1:25. The panoramic images were in a JPEG format with a resolution of 1935x1054 pixels, 96 dpi and 24 bits. All the measurements were carried out using Image J 1.48v software (National Institutes of Health, USA). All the radiographic evaluations were done on a 20-inch LCD monitor with a resolution of 1600x900 operating at 32-bits (HP 2011x, Hewlett-Packard Company USA) in a quiet room with subdued ambient lighting.

Radiographic evaluation

The evaluation of the radiographs was made independently by two Oral and Maxillofacial Radiology experts. The MI and PMI were measured on the panoramic radiographs. The measurements were made according to the following criteria: The MI was calculated as the distance of the mandibular cortical thickness on the line perpendicular to the bottom of the mandible in the middle of the mental foramen both for the right and the left side (10) (Figure 1). The PMI was calculated as both PMI-s and PMI-i for the right and left side as (3-11): PMI-s: mandibular cortical thickness/distance from the superior margin of the mental foramen to the inferior border of the mandible. PMI-i: mandibular cortical thickness/distance from the inferior margin of the mental foramen to the inferior border of the mandible (Figure 2). Where possible, all the measurements in the control group were made on both the left and right sides. At least two weeks later, both observers repeated their measurements on 40 digital panoramic radiographs (20 patient group, 20 control group) to evaluate the intra-observer agreement level.

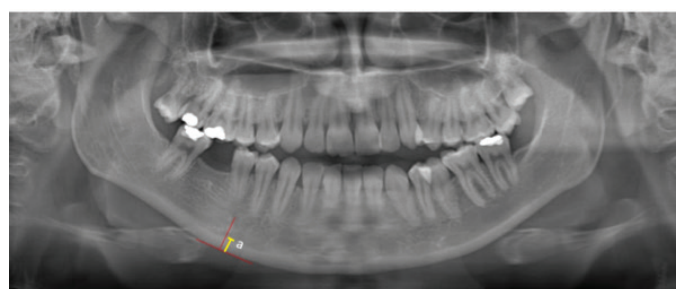


Figure 1. Mental Index (MI). Length of "a" in mm.

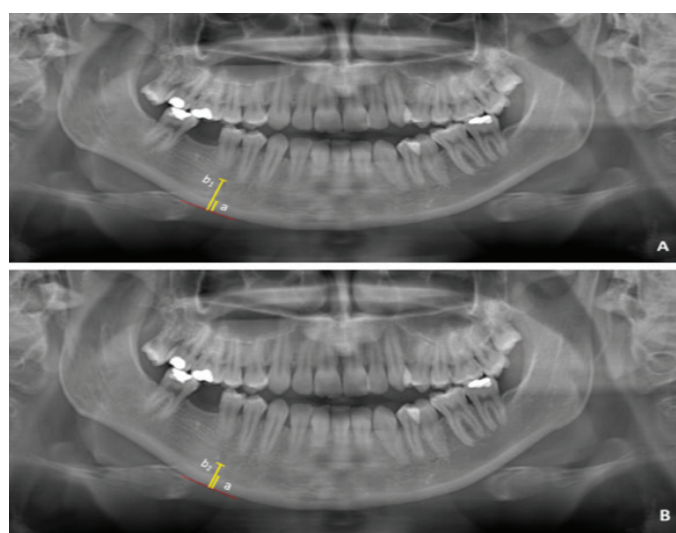


Figure 2. Panoramic Mandibular Index (PMI). PMI-s = ratio of a/b_1 (A), PMI-i = ratio of a/b_2 (B).

Statistical analysis

IBM SPSS Statistics 23 software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY, USA) was used in the present study. The normality of the data was assessed with the Kolmogorov Smirnov and Shapiro-Wilk

tests before the analysis of the index measurements. When the data showed a normal distribution, the parametric t-test was used and when not, the Mann Whitney-U test was used for the analysis of the indices. The first and second measurements of the two observers showed a normal distribution. The paired t-test and Pearson correlation coefficient were therefore used for the evaluation of the intra-observer agreement level. Any difference between the genders was assessed with the t-test. Confidence level was set to 95% and $p < 0.05$ was considered significant.

Results

The mean age of the patient group was 27.61 (Sd:4.86) and the control group was 27.04 (Sd:4.67). The descriptive statistics and gender distribution of the age and p-values regarding the t-tests are given in Table 1.

3-premolars+molars. Any difference between these tooth groups was analyzed with the ANOVA test. Depending on the test results, no significant differences between tooth groups for the MI ($p=0.144$), PMI-s ($p=0.719$), PMI-i ($p=0.945$).

The difference between the first and second measurements of the first and second observer for the MI was non-significant. ($p = 0.356 > 0.05$; $p = 0.481 > 0.05$, respectively) The intra-observer agreement for the MI was found to be $r = 0.943$ and $r = 0.652$ for the first and second observer respectively according to the 95% confidence interval. No significant difference was detected between the first and second measurements of the first and second observer for the PMI-s ($p = 0.301 > 0.05$; $p = 0.610 > 0.05$, respectively). The intra-observer agreement for the PMI-s was found to be $r = 0.922$ and $r = 0.680$ for the first and second observer respectively according to the 95% confidence interval. The difference between the first and second measurements of the first and second observer for the

Table 1. Descriptive statistics of gender distribution regarding age and statistical analysis results according to t-test.

Groups	Gender	N (%)	Mean (Sd)	p-value	95% Confidence Interval of the Difference	
					Lower	Upper
Control	Female	23 (51.1%)	27.83 (5.25)	0.256	-4.400	1.203
	Male	22 (48.9%)	26.23 (3.94)			
	Total	45 (100%)	27.04 (4.68)			
Patient	Female	21 (45.7%)	29.00 (3.92)	0.075	-5.387	0.267
	Male	25 (54.3%)	26.44 (5.32)			
	Total	46 (100%)	27.61 (4.86)			

Sd: Standard deviation

Table 2. Descriptive statistics of the extracted tooth/teeth according to location and gender.

	Female N (%)	Male N (%)	Total N (%)
Right-Side	8 (42%)	11 (58%)	19 (41%)
Left-Side	3 (27%)	8 (73%)	11 (24%)
Bilateral	10 (62%)	6 (38%)	16 (35%)

Nineteen (41%) of the extracted teeth belonged to the right side, 11 (24%) belonged to the left side, and 16 (35%) belonged to the bilateral sides of the posterior region of the mandible. The details are given in Table 2. The most frequent extracted teeth were the first molars on both sides of the mandible among both genders. None of the first premolars were extracted. The descriptive statistics of the extracted tooth/teeth and gender are given in Table 3.

No significant difference was observed between age and the measurements made for all the indexes for both observers ($p > 0.05$). When all the groups (right side, left side, and bilateral) were assessed, no significant difference was found between the MI, PMI-s, and PMI-i from the patient group and the control group ($p > 0.05$). The extracted teeth were divided into three groups as: 1-premolars, 2-molars and

Table 3. Descriptive statistics of the extracted tooth/teeth according to tooth/teeth type and gender.

Teeth No	Female N (%)	Male N (%)	Total N (%)
44	-	-	-
45	2 (4%)	3 (6%)	5 (7%)
46	15 (5%)	15 (5%)	30 (39%)
47	4 (67%)	2 (33%)	6 (8%)
34	-	-	-
35	3 (5%)	3 (5%)	6 (8%)
36	12 (48%)	13 (52%)	25 (33%)
37	3 (75%)	1 (25%)	4 (5%)

PMI-i was non-significant ($p = 0.944 > 0.05$; $p = 0.964 > 0.05$, respectively). The intra-observer agreement for the PMI-i was found to be $r = 0.921$ and $r = 0.627$ for the first and second observer respectively according to the 95% confidence interval. No significant difference was detected for the measurements made for the MI, PMI-s and PMI-i for the right side, the left side, and the total for both observers ($p > 0.05$). The correlation coefficients for the inter-observer agreement level were statistically significant ($p < 0.05$).

Table 4. Comparison of the mean index values of the control group (a) and patient group (b).

	Groups	Mean (Sd)	p-value	95% Confidence Interval of the Difference	
				Lower	Upper
Right-MI	Control	10.60 (1.64)	0.336	-0.374	1.085
	Patient	10.25 (1.70)			
Right-PMI (sup)	Control	0.27 (0.06)	0.659	-0.018	0.029
	Patient	0.27 (0.05)			
Right-PMI (inf)	Control	0.34 (0.08)	0.697	-0.037	0.025
	Patient	0.34 (0.07)			
Left-MI	Control	10.25 (1.98)	0.712	-0.660	0.962
	Patient	10.10 (1.74)			
Left-PMI (sup)	Control	0.27 (0.08)	0.817	-0.021	0.027
	Patient	0.26 (0.05)			
Left-PMI (inf)	Control	0.32 (0.07)	0.514	-0.041	0.021
	Patient	0.33 (0.07)			
Total-MI	Control	10.43(1.81)	0.356	-0.375	1.085
	Patient	10.17 (1.71)			
Total-PMI (sup)	Control	0.27 (0.07)	0.698 (mw)	-0.074	0.034
	Patient	0.27 (0.05)			
Total-PMI (inf)	Control	0.32 (0.07)	0.460 (mw)	-0.037	0.025
	Patient	0.33 (0.07)			

Table 5. Comparison of the mean index values of the control group (a) and patient group (b) according to gender.

a. Control Group	Gender	Mean (Sd)	p-value	95% Confidence Interval of the Difference	
				Lower	Upper
Right-MI	Female	10.57 (1.64)	0.901	-0.987	1.117
	Male	10.64 (1.69)			
Right-PMI (sup)	Female	0.28 (0.06)	0.272	-0.055	0.016
	Male	0.27 (0.05)			
Right-PMI (inf)	Female	0.35 (0.08)	0.209	-0.078	0.017
	Male	0.32 (0.06)			
Left-MI	Female	9.77 (1.66)	0.111	-0.235	2.206
	Male	10.76 (2.18)			
Left-PMI (sup)	Female	0.27 (0.06)	0.507	-0.048	0.024
	Male	0.26 (0.06)			
Left-PMI (inf)	Female	0.33 (0.07)	0.625	-0.054	0.035
	Male	0.32 (0.07)			

a. Patient Group	Gender	Mean (Sd)	p-value	95% Confidence Interval of the Difference	
				Lower	Upper
Right-MI	Female	9.82 (1.74)	0.144	-0.275	1.821
	Male	10.60 (1.62)			
Right-PMI (sup)	Female	0.29 (0.23)	0.097	-0.060	0.003
	Male	0.26 (0.06)			
Right-PMI (inf)	Female	0.36 (0.07)	0.067	-0.078	0.002
	Male	0.32 (0.06)			
Left-MI	Female	9.77 (1.77)	0.259	-0.470	2.206
	Male	10.38 (1.69)			
Left-PMI (sup)	Female	0.27 (0.06)	0.536	-0.048	1.697
	Male	0.26 (0.05)			
Left-PMI (inf)	Female	0.34 (0.08)	0.512	-0.043	0.022
	Male	0.33 (0.06)			

As a main result, no significant difference was found between the MI, PMI-s, and PMI-i of the patient group and control group for the right side and the left side of the mandible. In addition, bilateral teeth loss did not influence the indices ($p > 0.05$). No significant difference was observed between females and males ($p > 0.05$). The details are given in Tables 4 and 5.

Discussion

Mandibular bone quality may be influenced by age, sex, and dental status (9). According to our results, we did not find any difference between the MI, PMI-s, and PMI-i values for the subjects aged between 18 and 35 years old. The increase of age from 18 to 35 did not lead to a difference in the mean MI, PMI-s, and PMI-I index values. This means that these indices were not influenced by age in young adults. It was reported that there was an age-related decrease for the MI values in the females and males (3,6,8,9). Previous studies investigated the interactions of the radiomorphometric indices with age and loss of dentition (8,12,13). Zlataric et al. (14) evaluated the MI values in middle aged and elderly individuals and showed that MI values decreased in both genders until 78 years. Ledgerton et al. (8) reported that in British women, MI underwent a gradual reduction until the sixth decade, and then decreased sharply. They also reported that PMI-s and PMI-i were negatively correlated with age and that there was a significant difference between the index values between ages <55 and >65 years old. The difference between our results and these studies could be related to the difference in the age groups. We evaluated young adults and others evaluated middle aged adults and older in their studies. Middle aged adults and older adults could be affected by osteoporosis, which is reported to decrease the MI and PMI values (15-17).

To the best of our knowledge, there are limited studies concerning the effect of tooth loss on MI and PMI in young adults. In our study we found that tooth/teeth loss did not have any effect on the MI, PMI-s and PMI-i values in young adults. Moradi et al. (12) reported no effect of tooth loss on MI and PMI among adults and older patients. In their study, the dental status of the patients was classified as full dentate, partially dentate (having all teeth except molars), and completely dentate. Mostafa et al. (13) evaluated the effect of edentulism on the MI and PMI. They recorded both maxillary and mandibular dentitions (excluding third molars) as full dentition, partial dentition (any tooth missing), and complete edentulous. According to their results, they found no effect of dentition on the indices. In our study, we only evaluated the right, the left, and the bilateral mandibular posterior region according to one or more extracted teeth and did not divide the individuals into the groups mentioned in the two studies above. We also gave detailed information regarding the extracted tooth type, extracted tooth number, and location of the extracted tooth. However, as this was a retrospective radiographic study, we were unable to obtain the information regarding the time period since the tooth/teeth have been extracted. Although the age groups and dental status were classified differently, our results showed a similarity with both studies. According to the results of our study and the other studies conducted on different age groups, one may conclude that edentulism; both among young individuals and older

individuals, does not have an effect on these indices. When a tooth/teeth are extracted from the jaws, the structure and function of the muscles of the mastication change. Thus, the atrophy in the insertion regions of these muscles may happen. However, these results may be related to the phenomena of 'no atrophy occurring in the inferior cortex of the mandible and mental foramen regions due to tooth extraction' (13,18).

Panoramic radiographs are available in most dental clinics. It is an easy radiographic technique to perform. The resultant image shows the maxillary and mandibular teeth and the mandible and maxillae. The cortical border of the mandible and foramen mentale is also visible. One disadvantage is that in some cases the inferior and the superior border of the foramen mentale and inferior mandibular cortex borders cannot be visible enough for the selection of the points to be measured. In our study the four radiographs belonging to the patient group and the four radiographs belonging to the control group were excluded due to undefended mental foramen and /or inferior mandibular cortex borders.

MI and PMI are indices used worldwide. They are both repeatable and reproducible on digital panoramic images (19). They are easy to interpret as they rely on the measurement of the specific points on panoramic radiographs. The measurements could be done with digital rulers on digital panoramic images or they could be done with digital calipers on conventional radiographs. One problem with the MI is that the result is a direct linear measurement made on the panoramic radiograph; thus, one could not compare the MI values of the panoramic images taken with different machines as the magnification value differ among panoramic machines. The PMI is the ratio of two linear measurements thus, the differences in magnification between the different panoramic machines does not affect the results, and a comparison between the images is possible (6,13). At this point, the PMI differs from the MI. We found no significant difference between the genders in terms of tooth/teeth loss. Thus, we can conclude that gender does not influence early tooth/teeth extraction on the MI and PMI-s and PMI-i. Our results show a similarity with Mostafa et al. (13).

Gender is an important factor affecting the bone metabolism. Osteoporosis is seen more frequently among females due to hormonal changes in menopause. Osteoporosis is a pathology affecting the skeletal system. It is characterized by 'low bone mass, compromised bone strength, and microarchitectural deterioration predisposing to an increased risk of fracture'. Osteoporosis is the most common metabolic bone disease seen among individuals (20). Studies report that the thickness of the inferior cortex of the mandible may be useful to predict the bone mass from panoramic radiographs with these indices (9). Radiomorphometric indices and their relationship to gender and age are studied. The cortical bone in the mental region is significantly thinner in individuals with osteoporosis. PMI and MI may be used as an indicator of bone mineral changes (15-17).

Tooth or teeth extraction on the mandibular posterior region either on the right or left side or both sides did not influence the MI or PMI-s and PMI-i. Dölekoğlu et al. (21) investigated the effect of disorders affecting the skeletal metabolism among total edentulous patients and reported that the PMI-i and PMI-s did not differ significantly on the right or left side of the mandible.

Conclusion

According to this radiographic study mandibular premolar and/or molar teeth loss did not have any effect on the MI, PMI-s and PMI-i among young adults in both genders.

Türkçe Öz: Alt çene arka bölgedeki diş kaybının mental indeks ve panoramik mandibular indeks üzerindeki etkileri. Amaç: Mental indeks (MI) ve panoramik mandibular indeks (PMI) mandibulanın kemik kalitesini değerlendirmek için kullanılan önemli radyomorfometrik indekslerdir. Bu çalışmanın amacı genç erişkinlerde alt çene arka bölgedeki diş / dişlerin kaybının MI ve PMI (superior panoramik mandibular indeks: PMI-s ve inferior panoramik mandibular indeks: PMI-i) üzerine etkisinin araştırılmasıdır. Gereç ve yöntem: 18-35 yaşları arasındaki 253 hastaya ait dijital panoramik radyografiler incelendi. Çalışmanın dahil edilme kriterlerine göre hasta ve kontrol grubu oluşturuldu. Mandibula posterior bölgesinde en az bir eksik dişe sahip olan bireyler hasta grubunu (n = 46), tüm dişleri mevcut olanlar kontrol grubunu (n = 45) oluşturdu. Tüm bireyler için MI ve PMI (PMI-i ve PMI-s) hesaplandı. Bulgular: Hasta grubu ve kontrol grubunda her iki cinsiyet arasında MI, PMI-i ve PMI-s indeksleri arasında anlamlı bir fark bulunamadı (p > 0.05). Sonuç: Genç erişkinlerde mandibular premolar ve molar diş veya dişlerin kaybı, her iki cinsiyette de MI, PMI-i ve PMI-s indekslerinde herhangi bir değişikliğe yol açmamıştır. Anahtar Kelimeler: Mandibula; mental indeks; panoramik mandibular indeks; panoramik radyografi; diş kaybı

Ethics Committee Approval: The study protocol has been approved by the ethical committee of Gazi University (project no:2017-368).

Informed Consent: Informed consent was waived due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.

Author contributions: GA, ZA and KG participated in designing the study. GA and ZA participated in generating the data for the study. GA and ZA participated in gathering the data for the study. OK participated in the analysis of the data. ZK wrote the majority of the original draft of the paper. GA and ZA participated in writing the paper. All authors approved the final version of this paper.

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

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Relationship between dental plaque formation and salivary cortisol level in pregnant women

Purpose

Understanding how increased level of salivary cortisol contributes to the development of dental biofilm during pregnancy can help in the prevention of dental caries and periodontal diseases. This study aims to evaluate the relationship between salivary cortisol level and dental biofilm formation in pregnant women.

Patients and Methods

This descriptive-analytic study was conducted in Hamadan, Iran in 2011. Forty consecutive pregnant women with no history of abortion, stillbirth, or any known physical or psychological disorders at weeks 25 and 33 of gestation were included. Salivary samples were collected for measurement of cortisol levels by Enzyme Linked Immunoabsorbent Assay (ELISA) method. The amount and extension of dental biofilms were determined by using a disclosing agent. Data were analyzed using descriptive and analytical statistics in SPSS version 16.

Results

The mean levels of salivary cortisol at weeks 25 and 33 of gestation were respectively, $2.45 \pm 1.56 \mu\text{g/dl}$ and $5.24 \pm 4.07 \mu\text{g/dl}$ which demonstrates a significant difference ($P < 0.001$). Evaluation of dental biofilm at two time intervals revealed a significant increase in amount of dental biofilm at week 33 of gestational period ($34.65 \pm 10.9\%$ vs. $42.45 \pm 12.35\%$, $P < 0.001$). Elevated levels of dental biofilm were significantly correlated with salivary cortisol levels at week 33 ($r = 0.494$, $P = 0.001$), however, it was not significant at week 25 of gestation ($r = 0.148$, $P = 0.361$).

Conclusion








The findings suggested that increased levels of salivary cortisol can predict dental biofilm formation and accumulation in pregnant women in the last weeks of gestation.

Keywords: Cortisol; dental; plaque; biofilm; saliva; pregnancy

Introduction

It has been proven that changes occur in oral cavity and stomatognathic system during pregnancy which may lead to the periodontal disease, dental caries, oral mucosal changes, chloasma, tooth loosening and erosion (1-3). Although it has been suggested that poor oral health is the most important reason for these oral complications, some other causes including physiological and hormonal changes particularly in saliva during this period may lead to these oral diseases (4). In this context, significant hormonal changes occur in pregnant women that can directly affect the salivary hormones.

Stressful conditions within pregnancy can be induced by increased levels of cortisol and lead to decreased number of IgA and IgG antibodies which supports the growth of oral bacteria and the occurrence of local

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inflammation (5). Although the mechanisms of stress and dental plaque formation are not clear, stress may reduce individual resistance to dental disease causing bacteria. These microorganisms produce inflammatory and immune responses in the host tissue (6,7). Corticosteroids released during stress impede the immune response, which inhibits salivary immunoglobulins (especially IgA) and other antimicrobial proteins present in the saliva such as lactoferrin, lysozyme and lactoperoxidase. Catecholamines can have a direct effect on plasma cells by reducing the synthesis of secretion of immunoglobulin A. Simultaneous changes in the quality and quantity of saliva may lead to increased adherence capability and production of biofilm on dental surfaces and increased sensitivity to decay (8).

As dental biofilm causes tooth decay and periodontal disease, it is one of the most important indicators of the clinical progress of both condition (9). Studies in different groups such as children and women, have shown a positive association between cortisol and dental biofilm (10-13). However, Kambalimath et al. (14) who had investigated 4- to 5-year-old children, reported that no correlation between cortisol and dental biofilm can be found. Considering these conflicting arguments in the relationship between cortisol and dental biofilm, the importance of oral health during pregnancy, we aimed to investigate possible relationship between dental biofilm formation and cortisol levels in pregnant women. The null hypothesis tested in this study is that there is no correlation between biofilm formation and salivary cortisol levels in any examined period of pregnancy.

Patients and Methods

Sample characteristics

40 pregnant women were included in this cross-sectional study with simple sampling, which has been conducted in Hamadan, Iran, 2011. Inclusion criteria were nulliparous, gingival Index < 1 (15) and Beck anxiety test < 19 (16). Exclusion criteria were history of abortion, stillbirth, gestational diabetes, any known physical or psychological disorder, smoking and unwanted pregnancy. Also, subjects had to be between 18 and 35 years of age and had to have complete recorded data at the institutional healthcare centers. Pregnant women at week 25 of gestation who were experiencing their first pregnancy under normal sociologic and behavioral conditions were enrolled and they signed the informed consent approved by Institutional Ethics Committee (Ethic code: IR.UMSHA.REC.1396.428) at Hamadan University of Medical Sciences, Iran.

Sample size calculation

Based on previous studies, if the standard deviation of cortisol levels and salivary growth in pregnant women is considered to be about 0.45, then the minimum difference between cortisol groups is 0.3. When the error level is 0.05 and the power is 90%, the minimum number of subjects required per group was calculated as 38 which was rounded up to 40 for practical purposes.

Studies have shown that cortisol level increases between

25-33 weeks of pregnancy (17). After obtaining informed consent, in the first stage, all subjects were examined by one periodontist to determine gingival index in 25th week of pregnancy and rule out gestational diabetes by OGTT Test. Also, Beck Stress Test was performed by one psychologist. Subjects with gingival index > 1 and Beck Stress Test \geq 19 were excluded. At stage two, after 8 weeks, all participants were invited to healthcare centers and were reexamined in terms of amount and extension of dental biofilms and collection of second salivary samples to measure their salivary cortisol level.

Determination of gingival index

Gingival Index was considered for the assessment of gingival condition. It is scored on the basis of 0.0 to 3.0. The score 0.0 means normal gingiva; 1.0 means mild inflammation or slight change in color and slight edema but no bleeding on probing; 2.0 means moderate inflammation or redness, edema and glazing, bleeding on probing, and score 3.0 means severe inflammation or marked redness and edema, ulceration with tendency to spontaneous bleeding. The bleeding was assessed by probing gently along the wall of soft tissue of gingival sulcus. The scores of four areas of the tooth were summed and divided by four to give the gingival index for tooth (15). In our study, mothers with a score of over 1 were identified as being infected and excluded from the study.

Performance of Beck stress test

A questioner consisting of twenty-one questions that expressed common symptoms of stress and anxiety. Each question has the same set of four possible answer choices including; not at all (Score 0.0), mildly (Score 1.0), moderately (Score 2.0), and severely (Score 3.0) (18).

Determination of salivary cortisol level

This examination was carried out by Enzyme Linked Immunosorbent Assay (ELISA) method using commercial saliva cortisol kit produced by Germany. Forty selected participant's saliva samples were collected from each case based on the standard protocol that was described by Dr. Navazesh (19) to determine levels of salivary cortisol. In this method, saliva samples were collected by expectoration between 9 a.m. and 11 a.m. to avoid circadian variation. Participants were asked to avoid eating, drinking, and brushing for at least 2 hours. The saliva sample was poured over the first minute and the saliva sample of fifth minute was collected. Saliva was collected in a laboratory plastic container and transferred to the laboratory within 2 hours of sampling.

Determination of dental biofilm

Dental biofilm is the most important cause of gum and periodontal disease. Microbial plaque is a thin layer of germs that constantly sit on the surface of the teeth and is contained in the mouth of all adults. This layer has protein substances and Coverage cells and other substances of salivary origin, but its main building is from microbes, so plaque control is

one of the key components in dentistry. It is difficult to find a dental plaque. Normally, microbial plaque is not visible. Therefore, for observation, disclosing agents are used as chemical pills that can stain dental plaque. The participants received disclosing agent and were asked to chew the disclosing tablet and use language gestures by rubbing the tablet on the tooth surface. Then the tonality of the tooth was determined in facial, lingual, distofacial and mesiofacial surfaces and calculated according to the formula of Sillness & Loe (15).

Statistical analysis

Data were analyzed by descriptive (frequency distribution, percentage, and mean) and analytical statistics (paired T test, Pearson's Correlation Coefficient and Z-Fisher's correlation coefficient Test) using SPSS version 16 software (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, IL, USA). Confidence level was set to 95% and the probability values $p \leq 0.05$ were considered statistically significant.

Results

The mean age of participants was 23.65 ± 4.58 years. Regarding the socioeconomic status, all women were housewives, 7.5% were illiterate, 25.0% had primary education level, 52.5% had secondary education level, 12.5% had diploma, and only 2.5% had college degree. Also, 15.0% of women were categorized as low-economic and financial level, 67.5% as moderate economic level, and only 17.5% had appropriate economic level. Most of the women (95.0%) did not refer to dentist for periodical dental evaluation. With respect to dental health cares, 72.5% followed daily tooth brushing and 22.5% brushed twice a day and only 5% used both tooth brushing and dental floss. Kolmogorov-Smirnov Test showed that all variables followed a normal distribution. Paired T Test showed that the mean level of salivary cortisol was found to be significantly higher at week 33 of gestation than week 25 (5.24 ± 4.07 versus 2.45 ± 1.56 $\mu\text{g/dl}$, $P < 0.001$) (Table 1). Also, evaluation of dental biofilm at two time points revealed a significant increase in the amount of dental biofilm at week 33 of gestation compared to week 25 (42.45 ± 12.35 versus 34.65 ± 10.9 $P < 0.001$). Pearson's correlation coefficient analysis showed a positive correlation between salivary cortisol level and amount of dental biofilm at week 33 of gestation ($r = 0.494$, $P = 0.001$), whereas this correlation was not statistically significant at week 25 of gestation ($r = 0.148$, $P = 0.361$). In addition, Z-Fisher's correlation coefficient analysis showed that increased salivary cortisol level caused increase in the amount of dental biofilm ($r = 0.462$, $P = 0.001$).

Discussion

The aim of present study was determining the correlation between dental biofilm accumulation and salivary cortisol level during gestational period. The findings depicted that increased levels of salivary cortisol were directly associated with the level of dental biofilm in pregnancy. This is in line with the notion that high levels of salivary cortisol can provide the conditions in favor of pathogen-induced plaque formation (20). The results of our study were similar to those of Johansson et al. (13). In this study, 72 women (29 controls with a mean age of 54 years without disease, and 43 subjects with mental discomfort with a mean age of 42 years) were studied in order to find out the relationship between salivary cortisol and dental plaque and gingivitis. The mean cortisol in the study group was 3.25 ± 3.46 and in the control group 0.25 ± 0.30 . After measuring the dental plaque, the mean of this was higher in the study group. ($p = 0.003$) (13). In agreement with these data, Hugo et al, demonstrated that stress and salivary cortisol are significant risk indicators of plaque formation among individuals aged 50 years and older (21). But Kambalimath et al's study (14) found that there was no significant difference between case and control groups in terms of dental caries and stress in children. It seems that the main reason for the difference in the results of present study and Kambalimath et al's study (14) is that 1) stress as a phenomenon caused by the psychological and social pressures on the individual, is less effective in children, 2) children's dental checkup is not necessarily stressful, 3) and the process of dental caries is longer than the formation of dental biofilm.

A trilateral relation is suggested between stress, oral immunity, and microbial activity. Stressful conditions compromise oral immunity, as indicated by decreased levels of salivary immunoglobulin and salivary flow and increased microbial activity (22). It has been demonstrated that stressful conditions such as pregnancy are associated with elevated cortisol level (20, 21). Increased levels of blood and salivary cortisol had been shown during pregnancy because of gestational stress and decrease in glomerular filtration rate particularly after week 25 of pregnancy. In this regard, some studies indicated that high salivary cortisol levels were associated with periodontal disorders and these stress-induced periodontal diseases could be due to changes in immunological responses to periopathogens (20, 22). Our study is among the first studies that have reported an association between salivary cortisol and dental biofilm formation during pregnancy, hence this result should be interpreted with caution.

Findings suggested that increased levels of salivary cortisol can predict dental biofilm formation. Increase in salivary cortisol

Table 1: Salivary cortisol level and dental biofilm at 25 and 33 weeks of gestation (*paired t test).

Variable	Minimum	Maximum	Mean \pm SD	p value *
Cortisol level (25 week)	0 $\mu\text{g/dl}$	6.7 $\mu\text{g/dl}$	2.45 \pm 1.56	0.000
Cortisol level (33 week)	0.5 $\mu\text{g/dl}$	26 $\mu\text{g/dl}$	5.24 \pm 4.07	0.000
Dental biofilm (25 week)	20 %	66 %	34.65 \pm 10.9	0.000
Dental biofilm (33 week)	21 %	72 %	42.45 \pm 12.35	0.000

levels as a sign of prenatal stress is associated with dental biofilm formation particularly at week 33 of gestation. Although it needs to be confirmed by further studies using larger sample sizes and specifically by evaluating salivary immune markers in relation to periodontal disorders in pregnant women. Our study had limitations, we examined only one hormone which, if one or more of the other hormones were studied, could have a stronger judgment on our assumptions. As points of strength, in present study, the women with history of smoking, or those who have any evidence of gestational diabetes, psychological disturbances and other excluding criteria were excluded to eliminate potential effects of these confounders on dental biofilm formation.

Conclusion

Findings show a positive correlation between salivary cortisol and dental biofilm at week 33 of gestation. This finding is very important because health policy-makers should, with this correlation, endeavor to produce better plans for having less stress-induced maternal pregnancies because the risk of dental biofilm increases in pregnancy by increasing cortisol in stressed conditions. Because restoration of teeth during pregnancy is associated with some restrictions, especially in the third trimester, we may not be able to reduce the damaging effects of dental plaque and the prevention is better than cure.

Türkçe öz: Gebelerde tükürük kortizon seviyesi ile dental plak oluşumu arasındaki ilişkinin incelenmesi. Amaç: Tükürük kortizon düzeyinin gebelik sırasında dental biyofilmin gelişimine nasıl katkıda bulunduğunu anlamak, diş çürüğü ve periodontal hastalıkların önlenmesinde yardımcı olabilir. Bu çalışmada gebelerde tükürük kortizol düzeyi ile biyofilm oluşumu arasındaki ilişkinin değerlendirilmesi amaçlanmıştır. Hastalar ve yöntem: Tamamlayıcı-analitik çalışma, 2011 yılında İran'ın Hamadan kentinde gerçekleştirilmiştir. Gebeliğin 25 ve 33 haftaları arasında bulunup kürtaj, ölü doğum veya bilinen herhangi bir fiziksel veya psikolojik bozukluk öyküsü bulunmayan 40 gebe kadın dahil edilmiştir. Tükürük örnekleri kortizol düzeyinin ölçülmesi için ELISA (enzyme linked immunoabsorbent assay) yöntemi ile incelenmiştir. Dental biyofilmin miktarı ve yoğunluğu bir belirleyici ajan kullanılarak belirlenip veriler Spss 16 paket programında tanımlayıcı ve analitik istatistik analizler kullanılarak değerlendirilmiştir. Bulgular: Gebeliğin 25 ve 33 haftalarında ortalama tükürük kortizol düzeyleri sırasıyla 2.45 ± 1.56 mg/dl ve 5.24 ± 4.07 mg/dl idi. Bu farkın istatistiksel olarak anlamlı olduğu görülmüştür ($p < 0.001$). İki ölçüm zamanı arasında dental biyofilm miktarında anlamlı bir artış olduğunu görmüştür ($\%34.65 \pm 10.9$ ve $\%42.45 \pm 12.35$, $p < 0.001$). Yüksek düzeydeki biyofilm, 33 haftada tükürük kortizol düzeyi ile ilişkili olduğu görülürken ($r = 0.494$, $p = 0.001$), gebeliğin 25 inci haftasında anlamlı ilişki görülmemiştir ($r = 0.148$, $p = 0.361$). Sonuç: Bulgular, gebeliğin son haftalarında tükürükte artmış kortizol düzeyinin, biyofilm oluşumu ve birikimini öngörebileceğini göstermektedir. Anahtar kelimeler: Kortizol; diş; plak; biyofilm; tükürük; gebelik

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Hamadan University of Medical Sciences (Ethic code: IR.UMSHA.REC.1396.428)

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

Peer-review: Externally peer-reviewed.

Author contributions: AT and MS participated in designing the study. AT and PT participated in generating the data for the study. RO participated in gathering the data for the study. ARS participat-

ed in the analysis of the data. RO and MS wrote the majority of the original draft of the paper. ST participated in writing the paper. All authors approved the final version of this paper.

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Comparison of the effect of postoperative care agents on human gingival fibroblasts: a preliminary study

Purpose

The aim of this study is to compare effects of postoperative care agents; chlorhexidine, octenidine dihydrochloride and hyaluronic acid on human gingival fibroblasts' viability, proliferation, apoptosis and migration.

Material and Methods

After cell culturing; chlorhexidine, octenidine dihydrochloride and hyaluronic acid solutions were applied on cells and nothing was applied for control group. The cells were monitored to investigate cytotoxicity; the percentage of apoptotic, living and dead cells at the time of 24, 48, and 72 hours (h). A scratch wound assay was performed to detect cell migration and cells were monitored at baseline, at 24 and 48h.

Results

At 24h, chlorhexidine showed statistically lower percentage of total apoptotic cells' than octenidine dihydrochloride ($p=0.049$), hyaluronic acid ($p=0.049$) and control ($p=0.049$). At 48h, hyaluronic acid showed statistically lower percentage than chlorhexidine ($p=0.049$), and control ($p=0.049$). All agents were found to have statistically and significantly more cytotoxic than control. However, there was no difference between experimental groups for proliferation rate. Octenidine dihydrochloride showed statistically negative effects on cell migration than chlorhexidine and hyaluronic acid at 24h. Chlorhexidine and hyaluronic acid maintained migration ability of cells than octenidine dihydrochloride at 48h.

Conclusion

All agents have similar effects on cell behavior such as viability, apoptosis and cell proliferation. However, octenidine dihydrochloride showed statistically negative effects on migration ability than chlorhexidine and hyaluronic acid.

Keywords: Chlorhexidine; hyaluronic acid; octenidine dihydrochloride; cell viability; cell migration

Introduction

In oral surgery practice, mouth rinses are used for postoperative care to prevent complications caused by various risk factors including bacterial infection, surgical trauma, insufficient wound care, and poor oral hygiene (1). The healing process begins after oral surgical procedures, and wound care is a key factor for hindering healing complications caused by over inflammatory reactions or infection during the early wound healing process (2). Although bacterial invasion into the wound area is one of the reasons for postoperative infection and mouth rinses are commonly prescribed in order to prevent postoperative complications, oral mouth rinses have some cytotoxic activities that cause fibrinolysis, which can disrupt the wound-healing process (3). There are many postoperative care solutions such as chlorhexidine (CHX), octenidine dihydrochloride (OCT),

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povidone iodine, Meridol, and hyaluronic acid (HA). In the recent literature, there are studies about the cytotoxic and antimicrobial effects of these products, and each has its own advantages and disadvantages.

CHX is the most commonly prescribed antimicrobial mouth rinse after oral surgical procedures. CHX molecules are symmetrical cationic molecules composed of two 4-chlorophenyl rings and two biguanide groups (bisbiguanide) connected by a central hexamethylene chain (4,5). CHX is a substantive antimicrobial mouth rinse and maintains its activity for long periods. Nevertheless, CHX has been reported as having adverse effects such as causing alterations in the actin cytoskeletal assembly, and inducing apoptosis and autophagic and necrotic cell death (6).

OCT is a rarely studied mouth rinse, which is known for its lower cytotoxicity than CHX (3). OCT is a cationic surfactant and bis-(dihydropyridinyl)-decane derivative used as a postoperative care agent for mucosal and cutaneous wounds. Schmidt et al. demonstrated that OCT had a lower cytotoxic activity on human fibroblasts and epithelial cells (3).

Although these two mouth rinses, CHX followed by OCT, are widely used in clinical practice, their cytotoxic effects could impair the early wound healing process. Some postoperative care agents enhance healing, especially in wound healing; HA induces beneficial early granulation tissue formation, inhibits destructive inflammatory reactions during the healing phase, and supports reepithelization and angiogenesis (7). HA is an anionic, non-sulfated glycosaminoglycan molecule and is the major carbohydrate component of the extracellular matrix of many biologic structures such as connective, epithelial, and neural tissues. HA is a multifunctional biologic structure. Its synthetic form has been used in many different medical fields, in ophthalmology for dry eyes and postoperative care, in dermatology as a dermal filler and for promoting wound healing, and in rheumatology for joint fluid replacement. In addition to these clinical uses, HA's synthetic form for topical oral use for enhancing postoperative wound healing has also been reported (7,8). Furthermore, Al-Bayaty et al. investigated HA's antimicrobial activity and concluded that HA was antimicrobial, but when compared with CHX, its antimicrobial activity was very low (8).

Fibroblasts are crucial to the wound healing process. These cells are widely used in *in vitro* studies in order to examine cell behaviors during the wound healing process (3,9). The purpose of this study was to investigate and compare the *in vitro* effects of CHX, OCT, and HA on human gingival fibroblasts' (HGFs) viability, apoptosis, proliferation, and migration in the early wound healing period using MTT assays, Annexin-V assays, and wound scratch assays.

Materials and Methods

Human gingival fibroblast (HGF) culture

HGFs were obtained from Erciyes University Betul-Ziya Eren Genome and Stem Cell Center (Kayseri, Turkey) and cultured in DMEM-Low Glucose (Biological Industries, Kibbutz Beit Haemek, Israel) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin and streptomycin, and incubated at 37°C in a 5% humidified CO₂ atmosphere. An ethical consideration was not required.

The samples of the study were divided into the four groups to compare effects of postoperative care agents. The groups were; chlorhexidine applied group (CHX), octenidine dihydrochloride applied (OCT) group, hyaluronic acid (HA) applied and nothing (control group) were applied on human gingival fibroblasts' cell.

Cell proliferation (MTT) assay

MTT assays were performed to monitor cell proliferation and viability (10,11). Cells were seeded at 5000 cells/cm² in 96-well plates in standard culture medium. The final concentration was 0.5 mg/mL MTT in standard culture medium after 24 h, 48 h, and 72 h of culturing. Following 4 h of incubation, the MTT solution was removed and dimethyl sulfoxide was added to dissolve the formed formazan crystals. The culture dishes were agitated in a shaker for 10 minutes to ensure the dissolution of formazan crystals. Absorbance was measured at a wavelength of 560-750 nm using a Glomax Multi Detection System microplate reader (Promega, USA) (twelve replicates for each treatment).

Annexin V and dead cell assay

Apoptotic, live, and dead cells were detected using a fluorescein conjugated annexin V kit with a Muse EasyCyte flow cytometer following the manufacturer's instructions (Merck, Millipore, USA) (12). The average of the measurements from triplicate experiments was used in the calculation of the final data.

Wound healing assay

HGFs were grown to 95% confluence in 6 well-plates. A scratch wound was made by scratching the cells with a pipette tip (13). The cells were rinsed with CHX (Andorex®, Pharmactive, Turkey), OCT (Octenidol®, Schülke, Germany) and HA (Gencigel®, Ricerfarma, Italy) solutions for 30 seconds. HGFs were incubated in standard culture medium at 37°C in a 5% humidified CO₂ atmosphere. The wound area was photographed at the beginning, at 24 h, and at 48 h, and cell migration was assessed by measuring the gap size in at least

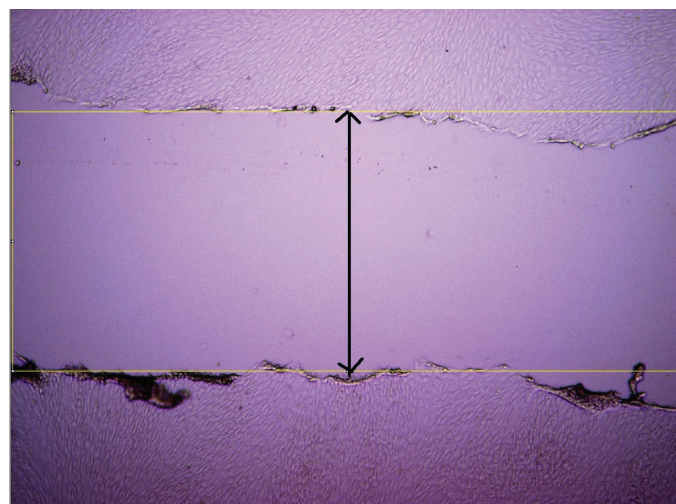


Figure 1. Calculating the width of the scratch wound by drawing and measuring vertical lines.

10 fields using Image J (National Institute of Mental Health, Maryland, USA) (Figure 1). The average of the measurements from three experiments was used in the calculation of the final data. The migration rate is expressed as the percentage of scratch closure on an initial area basis, according to the following equation ($A_t(\text{Baseline}) = \text{scratch width at time } 0$, and $A_t = \text{scratch width at } 24 \text{ h and } 48 \text{ h}$) (14):

Results

Cell proliferation

Graphical data of cell proliferation are shown in Figure 2. Twenty-four, 48, and 72 hours after administration of the solutions, there were statistically significant differences in cell proliferation between the control group and experimental groups (Table 1). However, there were no differences between the mouth rinse groups for proliferation rates according to the MTT assay. The cell proliferation value of the control group was significantly higher than in the CHX,

OCT, and HA groups ($p < 0.001$). There were no statistically significant differences in terms of cell proliferation between the CHX, OCT, and HA groups.

Cell viability

Graphical data of cell viability is presented in Figure 3. When both CHX and OCT solutions were applied, there were time-dependent statistically significant differences between the different times ($p = 0.027$) (Table 2). For both solutions, cell viability after 24 h was significantly higher than cell viability after 48 h and 72 h ($p = 0.049$). Cell viability in the CHX and OCT groups showed a decrease at 48 h and rose again after 72 h. In both groups, there were statistically significant differences for cell viability after 48 h and 72 h ($p = 0.049$). For the control and HA groups, there were no time-dependent statistically significant differences ($p = 0.148$).

Twenty-four and 48 hours after administration of the solutions, there was a statistically significant difference in cell viability between the solutions ($p < 0.05$). There were no

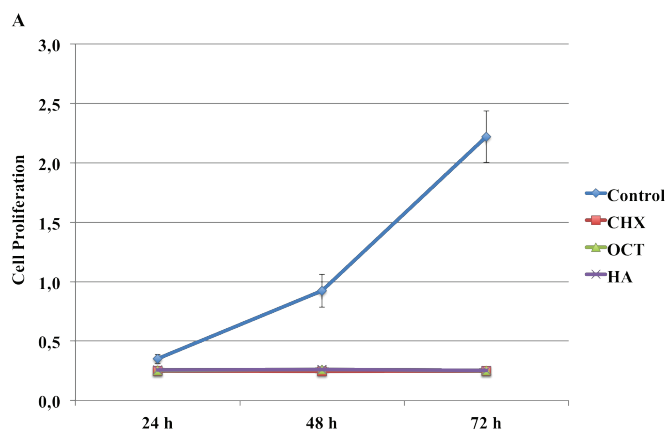


Figure 2. Time-dependent effects of CHX, OCT and HA on HGFs' cell proliferation.

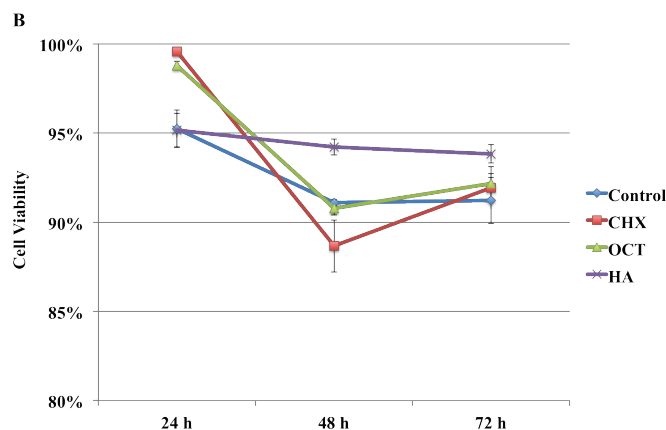


Figure 3. Time-dependent effects of CHX, OCT and HA on HGFs' Cell viability.

Table 1. Cell proliferation stratified by time periods (Post Hoc Tukey's test * $p < 0.05$).

MTT Assay	24 hours Mean±SD	48 hours Mean±SD	72 hours Mean±SD
Control	0.351±0.038	0.9242±0.138	2.2188±0.216
CHX	0.251±0.012	0.2459±0.013	0.2489±0.023
OCT	0.2515±0.016	0.2627±0.018	0.253±0.022
HA	0.2555±0.011	0.26±0.014	0.2539±0.023
p	0.002**	0.008**	<0.001***

Table 2. Cell viability in different time periods.

Living HGFs (%)	24 hours Mean±SD (median)	48 hours Mean±SD (median)	72 hours Mean±SD (median)	p
Control	95,25±1,05 (95,35)	91,10±0,18 (91,05)	91,23±1,29 (91,45)	0,061
CHX	99,58±0,20 (99,55)	88,67±1,46 (87,90)	91,93±0,80 (92,15)	0,027*
OCT	98,80±0,23 (98,75)	90,77±0,37 (90,90)	92,17±0,95 (92,10)	0,027*
HA	95,17±0,94 (94,95)	94,22±0,45 (94,15)	93,83±0,51 (93,70)	0,148
p	0,025*	0,022*	0,086	

statistically significant differences in terms of cell viability between the CHX, OCT, HA, and control groups after 72 h.

Total apoptotic cells

Graphical data of total apoptotic cells are shown in Figure 4. When both CHX and OCT solutions were applied, there were time-dependent statistically significant differences between the different times for total apoptotic cell percentages ($p=0.027$) (Table 3). For both solutions, apoptotic cell percentages after 24 h were significantly lower than living cell percentages after 48 h and 72 h ($p=0.049$). The apoptotic cell percentage in the CHX and OCT groups showed an increase at 48 h and declined again after 72 h. In both groups, there were statistically significant differences for apoptotic cell percentages after 48 h and 72 h ($p=0.049$). For the control and HA groups, there were no time-dependent statistically significant differences.

In the evaluation of the percentage of total apoptotic cells at 24 h, CHX showed a statistically lower percentage than OCT ($p=0.049$), HA ($p=0.049$), and the control group ($p=0.049$). At

48 h, HA showed a statistically lower percentage of apoptotic cells than the CHX ($p=0.049$) and control groups ($p=0.049$). CHX and OCT may prevent early apoptosis at 24 h; however, there were no statistically significant differences between the solutions at 72 h.

Gap closure rate

Graphical data of gap closure rates are given in Figure 5. CHX, OCT, and HA solutions had an inhibitory effect on HGF migration according to the wound healing assay. 24 and 48 hours after administration of the solutions, there was a statistically significant difference between the control group and the test groups ($p<0.001$) (Table 4). OCT showed a statistically worse effect on HGF migration than CHX ($p=0.01$) and HA ($p=0.01$) at 24 h. CHX and HA maintained the ability of HGF migration better than OCT at 48 h ($p=0.01$, $p<0.001$, respectively). CHX and HA had a similar effect according to the wound healing assay. Inhibition of cell migration was observed in the CHX, OCT, and HA groups in the scratch gap tests (Figure 6).

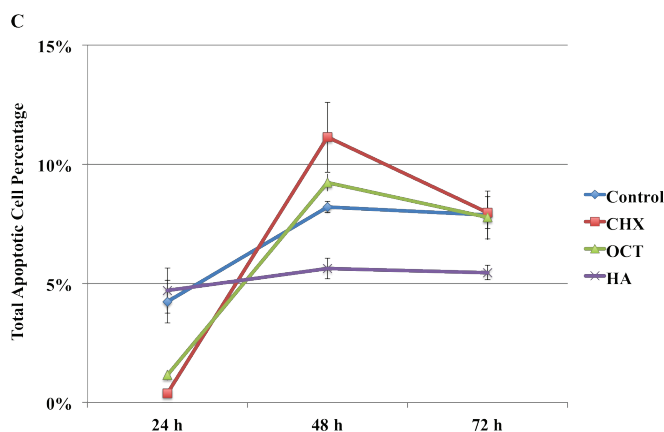


Figure 4. Time-dependent effects of CHX, OCT and HA on HGFs' total apoptotic cell percentage.

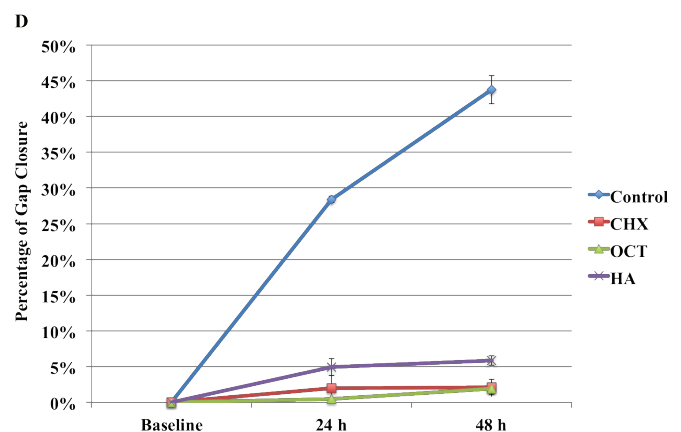


Figure 5. Time-dependent effects of CHX, OCT and HA on Gap Closure Rate in the wound scratch assay.

Table 3. Total apoptotic cell percentage for different time periods.

Total apoptotic HGFs (%)	24 hours	48 hours	72 hours	
	Mean±SD (Median)	Mean±SD (Median)	Mean±SD (Median)	
Control	4.23±0.90 (4.30)	8.20±0.23 (8.25)	7.87±1.01 (7.70)	0.061
CHX	0.38±0.16 (0.45)	11.13±1.47 (11.8)	7.97±0.67 (7.80)	0.027*
OCT	1.15±0.22 (1.25)	9.23±0.37 (9.10)	7.77±0.95 (7.80)	0.027*

Table 4. Gap closure rate (*statistically significant).

Wound scratch assay gap closure rate (%)	24 hours Mean±SD	48 hours Mean±SD
Control	28.42±0.81	43.74±3.44
CHX	1.99±3.11	2.15±1.90
OCT	0.50±0.72	1.91±1.91
HA	4.94±2.01	5.87±1.16
p	<0.001*	<0.001*

Discussion

In this study, HGFs were chosen to analyze the cytotoxic effects of CHX, OCT, and HA, because fibroblasts are essential to the wound healing process. After homeostasis and clot formation, wounds enter the proliferative step. Fibroblasts are one of the primary cell types that expand during this phase, driven by a burst of growth hormones supplied by the inflammatory response (15). The proliferative potential of

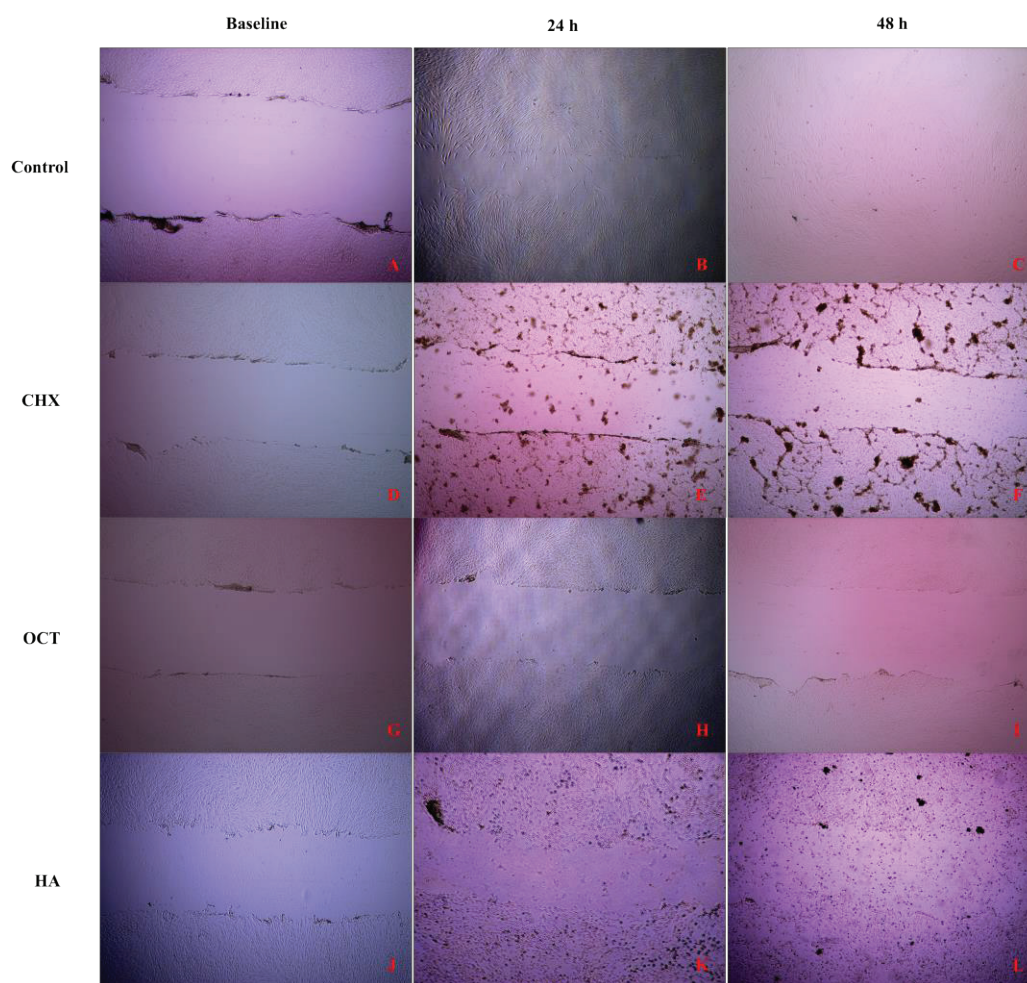


Figure 6. Gap closure views of control, CHX, OCT and HA groups at baseline, 24 h and 48 h.

fibroblasts in the wound bed appears to drive the course of the healing process (16).

CHX is the most widely used and verified agent, having a broad activity spectrum against oral pathogens with good tolerability (17). Löe and Schiott defined CHX as the gold standard of mouth rinses in 1970 (4). The most important advantage of CHX is the very high level of substantivity, which leads to prolonged adherence of the antiseptic on hard and soft oral tissue. Therefore, the antiseptic is gradually released at an effective dose, which assures the persistence of its antimicrobial effect (18).

However, negative effects of CHX on human alveolar bone cells, stem cells from buccal fat pads, and HGFs were observed in many studies (1, 9, 18). Cabral and Fernandes analyzed osteoblastic cells *in vitro* using MTT assays and suggested that CHX had a negative effect on cell growth of osteoblastic cells (1). Furthermore, Eick et al. demonstrated that commercially available CHX had a very strong cytotoxic effect on HGFs in MTT assays at different concentrations (9). Park et al. analyzed cell viability of stem cells derived from buccal fat pads using cell counting kit-8 (CCK-8) assays and found that both CHX and Listerine had negative effects on cell viability and relative viability (19). Schmidt et al. compared the cytotoxic effects of CHX and OCT using MTT assays and found that OCT had a lower cytotoxic potential on HGFs and human nasal epithelial cells in the applied concentration compared with CHX (3). An *in vitro* study on L929 cells (ATCC CCL 1) derived from an immortalized mouse fibroblast cell line, which is routinely used in *in vitro*

cytotoxicity assessments, by Müller and Kramer reported that OCT was less cytotoxic than several antiseptic solutions including CHX, benzalkonium chloride, cetylpyridinium chloride, mild silver protein, polyhexamethylene biguanide, povidone iodine in solution, povidone iodine in ointment, silver (I) sulfadiazine, and triclosan (20). In the present study, all of the studied postoperative care agents showed a statistically significant negative effect on cell viability compared with the control group in the MTT assays. A comparison of the experimental groups showed no statistically significant differences between them.

In the literature, it was suggested that exogenous HA application could decrease the inflammatory response and prevent oxygen free-radical damage after tooth extraction (21, 22). HA has a moderating effect by erasing free radicals (23). Mendoza et al. reviewed all available data on the features and clinical profile of HA and they claimed that HA could erase free radicals (24). In a study conducted by Ye et al., it was suggested that high-molecular-weight HA could be an effective protective agent that had antioxidant properties (25). Gocmen et al. investigated the antioxidant and anti-inflammatory effect of HA and reported that it had an anti-inflammatory effect following wisdom tooth extraction. However, the oxidative stress levels and clinical outcomes were similar after one week. The authors suggested that according to their histologic data, HA application showed a lower inflammatory response. However, the clinical outcomes after one week showed no significant differences between the

groups. The authors claimed that the reason for this difference was that postoperative sequelae generally resolve within one week (2). The better cell viability and apoptosis findings of the HA group in this study compared with other groups might be related to this anti-inflammatory characteristic.

In this present study, on comparing cell viability and apoptosis, the findings showed concordance with each other. At 24 h, CHX showed better results compared with OCT and HA, but at 48 h, HA showed better results than all groups including the control group. These data suggest that HA might have a positive effect on cell viability and apoptosis at 48 h.

Conclusion

All the mouth rinses had similar effects on cell behavior such as viability, apoptosis, and cell proliferation. Although the results of the present study support the hypothesis that CHX, OCT, and HA prevent cell migration and maintain HGF viability, OCT showed a greater statistically negative effect for HGF migration ability than CHX and HA. The reason of the negative effect of HA on cell migration and viability could be the concentration used in this experiment. Different concentrations at different times for these agents should be further studied. Additional experiments that investigate other parameters such as cell differentiation, collagen synthesis and breakdown, inflammatory response, and growth factor release should be conducted in order to understand the effects of these agents at a molecular level. Additionally, in this experiment, the antimicrobial effects of these agents were not evaluated. Finding the balance between bactericidal effects without cellular toxicity is important for long-term postoperative treatment. Further experimental and clinical studies are needed to evaluate the antimicrobial effects of CHX, OCT, and HA; therefore, it would be more suitable to combine these findings with the cellular effects of these agents in order to understand their clinical effects.

Türkçe Öz: Ameliyat sonrası kullanılan bakım ürünlerinin insan diş eti fibroblastlarına etkilerinin karşılaştırılması: ön çalışma. Amaç: Bu çalışmanın amacı postoperatif bakım ajanları olan; klorheksidin, oktenidin dihidroklorür ve hyalüronik asitin insan gingival fibroblastlarının canlılığı, proliferasyonu, apoptozisi ve migrasyonu üzerine etkilerini karşılaştırmaktır. Gereç ve Yöntem: Hücre kültüründen sonra; klorheksidin, oktenidin dihidroklorür, hyalüronik asit çözeltileri hücrelere uygulandı ve kontrol grubu için hücrelere hiçbir şey uygulanmadı. Hücrelerin apoptotik, canlı ve ölü hücre yüzdeleri sitotoksisiteyi araştırmak amacıyla 24, 48 ve 72 saat izlendi. Hücre migrasyonunu saptamak için yara tahmini testi yapıldı ve hücrelerin başlangıç, 24. saat ve 48. saat takipleri yapıldı. Bulgular: 24 saat sonra, klorheksidin; oktenidin dihidroklorür ($p = 0.049$), hyalüronik asit ($p = 0.049$) ve kontrol ($p = 0.049$)'dan istatistiksel olarak daha düşük toplam apoptotik hücre yüzdesi gösterdi. 48. saatte, hyalüronik asit; klorheksidin ($p = 0.049$) ve kontrol ($p = 0.049$)'dan istatistiksel olarak daha düşük apoptotik hücre yüzdesi gösterdi. Tüm ajanlar kontrol grubuna göre istatistiksel olarak daha fazla sitotoksik bulundu. Bununla birlikte, çoğalma oranı açısından deney grupları arasında fark yoktu. Oktenidin dihidroklorür, hücre göçünde 24. saatte klorheksidin ve hyalüronik asitten istatistiksel olarak daha negatif etki gösterdi. 48. saatte klorheksidin ve hyalüronik asit oktenidin hücre migrasyon kapasitesi oktenidin dihidrokloritten daha fazla bulundu. Sonuç: Tüm ajanlar canlılık, apoptoz ve hücre proliferasyonu gibi hücre davranışları üzerinde benzer etkilere sahiptir. Bununla birlikte, oktenidin dihidroklorür; klorheksidin ve hyalüronik aside göre, hücre göç etme kapasitesi üzerinde istatistiksel olarak daha olumsuz bir etki

gösterdi. **Anahtar Kelimeler:** Klorheksidin; hyalüronik asit; oktenidin dihidroklorit; hücre canlılığı; hücre göçü

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: UM, ZBG and HS participated in designing the study. UM and ZBG participated in generating the data for the study. ZBG, GMYU and DGM participated in gathering the data for the study. UM participated in the analysis of the data. UM, ZBG, GMYU and DGM wrote the majority of the original draft of the paper. UM, ZBG, HS and GMYU participated in writing the paper. All authors approved the final version of this paper.

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

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Retrospective evaluation of patients admitted to Karadeniz Technical University Pediatric Dentistry clinic due to trauma

Purpose

Traumatic dental injuries are among the commonly observed problems in the primary and permanent teeth. The rate of prevalence of dental trauma varies globally. In this study, we investigated the type of dental trauma, related factors, and treatment procedures in children.

Subjects and Methods

During a 5-year period (January 2011–January 2016), 416 children aged in the range of 1–15 years were admitted to our clinic with dental trauma. The cause and type of the dental trauma in the primary and permanent teeth and their relation with gender and age were evaluated using the chi-square test, and their distribution by age was evaluated using regression analysis.

Results

Overall, girls and boys comprised 37% and 63% of the study population, respectively. The mean age was 8.5 years. Falls (61.1%) were the most common cause of traumatic dental injuries, and enamel–dentin fracture (26%) was the most common dental trauma type.

Conclusions






Traumatic dental injuries in children are common. A large proportion of patients without any clinical symptoms (15.8%) did not seek any treatment after the trauma. Teachers, parents, and children should be informed about the action to be taken when dental trauma occurs and about the importance of immediately taking the child to a dentist after the trauma to ensure an accurate diagnosis, an optimal treatment plan, and positive outcome.

Keywords: Dental injuries; retrospective studies; pediatric dentistry

Introduction

Following dental caries, dental traumas in children cause the most significant damage to the teeth. Traumatic dental injuries frequently occur in children, and those in young adults constitute 5% of all injuries that require treatment (1, 2). The literature has shown that 3%–80% of emergency dental treatments are because of tooth injuries and the incidence of dental trauma within 1 year is 0.4% in every age group and 1.3%–4% in school-age children. It has been reported that 25% of all school-age children have had a dental injury, and 33% of adults, mostly at the age of 19 years, have experienced trauma in their permanent teeth (3).

A majority of the dental trauma cases occur during childhood bicycle accidents or sports events or as a result of a fall. Additionally, collisions, traffic accidents, home accidents, and child abuse also cause dental traumas. Traumatic injuries, such as luxation, cause dental traumas of the permanent teeth in 15%–61% cases and of the primary teeth in 62%–73% cases. Trauma types are classified into five groups based on the

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treatment and healing: concussion, subluxation, extrusion, lateral luxation, and intrusion (4). Luxation injuries are the most common traumatic injuries in the primary teeth, whereas crown fractures are more frequently reported in the permanent teeth. Besides, the central incisors of the upper jaw are the most affected teeth by traumatic dental injuries in both the primary and permanent teeth (5-7).

The results obtained from studies have varied depending upon the countries and regions where the studies were conducted and the age groups of the children included in the studies. Apart from the functional, phonetic, and aesthetic disorders, psychological problems emerge in children and their families after the children frequently experienced dental injuries. Traumatic dental injuries are challenging for all physicians. Therefore, an accurate diagnosis, treatment plan, and follow-up are critical for ensuring a positive treatment outcome. The etiology of trauma, its distribution, and its consequences must be known for taking protective measures against dental traumas, preventing physical and psychological problems that may occur, and deciding upon an effective and appropriate treatment (8-11). Studies investigating the frequency of dental traumas with the causes and consequences in Turkey are needed (12, 13). Particularly, regional studies will be more comprehensive and useful in our country, with different cultures and lifestyles in each region. In the present study, the incidence of dental traumas, related factors, and treatment procedures in children admitted to Karadeniz Technical University, Faculty of Dentistry, Department of Pedodontics, from Trabzon and the surrounding cities between 2011 and 2016 were investigated.

Subjects and Methods

This study was reviewed and approved by the Ethical Committee of Karadeniz Technical University, Faculty of Medicine (2017/227). In total, 416 children aged 1–15 years with a history of dental trauma were admitted to the Karadeniz Technical University, Faculty of Dentistry, Department of Pedodontics from Trabzon and the surrounding cities between January 2011 and 2016. Trauma records were obtained based on the Andreasen and Andreasen classification (14). Standard trauma registry forms were used to obtain all the patients' data (age, gender, affected tooth, trauma, time between the trauma and onset of treatment, cause of trauma, type of treatment, and treatment procedure). The cases of multiple trauma types and associated soft tissue injuries were recorded as a combined trauma.

Statistical analyses

The data collected from all groups were imported to the Statistical Package for Social Sciences (SPSS) for Windows software, version 16.0 (SPSS Inc., Chicago, IL, USA). The type of trauma in the primary and permanent teeth, difference between gender and age groups, and cause of trauma were analyzed using the chi-square test. The distribution of dental traumas by age was calculated using the regression analysis because data distribution did not meet the requirements for normality and homogeneity of variance assumptions. The confidence interval was set to 95%, and a p value of <0.05 was considered statistically significant.

Results

In total, 416 children aged 1–15 years were admitted to our clinic from Trabzon and its surrounding cities because of dental trauma during the 5 year follow-up period (January 2011–January 2016). Table 1 shows the distribution of gender, age, and tooth type of the traumatized children; 37% of the patients were girls, and 63% were boys. The mean age was 8.5 years. It was determined that 102 and 314 of these traumas were in the primary and permanent teeth, respectively. Furthermore, dental trauma was more frequently encountered in boys (63%).

Table 1. The distribution of traumatized children by age, gender and trauma etiology

		Number (n)	Percentage (%)
Gender	G	154	37
	B	262	63
Age	0-3	52	12.5
	4-6	39	9.4
	7-9	137	32.9
	10-12	147	35.3
	13-15	41	9.9
Type of tooth	Primary	102	24.5
	Permanent	314	75.5

The distribution of the causes of traumatic dental injuries by age and gender demonstrated that the most common cause of trauma was falls (61.1%) in both the girls and boys. This was followed by collisions (11.3%) and bicycle accidents (8.2%; Figure 1).

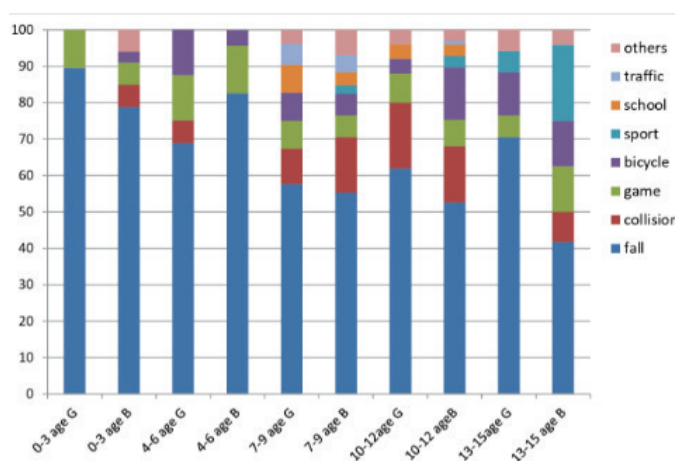


Figure 1. Overall percentage distribution of the causes of traumatic dental injuries by age and gender.

The overall percentage distribution of traumatic dental injuries by age and gender showed that enamel–dentin fracture had the highest ratio (26%) and it was followed by the combined trauma type with a rate of 21.6% (Figure 2).

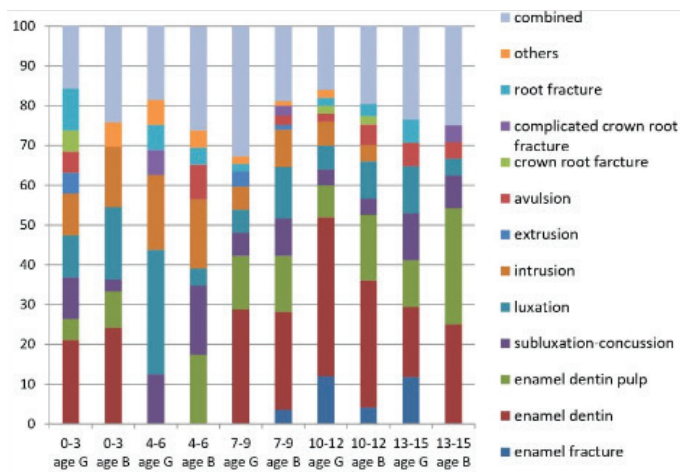


Figure 2. Overall percentage distribution of types of traumatic dental injuries by age and gender.

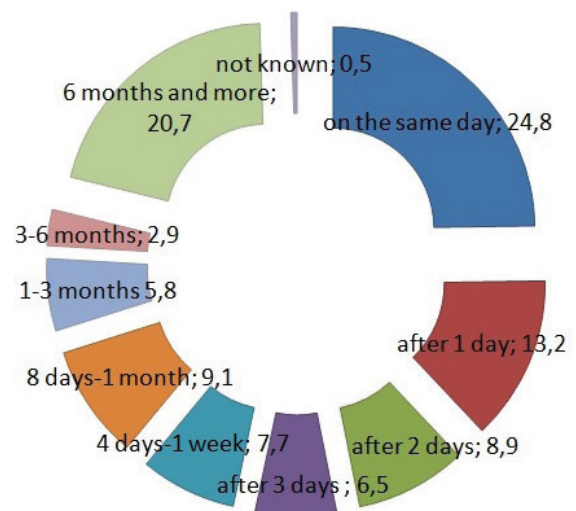


Figure 3. The time interval between the moment of trauma and the onset of treatment.

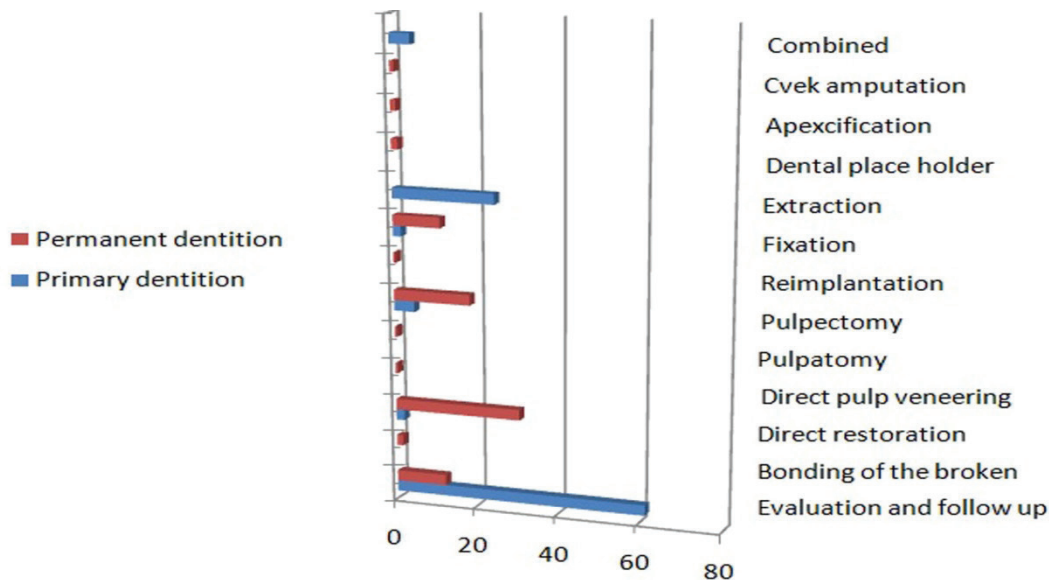


Figure 4. Distribution of post-traumatic treatment type according to dental type.

When the time interval between the trauma and onset of the treatment was evaluated, the percentage of the patients who initiated treatment on the same day of the trauma was the highest (24.8%), whereas those who initiated treatment at an unknown time had the lowest percentage (0.5%) (Figure 3).

Considering the distribution of the treatment procedure for the permanent and primary teeth, follow-up in the primary teeth (61.4%) and direct restoration in the permanent teeth

(30.8%) were determined to have the highest percentages (Figure 4).

The combined trauma and treatment types were separately evaluated, and it was found that the combined trauma type with enamel and enamel–dentin fractures coexisted (1.2%). The combined treatment type (1.2%) involving follow-up and direct restoration was more frequent than that involving the other treatment types (Tables 2 and 3).

Table 2. Percentage and frequency of the combined types of trauma

Type of Combined Trauma	%	Frequency
Enamel fracture-Enamel Dentin fracture	1.2	5
Enamel Dentin –Enamel Dentin Pulp Fracture	1.7	7
Enamel Dentin Fracture – Subluxation, Concussion	1.4	6
Enamel Dentin Fracture – Luxation Injury	1.2	5
Luxation – Intrusion Injury	1	4
Luxation – Extrusion Injury	1.4	6
Luxation – Avulsion Injury	1.4	6

Table 3. Percentage and frequency of combined treatment types

Type of Combined Trauma	%	Frequency
Follow up , Direct Restoration	1.2	5
Direct Restoration , Direct Pulp Veneers	1.7	7
Direct Pulp Veneers, Pulpectomy	7.5	31
Direct Restoration, Cvek Amputation	0.5	2
Pulpectomy, Fixation	0.7	3

Discussion

Although traumatic dental injuries can occur at any time in our lives, children and adolescents are exposed to these injuries more frequently. Young children are commonly affected by traumatic injuries of the primary teeth. Particularly, in the front teeth, the injuries can adversely affect the development of the underlying dental germ (5). The probability of developmental anomalies in the permanent incisors after a traumatic injury has been reported to be between 12% and 74%. These anomalies are classified as enamel pigmentation, enamel hypoplasia, crown dilaceration, odontoma-like malformations, root dilaceration, and eruption disorders (9). Andreassen et al. reported that traumatic dental injuries were more frequent during 2–3 years and 9–10 years of age in the primary and permanent tooth dentition, respectively (11).

Children aged 2–3 years need to test their independence as they develop their mobility skills. However, their physical abilities are insufficient to meet these demands; hence, they often have accidents (15). The fact that the incidence of trauma increases at 2–3 years of age can be related to this situation. In the age group of 9–10 years, children are more willing to perform social and sporting activities, and they become aware of their bodies and abilities. Thus, dental traumas may more frequently occur in this age group (16, 17). Otuyemi et al. (18) stated that the prevalence of trauma in the primary dentition was higher than that in the permanent dentition. However, in many studies, a dental trauma was reported to more frequently occur in the permanent dentition (19). Also, the prevalence of dental trauma in the permanent teeth was higher than that in the primary teeth in the present study. This is probably because children in the primary dentition period spend more time under parental control; in the transition to permanent dentition, they socialize with the other children during school age, become aware of their bodies and abilities, and increase the sportive activities, which can lead to traumatic injuries (16, 17).

In some studies, it has been reported that gender does not have a significant difference in the frequency of dental trauma. However, in many studies, age and gender were found to have an impact on the frequency of dental traumatic injuries (20, 21). Epidemiological studies have demonstrated that boys are more likely to have dental injuries than girls in both the primary and permanent dentition periods (20, 22, 23). In our study, a higher number of dental injuries were found in boys.

Unsafe swings on playgrounds and in wooden blocks in daycare nurseries increase the risk of trauma incidence. As children grow and mature, they become aware of the current

risks and can develop reflexes to protect themselves. Injuries in the face and chin area are frequently caused by falls at home or in closed areas at the age of 1–3 years, game and bicycle accidents during school age, and sports or traffic accidents during puberty (24). Dental injuries caused by falls are more frequent in those with mental retardation because of malfunction in motor coordination. In epileptic patients, trauma-related dental injuries were observed in 52% of the cases (25–27). Frequent dentoalveolar injuries occur because of various traumas, such as fights, falls, traffic accidents, sports injuries, and game accidents (6). The etiology of traumatic dental injuries in our study showed that falls were the most important factor. Gabris et al. (28) and Gassner et al. (29) stated that traumatic injuries generally occur as a result of sports injuries. Avşar (30) reported that dental traumas were more frequently caused by falling off bicycles and falling down the stairs.

Traumatic dental injuries in children were examined according to the type of injury in our study, and enamel–dentin fractures were determined to be encountered the most. When studies in literature were reviewed, it was found that the frequency of crown fractures varied between 26% and 90% (1, 6, 12). Although many investigators have stated that crown fractures are more common than luxation, Rocha and Cardoso indicated that the frequency of both types of injuries are nearly similar (51.5% and 48.5%, respectively) and there was no statistically significant difference between them (31). Dental injuries often have a slight impact on the oral soft tissues and teeth and on the periphery depending on the severity of the trauma. However, it is a serious problem that has a negative impact on patients with regard to factors, such as pain, function, aesthetics, and psychology. The severity of trauma on the chin–face area, flexibility, shape and direction of the colliding object, extent to which the lips and other soft tissues reduce strength, strength of the tooth, and chin structure are the main determinants of trauma that can occur in dento alveolar injuries (32). The post-trauma damage to the primary teeth more frequently occurs in the peripheral supporting tissues (periodontal ligament and alveolar bone) than that in the hard tissues of the teeth because of the excessive flexibility of the tissues surrounding the primary teeth and shortness of the primary tooth root. Therefore, it is observed that an adverse impact on the primary teeth usually causes luxation. In the trauma of the primary teeth, the treatment and prognosis of the trauma should be evaluated in terms of the health of the permanent teeth because of their close proximity to the underlying permanent dental germ (3, 21). The injuries of the supporting tissues in the permanent teeth become more frequent around 7–10 years of age when alveolar bone resilience is high, and they become less frequent at the age of 11–13 years (2, 4, 21).

Dental trauma is an event that requires elaborating on the application to the dentistry department. An accurate diagnosis, an appropriate treatment plan, and follow-up are important to achieve a positive treatment outcome. The most important factor affecting the prognosis of the traumatized teeth is the time interval between the trauma and onset of the treatment (12, 17). A literature review demonstrated that the percentage of patients who consulted a dentist on the same day after trauma ranged 9%–48% (28), whereas it was determined to be 24.8% in our study.

The age of trauma is very important for a dentist for two reasons. First, the pulp of the primary teeth, which is wider than that of the permanent teeth, has a better potential for blood circulation and healing. Second, pulp destruction of the immature permanent teeth can cause the roots to become weak and thin or to stop root formation. In this regard, efforts should be made to protect the vitality of the pulp in children with dental trauma (3, 21, 33). The distribution of the treatment procedure in the permanent and primary teeth revealed that the evaluation and follow-up in the primary teeth and direct restoration in the permanent teeth had the highest percentages in our study. Further, the combined treatment type involving follow-up and direct restoration was the most frequent treatment type.

Dental trauma treatment in children is not only a difficult situation for physicians, but it is also associated with a significant responsibility (3,12). Young children naturally act to discover the environment (3). Research has shown that individuals who spend time with children throughout the day often have no idea of dental injuries. The primary task of families and caregivers is to be prepared for dental traumas. This preparation includes knowing the strategies for protecting from dental traumas and plan of action in an emergency, being aware of the importance of keeping the permanent teeth in milk or saliva, and taking the child to a dentist, and elaborating on this. Dental trauma is a major financial and moral health problem for children and their families. Currently, the most important duty of dentists is to inform and warn child patients, their families, pediatricians, teachers, and sports instructors regarding the issue (22,30,34).

Conclusion

Our study revealed that boys were exposed to more traumatic injuries, and the most important factor in traumatic injuries is falls. Therefore, teachers, pediatricians, parents, and children should be warned about possible dangers and emergency treatments for all age groups on dental trauma. The percentage of families taking their children to a dentist on the trauma day was found to be very low. It should be explained to the families that it is of great importance to calmly perform an emergency intervention and subsequently visit the nearest dentist at the earliest without losing time for the prognosis of the tooth. Traumatic dental injuries are very common, particularly in school age children. It is necessary to ensure that timely and appropriate treatments are undertaken by informing both families and freelance dentists about traumatic dental injuries.

Türkçe Öz: Karadeniz Teknik Üniversitesi Çocuk Diş Hekimliği Bölümüne Travma Nedeniyle Başvuran Hastaların Değerlendirilmesi: Retrospektif Araştırma. Amaç: Dental travmatik yaralanmalar, süt ve daimi dişlerde sık gözlenen problemler arasında yer almaktadır. Dental travma yaygınlığı dünya genelinde değişkendir. Bu çalışmada, çocuklarda diş travmasının türü, ilişkili faktörler ve tedavi prosedürleri incelenmiştir. Bireyler ve Yöntem: Beş yıllık (Ocak 2011-2016) bir periyotta, Trabzon ve çevre illerden 1-15 yaş arası 416 çocuk hasta, dental travma şikayetiyle kliniğimize başvurdu. Süt ve daimi dişlerde travmanın nedeni, tipi, cinsiyet ve yaş grupları ile ilişkisi ki-kare testi ile yaşlara göre dağılımı ise regresyon analizi ile hesaplandı. Bulgular: Hastaların% 37'si kız,% 63'ü erkektir. Yaş ortalaması 8.5 yıl olarak hesaplandı. Travmatik dental yaralanmaların en sık nedeni düşme (% 61.1) ve en sık görülen dental travma tipi "mine-dentin kırığı" idi (% 26). Sonuç: Çocuklarda dişlerin travmatik yaralanmalarına sıklıkla rastlanılmaktadır. Travmadan sonra

hastaların önemli bir bölümünün (%15.8) herhangi bir klinik semptom oluşmadan tedaviye başvurmadıkları saptanmıştır. Doğru bir teşhis, tedavi planlaması ve olumlu bir tedavi sonucu için öğretmenler, ebeveynler ve çocuklar dental travma gerçekleştiğinde ne yapılması gerektiği ve travma sonrası hemen bir diş hekimine başvurmanın önemi hakkında bilgilendirilmelidir. Anahtar Kelimeler: Diş yaralanmaları; retrospektif çalışmaları; pediatrik diş hekimliği.

Ethics Committee Approval: This study was reviewed and approved by the Ethical Committee of Karadeniz Technical University, Faculty of Medicine (2017/227).

Informed Consent: Informed consent was waived due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.

Author contributions: AK, OFG, SME, OB and TT participated in designing the study. AK, OFG, SME, OB and TT participated in generating the data for the study. AK, OFG and SME participated in gathering the data for the study. AK and TT participated in the analysis of the data. AK wrote the majority of the original draft of the paper. AK and OFG participated in writing the paper. All authors approved the final version of this paper.

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

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

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Fracture resistance of different implant supported ceramic abutment/crown systems

Purpose

The purpose of this study was to investigate the fracture resistance and failure modes of different non-aged and aged abutment/crown systems.

Materials and Methods

One hundred dental implants (diameter 4.3 mm and length 11.5 mm) were restored with five abutment/crown systems: G1: a lithium disilicate hybrid abutment crown, G2: a lithium disilicate crown cemented on a lithium disilicate hybrid abutment, G3: a lithium disilicate crown cemented on a zirconia hybrid abutment, G4: a direct veneer porcelain layering on a zirconia hybrid abutment, and G5: a lithium disilicate crown cemented on a prefabricated all-zirconia abutment. Each group was divided into two groups (n=10) as control (non-aged) and thermomechanically aged. The fracture resistance test was performed. Failures during the aging process and after the fracture resistance test were examined.

Results

Both of the factors (restoration type and aging) affected the fracture resistance values and there was not an interaction between the factors ($p>0.05$). When fracture resistance values were compared regardless of aging, the highest values were observed in G3 and G4, respectively ($p<0.05$). When comparing the fracture resistance values, regardless of the restoration type, the aged group showed a significant lower fracture resistance value than control group ($p<0.05$).





Conclusion

A titanium base enhanced the fracture resistance of zirconia abutments. Thermomechanical aging decreased the fracture resistance of the tested ceramic abutment/crown systems. The major failure mode was the abutment fracture.

Keywords: Dental implant-abutment design; Yttria-stabilized tetragonal zirconia polycrystals ceramic

Introduction

The ultimate goal in implant dentistry is not only to achieve a functional result, but also to create pleasing esthetics that consider the proper proportions and natural relationships among the peri-implant soft tissue, bone, and restorative material (1,2). Abutment, which is an intermediate component between implant and restoration, is important for mechanical stability and the esthetic result of an implant restoration. In this context, the present study has focused on the abutment material, abutment design, and crown material to provide reliable and esthetic implant-supported restorations (2). Biocompatibility, mechanical properties, and clinical success of implant abutments fabricated from commercially pure titanium have been well-documented (3-5). However, the metallic color of the titanium may reflect through soft tissue and impair the esthetics.

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To achieve optimal esthetics, especially in the anterior region, all-ceramic abutments have been introduced due to their tooth-like color and possible biological advantages (6). Furthermore, developments in Computer Aided Design-Computer Aided Manufacturing (CAD-CAM) technology have led clinicians to design case-specific, esthetic implant-restorations and to fabricate these restorations from various materials (7). Zirconia and lithium disilicate ceramics have been used recently as high strength implant supported superstructure materials.

Zirconia abutments can be fabricated as an one-piece which is entirely made of zirconia and as a two-piece consisting of a titanium base and a transmucosal zirconia part. This zirconia part connects to the dental implant via the titanium base. The connection element of the one-piece zirconia abutments has been reported to be prone to fracture (8). Moreover, the precise fit of the connection interface is questionable (9) and wear has been reported at the titanium implant (10). The two-piece zirconia abutments, which provide a titanium-titanium interface at the implant abutment connection, revealed a higher fracture strength compared with one-piece zirconia abutments and reduced the risk of implant platform damage under occlusal forces (11). Therefore, the two-piece zirconia abutments have currently attracted significant interest with high fracture resistance, good esthetics, providing a precise fit with the implant, and biocompatibility (1,6,12). However, the high optical opacity and white appearance of the zirconia ceramic are well known (13). To mimic the translucent appearance of natural dentition, conventional zirconia is veneered with glass ceramics in dental restorations (14). Veneering zirconia abutments can be achieved by cementing a ceramic crown on the zirconia abutment bonded on a titanium base or direct ceramic processing on the abutment bonded on a titanium base. Nevertheless, zirconia may fail to provide optimal esthetics because of its opacity in some clinical situations.

Lithium disilicate (LDS), the strongest glass ceramic, has a higher translucency and can provide better shade matching with natural dentition compared with zirconia (9,15). Recently, prefabricated LDS has been considered as an esthetic abutment material while the material has been widely used in fixed prosthodontics. LDS abutments are used with titanium bases. There are two restorative possibilities using LDS abutments including cementing a ceramic crown on the LDS abutment and fabricating the abutment and crown in one-piece and bonded to a titanium base (9). LDS abutments, especially one-piece restorations which are a combination of abutment and crown, can provide some advantages over zirconia abutments including less interocclusal space requirement, higher translucency, and elimination of layered structure and its interfacial bond problems.

Literature research revealed that several studies were conducted on the mechanical performance of zirconia abutments with different designs. However, limited research has been conducted on mechanical performance of differently designed two-piece ceramic abutments including lithium disilicate implant abutments (2,9). The purpose of this study was to investigate the fracture resistance and failure modes of non-aged and aged zirconia and LDS ceramic abutments with different crown designs. The null hypotheses of the study were that there would be no difference between the fracture

resistance of the different ceramic abutment/crown systems and thermomechanical aging would not affect the fracture resistance of these abutments.

Materials and Methods

Sample characteristics and preparation

One hundred dental implants (diameter 4.3 mm and length 11.5 mm) (NobelReplace, Nobel Biocare, Gothenburg, Sweden) were restored with five ceramic implant abutment/crown systems simulating the restoration of a maxillary right central incisor. The groups were as follows: Group 1 (G1): A Lithium disilicate hybrid abutment crown, Group 2 (G2): A Lithium disilicate crown cemented on a lithium disilicate hybrid abutment, Group 3 (G3): A Lithium disilicate crown cemented on a zirconia hybrid abutment, Group 4 (G4): A direct veneer porcelain layering on a zirconia hybrid abutment, Group 5 (G5): A Lithium disilicate crown cemented on a prefabricated all-zirconia abutment. Ceramic implant abutment/crown systems were designed and manufactured using a CAD-CAM system (Cerec, Sirona Dental Systems, Bensheim, Germany) (Figure 1).

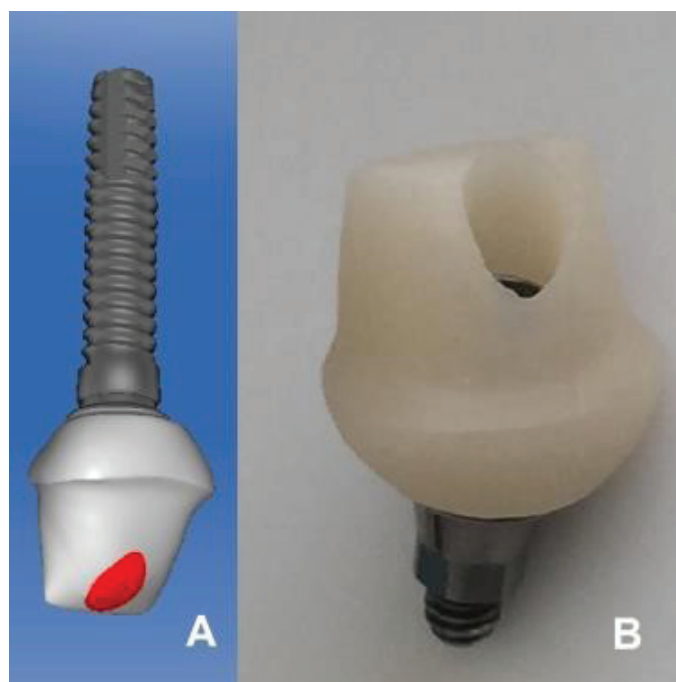


Figure 1. Custom ceramic abutment A: Design of abutment B: Milled and crystallized lithium disilicate abutment.

G1 (which consisted of a monoblock abutment and crown combination bonded to the titanium base) was milled from lithium disilicate (IPS e.max CAD, Ivoclar Vivadent, Schaan, Liechtenstein). For G2, G3, and G4, abutments were bonded to the titanium base. Using this abutment design, identical abutment parts were fabricated from lithium disilicate for G2, and from a presintered Y-TZP material (incorisZI mesoblocks, Sirona Dental Systems) for G3 and G4. After the milling process, the lithium disilicate abutments were fully crystallized in a porcelain furnace (Programat P300, Ivoclar Vivadent) and zirconia abutments were dried and sintered in a calibrated sintering furnace (inFire HTC, Sirona Dental Systems). CAD-CAM fabricated parts were produced in a milling unit

(Cerec MC XL, Sirona Dental Systems). After the fabrication process of the ceramic parts (lithium disilicate or zirconia), the hybrid abutments, ceramic part and titanium base of the abutments were bonded using a resin cement (Multilink Hybrid Abutment, Ivoclar Vivadent). Luting space was directly determined by the software. Prior to cementation, the titanium bases were screwed to the implants with a torque of 35 Ncm and appropriate manufacturer recommended surface treatments were applied to the bonding surfaces. The screw holes in the titanium bases were sealed with heavy body impression material (Hydrorise Maxi Heavy, Zhermack Spa, Badia Polesine, Italy). The bonding surfaces of the titanium bases were air-borne particle abraded with 50 microns aluminum-oxide particles at 2 bars pressure for 20 seconds. The bonding surfaces of the lithium disilicate ceramics were etched with 5% hydrofluoric acid (IPS Ceramic Etching Gel, Ivoclar Vivadent) followed by applying silane (Monobond Plus, Ivoclar Vivadent). Bonding surfaces of zirconia ceramics were air abraded with 50 microns aluminum-oxide particles at 2 bars pressure for 20 seconds. Both titanium bases and ceramic parts were cleaned in an ultrasonic bath of distilled water and dried prior to the cementation. After cleaning, the bonding surfaces were protected from contamination. A self curing resin cement (Multilink Hybrid Abutment, Ivoclar Vivadent) was used to bond titanium bases and ceramic parts of the restorations. Careful insertion of the ceramic part was provided considering the rotation and position stops. Two components were seated and pressed together by hand, using a constant pressure. Excess cements were removed. Then, as recommended by the cement manufacturer, the specimens were left to self-cure for 7 minutes. The specimens were stored in a humidifier at room temperature for 24 hours. Twenty specimens were prepared for each abutment group, 10 of which were assigned to the control (non-aged) group and thermomechanical aging group.

To prepare the crown part of the restorations, digital impressions of G2, G3, and G5 abutments were taken with the intraoral camera. The crown design of the G1 restoration was copied to each restoration design to prepare standardized crowns. The crowns were milled from lithium disilicate and full crystallization was provided. Crowns were seated on the abutment under finger pressure and cemented with dual cure self-adhesive resin (Multilink Automix, Ivoclar Vivadent) according to the manufacturer's instructions. Any excess cement was removed. The restorations were cured for 20 s from each side and all margins were finished and polished with abrasive disks. In G4 (the direct veneer porcelain layering on a zirconia hybrid abutment group), feldspathic ceramic (Vita VM9, Vita Zahnfabrick, Bad Sackingen, Germany) was processed directly on to the abutment. After specimen fabrication, the ceramic abutment/crown system-implant assemblies were embedded into autopolymerizing acrylic resin in a 30-degree off-axis loading platform by using a custom-made positioning device (2).

Aging and fracture resistance protocols

Ten specimens from each group were exposed to thermomechanical aging in an artificial chewing simulator (Mastication Simulator, Esetron Smart Robototechnologies, Ankara, Turkey). The aging process included 500000 loading

cycles under a dynamic loading force of 100 N load which was vertically applied on the cingulum of the crowns with a 6-mm-diameter steel ball and at a 0.5 mm/min crosshead speed and simultaneous thermocycling performed for 2000 cycles (1 minute each cycle) in 5°C and 55°C water. The specimens that survived at the end of the aging were tested for fracture resistance. The remaining 10 specimens in each of the five groups did not undergo the aging process, however, they underwent the fracture resistance test. The fracture resistance test was performed with a universal testing machine (Compression/Tension Device, Esetron Smart Robototechnologies) (Figure 2). The load was vertically applied below the incisal edge on the lingual aspect of the crown with a 6-mm-diameter steel ball and at a crosshead speed of 0.5 mm/min. The load at fracture (N) was recorded, and fractures during the simulation process and after the fracture resistance test were examined and analyzed under magnification (Loupe opt-on; Orange Dental, Biberach, Germany).

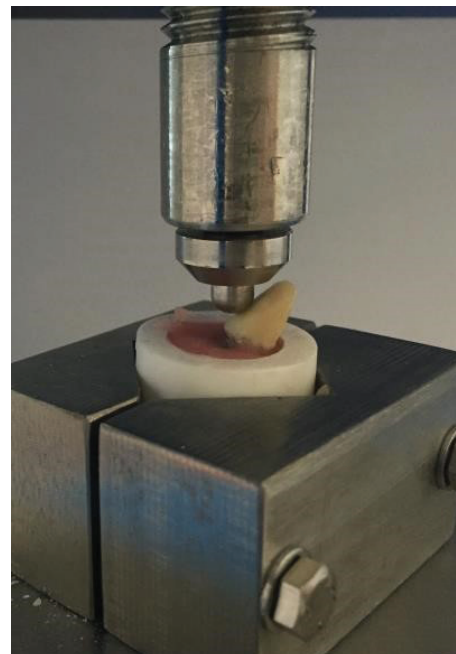


Figure 2. Specimen positioned in universal testing machine.

Statistical analysis

The data was analyzed with statistical software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM). The fracture resistance values were statistically analyzed using two-way analysis of variance (ANOVA), with the restoration type and thermomechanical aging as the independent variables followed by the Tukey HSD test.

All p values less than 0.05 were considered to be statistically significant.

Results

One specimen from Group 5 (thermomechanically aged lithium disilicate crown cemented on prefabricated all-zirconia abutment) was fractured (abutment fracture) during the aging process and this specimen was excluded from the statistical analysis. The fracture resistance values of both

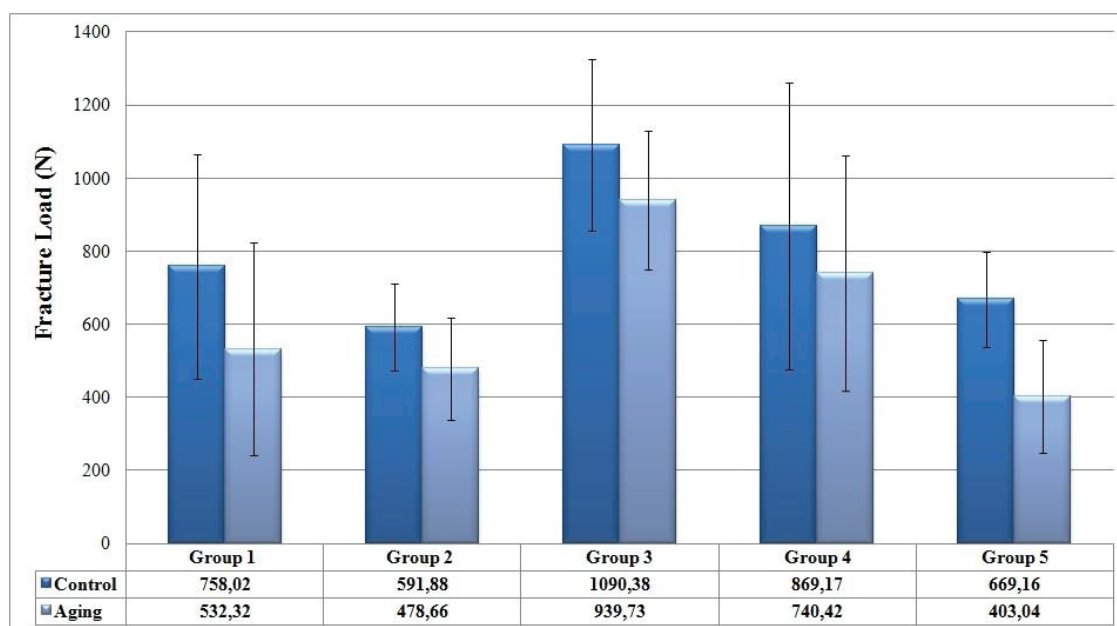


Figure 3. Fracture resistance values of the tested groups

*Group 1: Lithium disilicate hybrid abutment crown, Group 2: Lithium disilicate crown cemented on lithium disilicate hybrid abutment, Group 3: Lithium disilicate crown cemented on zirconia hybrid abutment, Group 4: Direct veneer porcelain layering on zirconia hybrid abutment, Group 5: Lithium disilicate crown cemented on prefabricated all-zirconia abutment.

Table 1. Fracture resistance values of the restoration types

Restoration type	Mean (\pm SD)*
Group 1 (n=20) Lithium disilicate hybrid abutment crown	645.17 (\pm 313.88) C
Group 2 (n=20) Lithium disilicate crown cemented on a lithium disilicate hybrid abutment	535.28 (\pm 139.21) C
Group 3 (n=20) Lithium disilicate crown cemented on a zirconia hybrid abutment	1015.05 (\pm 221.83) A
Group 4 (n=20) Direct veneer porcelain layering on a zirconia hybrid abutment	804.80 (\pm 355.90) B
Group 5 (n=19) Lithium disilicate crown cemented on a prefabricated all-zirconia abutment	543.10 (\pm 193.97) C

*SD: Standard deviation

Same capital letters indicate that the values were not statistically different among the restoration type groups.

control and aging groups in all restoration type groups are shown in Figure 3.

It was observed that the control group of Group 3 had the highest fracture resistance value among the groups and fracture resistance values were lower in all thermomechanically aged groups than the control groups. According to the two-way ANOVA, both of the factors (restoration type and aging) affected the fracture resistance values of the specimens and there was no interaction between the factors ($p=0.844$). The fracture resistance values of the groups by restoration type are shown in Table 1.

When fracture resistance values were compared according to the restoration type, the highest values were observed in Group 3 and Group 4, respectively ($p<0.05$). The lowest value was observed in Group 2, however, the results were not statistically different among Groups 1, 2, and 5. When comparing the fracture resistance values of the groups

Table 2. Fracture resistance values of control and aging groups

Aging	Mean (\pm SD)*
Control (Non-aged) Group (n=50)	795.72 (\pm 304.32) a
Aging Group (n=49)	623.24 (\pm 297.55) b

*SD: Standard deviation

Same small letters indicate that the values were not statistically different between the restoration type groups.

regardless of the restoration type, the aged group showed a significant lower fracture resistance value than the control group ($p<0.05$) (Table 2). The failure modes of the specimens were examined after the load at fracture test (Figure 4). The failure modes of non-aged and aged specimens are shown in Table 3 and 4, respectively.

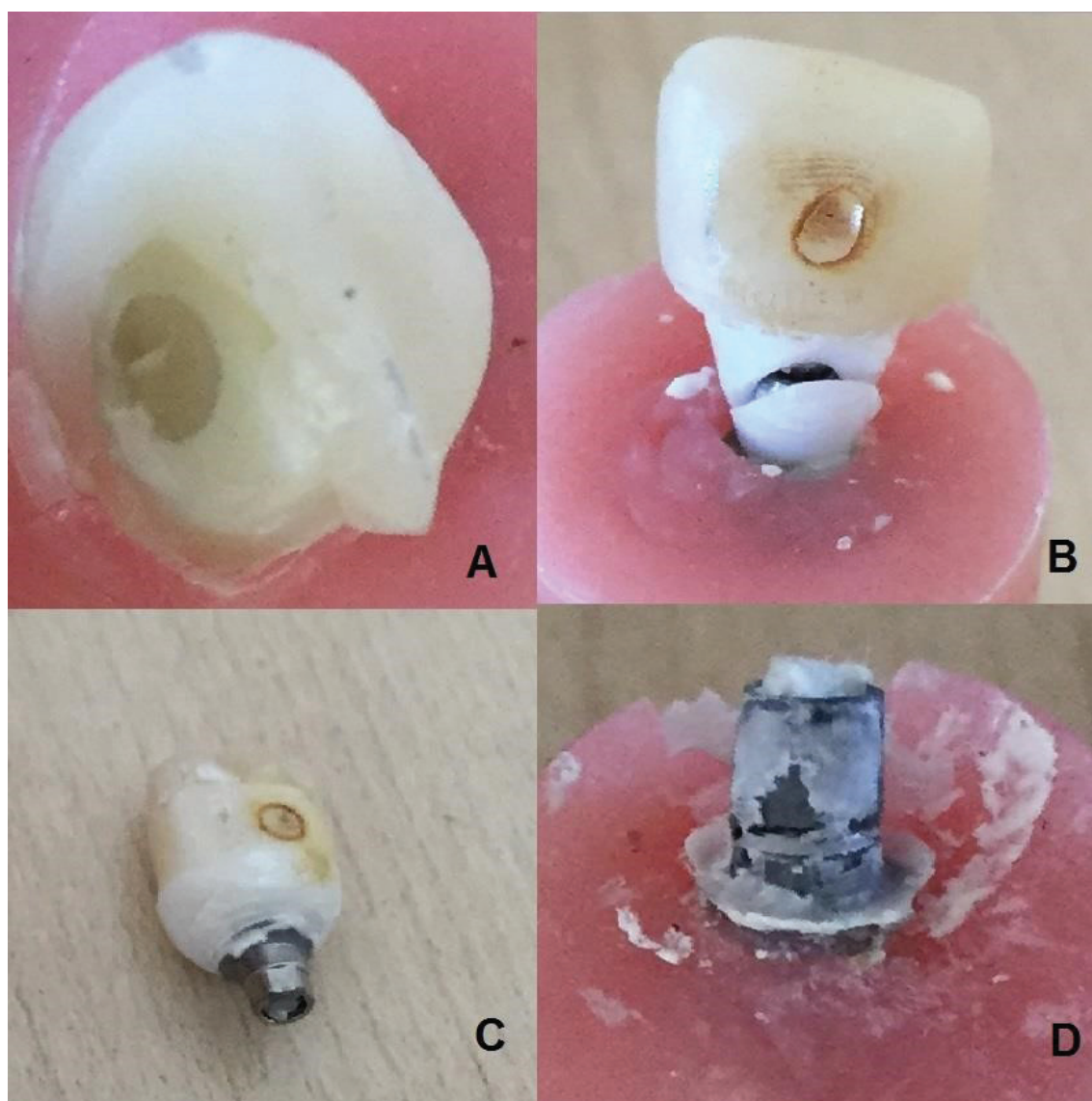


Figure 4. Failure modes A: Crown fracture B: Abutment fracture C: Fracture of screw of titanium base D: Deformation of titanium base .

Table 3. Failure modes of non-aged specimens

	Crown fracture	Abutment fracture	Fracture of screw of titanium base	Deformation of titanium base
Group 1	1	9	-	-
Group 2	6	3	1	-
Group 3	5	2	1	2
Group 4	3	3	4	-
Group 5	2	8	-	-

Table 4. Failure modes of the aged specimens

	Crown fracture	Abutment fracture	Fracture of screw of titanium base	Deformation of titanium base
Group 1	2	7	1	-
Group 2	6	4	-	-
Group 3	3	6	1	-
Group 4	3	5	2	-
Group 5	-	9	-	-

In both non-aged and aged specimens, the most observed failure was abutment fracture followed by crown fracture. Six non-aged specimens (one in G2, one in G3, and four in G4) showed a fracture of the screw in the titanium base. Four aged specimens (one in G1, one in G3, and two in G4) showed a fracture of screw in the titanium base. Deformation of the titanium base was seen in only two specimens in the non-aged Group 3.

Discussion

The null hypotheses of the present study were rejected as significant differences were found between the fracture resistances of the different ceramic abutment/crown systems and thermomechanical aging resulted in significantly lower fracture resistance compared with non-aged restorations. The fracture resistance values found in the present study revealed that one-piece zirconia abutments showed lower fracture resistance than zirconia abutments with titanium bases. This finding is in agreement with previous studies (1,8,12). The implant abutment connection area has been reported as the weakest part of an internal connection in the one-piece zirconia abutment (8). The titanium base of the hybrid ceramic abutments functioned as a substitute for the weakest part of these abutments. Therefore, the titanium base can reinforce the fracture strength of a zirconia abutment. Furthermore, Stimmelmayer et al. (12) reported similar mechanical behavior for zirconia abutments with a titanium base and titanium abutments. Another reported problem with one-piece zirconia abutments was the greater wear that was generated on the implant platform in one-piece zirconia abutments compared with titanium abutments (10,16). Therefore, the two-piece zirconia abutment design provides significant advantages over one-piece zirconia abutments by generating a titanium-titanium interface at the implant-abutment connection which has been shown to reduce the risk of implant platform damage in use and to enhance fracture resistance.

The mechanical behavior of one-piece, two-piece, and differently designed zirconia abutments have been extensively studied. However, limited information exists on the more recently introduced lithium disilicate implant abutments (2,9). In the present study, the mean fracture resistance of both groups (Group 1 and Group 2) of lithium disilicate abutments was found to be lower than the two-piece zirconia abutment groups and no statistical difference was found between Group 1 and Group 2 - similarly with previous researches (2,9). However, a seemingly positive difference between Group 1 and Group 2 was observed with regard to failure mode. In Group 1, generally catastrophic bulk fractures were observed while a fracture of the ceramic crown and an intact abutment was observed in Group 2. These results of the lithium disilicate abutment groups may reveal an advantage of the restoration type using a lithium disilicate abutment and cemented crown. The crown failure on an intact abutment can be easily reconstructed. Furthermore, in this design, the optimal implant angulation to position the screw hole in the palatal site of the restoration is less critical while it is important for lithium disilicate hybrid abutment crown restoration type.

Maximum bite forces in humans range from approximately

100 N to 300 N in the anterior region and 200 N to 900 N in the posterior region (17,18). Furthermore, bruxism and other parafunction can cause higher bite forces (19). The mean fracture resistances found in this study showed that zirconia abutments with titanium bases can withstand maximum bite forces in both anterior and posterior region. However, one-piece zirconia abutments and lithium disilicate abutment/crown systems which showed lower fracture resistance may not withstand higher levels of force in the posterior region, and so the use of these restorations should be limited in the anterior region.

In the present study, implant-supported anterior restorations with different designs and materials were tested under artificially aged and non-aged conditions. In-vitro testing of restorations under static load without artificial aging can provide information on indication and clinical limitations of a treatment modality. However, artificial aging has been considered as a reliable tool to predict clinical durability of restorations before recommending for clinical use (14,20). In the present study, all specimens were subjected to cyclic loading and thermal cycling to the mechanical behavior of different restorations under clinically approximated conditions. The parameters of mastication simulation were chosen taking previous studies into consideration (1,8,21). There are, however, no accepted standards of loading parameters for testing implant restorations in a mastication simulator. The thermomechanical aging performed in the study which simulated an approximately 2.5 years of clinical service period for a fixed prosthesis (22,23). The results of this study revealed a significant decrease in the fracture resistance of restorations tested as well as previous studies (2,8). This fatigue behavior of ceramic abutments might be attributed to the presence of micro defects and the slow growth of subcritical cracks within the material (24). In addition to the effects of mechanical loading, zirconia ceramics are sensitive to thermal aging in the presence of moisture in the oral environment (25).

Restorations with a titanium base showed high fracture resistance ranging from 740-1090 N in the universal testing machine. However, comparing the fracture resistance values of this study can not be possible because the test parameters including implant design, implant-abutment connection, abutment dimensions, restorative material, and loading conditions may affect the magnitude of the load that causes a fracture of an implant-supported crown (4,9).

Considering the failures during the study, one specimen in Group 5 failed during thermomechanical aging and the remaining specimens survived. However, deterioration related to aging generally occurs without any evidence of failure (26). The fracture resistance values and failure modes after a static fracture test may indicate weak points and deformed parts. In the present study, abutment fracture was generally observed in the one-piece zirconia abutment group especially at the implant-abutment connection in accordance with previous studies (8,27,28). Thin ceramic parts can be prone to fracture. In the two-piece zirconia abutment groups, the fracture of the crown part was prominent while fracturing in the zirconia abutment part generally occurred in aged specimens. This may be attributed to the negative effects of aging on zirconia.

The results of the present study may provide clinically relevant data for different implant-supported ceramic abutment/crown systems in anterior applications. However, invitro conditions do not simulate the clinical situation. Well-designed long-term randomized controlled clinical studies are required to evaluate survival and complication rates of these restorations in clinical use.

Conclusion

Zirconia abutments with a titanium base enhance the fracture resistance of zirconia abutments. Prefabricated zirconia abutments showed a lower fracture resistance than other zirconia abutments. Thermomechanical aging decreased the fracture resistance of the tested ceramic abutment/crown systems. All specimens withstood the thermomechanical aging except one specimen in the prefabricated zirconia abutment group. The major failure mode was the abutment fracture.

Türkçe Öz: Farklı implant destekli seramik abutment/kron sistemlerinin kırılma dayanıklılığı. Amaç: Bu çalışmanın amacı yaşlandırılmayan ve yaşlandırılan farklı abutment/kron sistemlerinin kırılma dayanıklılığını ve başarısızlık tiplerini belirlemektir. Gereç ve yöntem: Yüz adet dental implant (4.3 mm çapında ve 11.5 mm uzunluğunda), beş farklı abutment/kron sistemi ile restore edildi. Bu sistemler G1: lityum disilikat hibrit abutment kron, G2: lityum disilikat hibrit abutment üzerine simante edilen lityum disilikat kron, G3: zirkonya hibrit abutment üzerine simante edilen lityum disilikat kron, G4: veneer porseleni uygulanan zirkonya hibrit abutment ve G5: prefabrike zirkonya abutment üzerine simante edilen lityum disilikat kron grubudur. Her grup, kontrol (yaşlandırmasız) ve termomekanik yaşlandırma olmak üzere iki gruba (n=10) ayrıldı. Kırılma dayanıklılığı testi yapıldı. Yaşlandırma süreci boyunca ve kırılma dayanıklılığı testinden sonra başarısızlık tipleri belirlendi. Bulgular: Her iki faktöründe (restorasyon tipi ve yaşlandırma) kırılma dayanıklılığı değerlerini etkilediği fakat faktörler arasında interaksiyon olmadığı belirlendi ($p>0.05$). Yaşlandırma faktörü olmadan kırılma dayanıklılıkları karşılaştırıldığında, en yüksek değerler sırasıyla G3 ve G4'te belirlendi ($p<0.05$). Restorasyon tipi olmadan kırılma dayanıklılıkları karşılaştırıldığında, yaşlandırma grubunda kontrol grubuna kıyasla daha düşük kırılma dayanıklılığı tespit edildi ($p<0.05$). Sonuç: Titanium kaide zirkonya abutmentlerin kırılma dayanıklılığını arttırmaktadır. Termomekanik yaşlandırma, test edilen seramik abutment/kron sistemlerinin kırılma dayanıklılığını azaltmaktadır. En sık görülen başarısızlık tipi abutment kırığıdır. Anahtar kelimeler: Dental implant-kaide tasarımı; Yitria-dengeli tetragonal zirkonyum polikristali seramiği.

Ethics Committee Approval: Not required.

Informed Consent: Not required.

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Author contributions: MBG and CA participated in designing the study. MBG and CA participated in generating the data for the study. MBG and CA participated in gathering the data for the study. MBG, SKN, HY and CA participated in the analysis of the data. MBG and SKN wrote the majority of the original draft of the paper. HY and CA participated in writing the paper. All authors approved the final version of this paper.

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

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The effect of different chelating agents on the push-out bond strength of proroot mta and endosequence root repair material

Purpose

This study aimed to evaluate the effects of 17% ethylenediaminetetraacetic acid (EDTA), 7% maleic acid (MA), and 10% citric acid (CA) on the push-out bond strength of ProRoot MTA and Endosequence Root Repair Material (ERRM) putty.

Materials and Methods

Eighty single-rooted extracted human teeth were instrumented to obtain a standardized immature teeth model. Based on the chelating agents tested, the specimens were randomly divided into three experimental groups: Group 1 (17% EDTA), Group 2 (7% MA), Group 3 (10% CA), and Group 4 (Positive Control) (n=20 for each group). Each group was further classified into two subgroups: Group A (ProRoot MTA) and Group B (Endosequence Root Repair Material (ERRM) putty) (n=10 for each subgroup). After irrigation and placement of cements, teeth were stored at 37°C and in 100% humidity for a week. A total of 240 dentine discs (three discs per teeth) were obtained and subjected to push-out assay. Data was analyzed using two-way analysis of variance and Tukey's post hoc t-test.

Results

Both types of chelating agent and calcium silicate-based cement were significantly associated with the push-out bond strength values. The push-out bond strength was significantly less for CA as compared to EDTA or MA. ERRM had higher bond strength values than ProRoot MTA (p<0.05).

Conclusion

The use of chelating agents increased the push-out bond strength of CSC. Regardless of tested chelating agents, ERRM had higher bond strength values than ProRoot MTA.

Keywords: Calcium silicate-based cement; chelating agent; Endosequence Root Repair Material; push-out bond strength

Introduction

Calcium silicate-based cements (CSC) have a wide variety of applications in endodontic therapy (1). CSC should exhibit high bond strength and display resistance to displacement forces that may occur owing to functional consequences or placement of restorative materials (2). Displacement, leakage, and micro fractures can occur in CSC because of these forces (3). Therefore, evaluating the effect of different variables that influence the bond strength of CSC to dentin is important for clinical success.

The smear layer is a non-homogenous structure composed of microorganisms, blood cells, residual pulp, odontoblast extensions and dentin chips (4). As the smear layer can penetrate up to 40 microns into the dentin tubules, the ability of intracanal medicaments and CSC to penetrate dentin is reduced, thereby adversely affecting the bond

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strength (5). Chelating agents are used to remove the smear layer in root canal therapies. However, these agents cause demineralization and structural changes in root canal dentin, thus affecting the bond strength of endodontic materials to root canal dentin (6).

Ethylenediaminetetraacetic acid (EDTA) is a frequently used chelating agent in removing smear layer and stimulates cellular differentiation and tissue formation, and increases the release of growth factors throughout the root canal (7). However, EDTA can cause weakness of the dentin structure in immature teeth of young patients, as it causes erosion in the dentin tubules (8). Because EDTA interferes with the hydration of MTA, it decreases microhardness, bond strength and biocompatibility of MTA (9). As alternatives to EDTA, citric acid (CA) and maleic acid (MA) can be used in endodontic procedures (10). Both agents cause wider opening in the dentin tubules (11), and increase the bond strength, resulting in an increased contact area between root canal dentin and endodontic cement (12). MA is suggested as an alternative to EDTA. It is highly acidic, less toxic than EDTA and has a greater ability to remove the smear layer than EDTA (10). As MA is a slightly organic acid, it is recommended that MA should be used in root canal irrigation at a concentration range of 5-15% (10). CA, another chelating agent, is used in different concentrations (1-50%) to remove the smear layer (13). CA, when used at a concentration of 1%, also presents a smear layer-removal effect similar to EDTA (14). Silveiro et al. (15) reported that 10% CA was effective in removing the smear layer because its pH was close to the neutral pH and was therefore more biocompatible.

Although mineral trioxide aggregate (MTA) is frequently used with numerous applications in endodontics, it has several disadvantages such as staining of the teeth, difficulty in clinical use, and long setting time (16). Because of these disadvantages, researchers are attempting to overcome the limitations of MTA. Recently, Endosequence Root Repair Material (ERRM), a bioceramic material was produced to overcome the disadvantages of MTA (17). It has similar uses like that of MTA and is available in mix-free, ready-to-use paste or injectable pat forms.

Exposure to irrigation solutions during chemomechanical irrigation changes the chemical and mechanical properties of the root canal dentin surface and so evaluating the effect of chelating agents on the bond strength of CSC should be investigated. Therefore, the purpose of this study was to examine the effects of 17% EDTA, 7% MA, and 10% CA on the push-out bond strength of ProRoot MTA and ERRM. The null hypotheses tested were as follows: (1) the chelating agent has no effect on the push-out bond strength of ProRoot MTA and ERRM; and (2) there is no difference between the push-out bond strength values of ProRoot MTA and ERRM.

Materials and Methods

Teeth Selection

Ethical approval was obtained from the Health Ethics Committee of the University of Cumhuriyet, Sivas, Turkey (ID: 2016-12/08). Based on the data from a pilot study, the values used in the power analysis were based on the following: $\alpha = 0.05$, $\beta = 0.10$, $1 - \beta = 0.90$. It was decided to take a sample of 80 teeth.

The present study was conducted on 80 single-rooted human teeth freshly extracted due to periodontal problems. The teeth were immersed in NaOCl (Wizard, Ankara, Turkey) for 3 hours and root surfaces were cleaned using a curette. Teeth were stored in 0.1% thymol solution at 4°C under the laboratory procedures. Multidimensional preoperative radiographs were taken to confirm the root curvature as less than 20° and also to confirm the presence of a single, non-complicated root canal.

Specimen Preparation

Each tooth was decoronated below the cemento-enamel junction using diamond burs and the root lengths were standardized to 15 ± 1 mm. Working length (wl) was determined by inserting a no. 10 K file (Dentsply Maillefer, Ballaigues, Switzerland) into each root canal until apically visible and then subtracting one mm from this point. Each root canal was instrumented with nickel titanium rotary Protaper Next files (Dentsply Maillefer, Ballaigues, Switzerland) up to size F5. During each file change, 1 mL of 2.5% NaOCl was applied with a side vented 27-gauge needle (Monoject, Tyco Healthcare, Mettawa, IL, USA) for 1 min. To provide an immature tooth model with a standard intracanal diameter, Peeso reamers (Mani Inc, Tochigi, Japan) from #1 to #6 were used sequentially. Finally, a #6 Peeso reamer protruded one mm beyond the apical foramen (3). Each root canal was irrigated with 5 ml of 5.25% NaOCl for 5 min. Finally, all roots were irrigated with 15 mL of bidistilled water. The root canals were then dried with paper cones (Dentsply, Maillefer, Switzerland).

The specimens were randomly divided into three experimental groups according to the chelating agents tested: Group 1 (17% EDTA (Wizard, Rehber Chemistry, Istanbul, Turkey)), Group 2 (7% MA (Merck Schuchardt, OHG, Hohenburn, Germany)), Group 3 (10% CA (Cumhuriyet University, Faculty of Medicine, Sivas, Turkey) and Group 4 (Positive Control) (n=20 for each group). Each group was further classified into two subgroups with regard to the type of CSC tested: Group A (ProRoot MTA) and Group B (ERRM) (n=10 for each group).

Irrigation Procedure and Placement of Cements

Each tooth was irrigated for 4 min and the total chelating agent volume delivered was 20 mL for each canal (18). Continuous irrigation was applied using a special irrigation device (VATEA, ReDent-Nova, Israel) that pumped the irrigants at the rate of 5 mL/min. Further, the chelating agents were removed by rinsing with 10 mL bidistilled water for 2 min. Approximately 4 mm of each type of cement tested (ProRoot MTA and ERRM) was placed in the coronal third of the root canals by using a MTA gun (MAP System, Dentsply Tulsa Dental, OK, USA) and compressed with hand plugs (Dentsply, Maillefer, Switzerland). ProRoot MTA was manually mixed using a metal spatula with a water to powder ratio of 0.33 following the manufacturer's recommendations. ERRM is premixed by the manufacturer. Each type of cement was gently applied to the dentinal walls with a moistened cotton pellet. The teeth were wrapped with a wet gauze and stored at 37°C and in 100% humidity for a week (2).

Push-Out Test

The teeth were embedded in acrylic blocks prepared as apical thirds in acrylic. Parallel transverse sections were obtained with a water-cooled low-speed IsoMet diamond saw (Buehler, Lake Bluff, NY, USA) from the coronal to the apical direction (three slices per tooth) (3). A total of 240 dentin slices (approximately 1 mm-thickness) were obtained. The thickness of each slice was measured using digital calipers (Teknikel, Istanbul, Turkey) with an accuracy of 0.001 mm. The homogeneity of the groups in terms of slice thickness was confirmed through analysis of variance (ANOVA) ($p > 0.05$).

A continuous load was applied to the center of the cements tested using a stainless steel cylindrical plunger of one mm in diameter, mounted onto a Lloyd LRX universal testing machine (Lloyd Instruments, Ltd., Fareham, UK). Loading was applied with a speed of 1 mm min⁻¹ from the apical to coronal direction until dislodgement of the cement occurred. All three slices of each teeth were tested using the push-out test and the mean was taken. The push-out bond strength was calculated in megapascals (MPa) by dividing the maximum load at failure (N) by the area of surface adhesion using the formula (19), $\text{area} = 2\pi r \times h$ (where $\pi = 3.14$, a constant value, r = radius of intraradicular space, and h = slice thickness in mm) (20).

Evaluation of Failure Patterns

After the push-out test, the fracture surfaces of all specimens were examined with a stereomicroscope (Zeiss) under 25 \times magnification. Photographs of different fracture types of the specimens were obtained with a stereomicroscope-based photographic machine (Canon EOS 1000D, Canon, Inc., Tokyo, Japan). Each sample was classified into one of the following categories: (i) adhesive failure at cement/dentin interface; (ii) cohesive failure within cement, and (iii) mixed failure in both cement and dentin. One representative specimen for each group was examined for scanning electron microscopy (SEM) analysis. Each specimen was dehydrated in graded ethanol series 25%, 50%, 75%, 90% for 25 min and finally in 100% ethanol for 1 h. The specimens were critically point-dried, mounted on aluminum stubs, sputter-coated with gold/palladium and examined with a scanning electron microscope (SEM) (Leo 440 CCD, Leica, Bensheim, Germany).

Statistical Analysis

Data was processed by SPSS for Windows, Version 22.0 (SPSS Inc., Chicago, IL, USA). The mean and standard deviation values of the push-out bond strength were calculated for each group. The effects of the type of chelating agents and endodontic cements on push-out bond strength were analyzed through two-way ANOVA and multiple comparisons were performed by Tukey's post-hoc test. A p-value less than 0.05 was considered statistically significant.

Results

Table 1 shows the mean values and standard deviations of the push-out bond strength (MPa). The use of chelating agents increased the push-out bond strength of endodontic cements. Both types of chelating agent and endodontic cement were significantly associated with the push-out bond strength values ($p < 0.05$). Regardless of the endodontic cements used, the push-out bond strength was significantly less for CA as compared to EDTA or MA ($p < 0.05$). There was no statistically significant difference between EDTA and MA ($p > 0.05$). Regardless of the chelating agents tested, ERM had higher bond strength values than ProRoot MTA ($p < 0.05$).

Table 1 shows the distribution of the failure patterns. Adhesive failure was the failure pattern mostly observed in the CA group, whereas, cohesive and mixed failures were the failure pattern mostly observed in the EDTA and MA groups, respectively. Fig. 1 shows the representative stereomicroscope and SEM images of failure modes: (a) adhesive failure, (b) cohesive failure, and (c) mixed failure.

Discussion

Previous studies examined the effects of various variables on the bond strength of different CSC (2, 3). These studies reported that the success of endodontic treatments was due to the well-adapted coronal restoration as well as the resistance of the repair agents to displacement forces generated during the condensation of permanent restorative materials. CSC are desired to show dislocation resistance to mechanical forces such as occlusion or condensation of restorative materials (2). It has been reported that the physical properties of endodontic cements change after root canal irrigation (21). Also, the removal of the smear layer causes a closer contact

Table 1. Push-out Bond Strength Values (MPa), and Distribution of Failure Modes for Each Group Groups

Chelating Agents	Calcium Silicate-Based Cement		Mode of Failure
	ProRoot MTA	Endosequence RRM	n (A/C/M)
17% EDTA	4.71 \pm 0.84 ^a	5.17 \pm 0.90 ^{a,*}	11 / 34 / 15
7% Maleic Acid	4.75 \pm 1.13 ^a	5.23 \pm 1.20 ^{a,*}	11 / 13 / 36
10% Citric Acid	3.99 \pm 0.89 ^b	4.50 \pm 0.86 ^{b,*}	36 / 12 / 12
Control (no agent)	3.97 \pm 0.70 ^b	4.23 \pm 0.81 ^b	15 / 19 / 26

Bond strength values are shown as Mean \pm SD. Same lower case letter represents no statistical significant difference within each column, and *represents statistical significant difference within each row, verified by two-way Anova and Tukey's test ($P > 0.05$). A: adhesive; C: cohesive; M: mix.

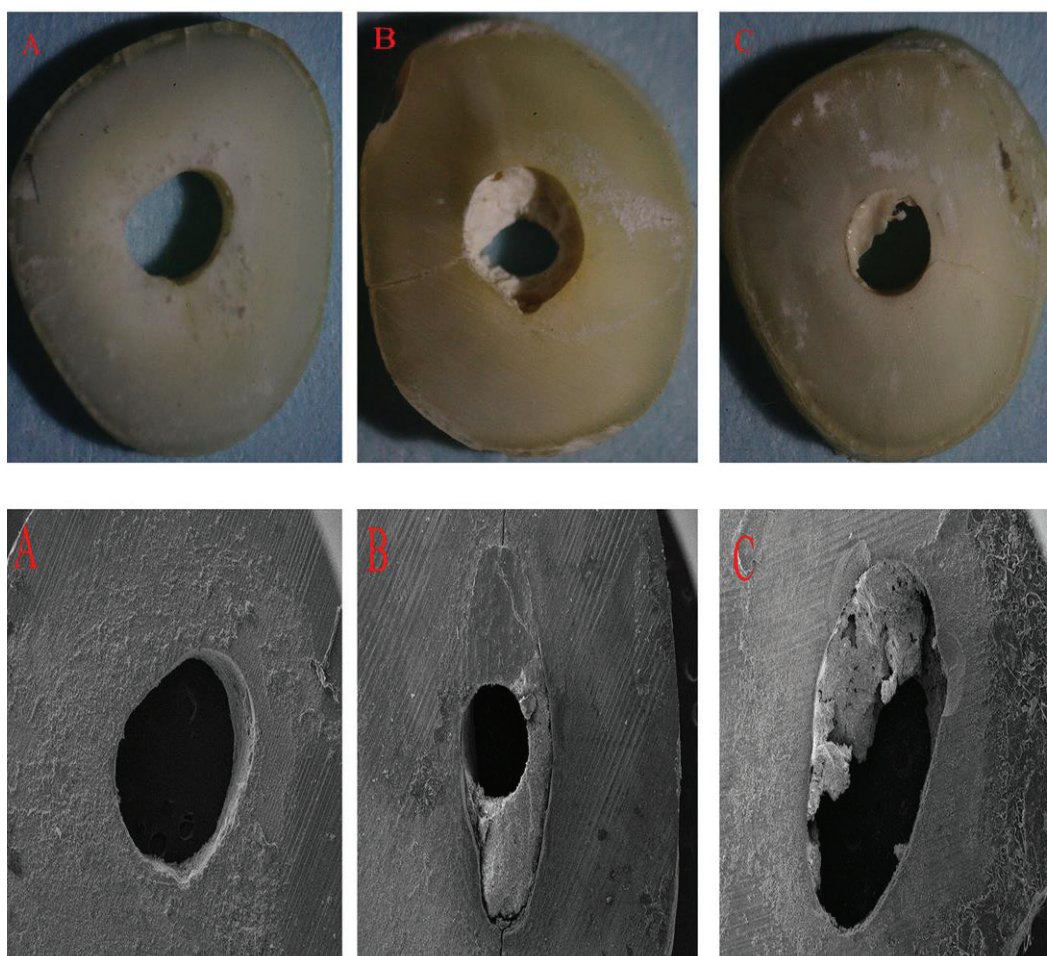


Figure 1. Representative stereomicroscope (25 \times) and scanning electron microscope (SEM) (54 \times) images of failure modes; (A) adhesive failure at cement/dentin interface; (B) cohesive failure within cement, and (C) mixed failure in both cement and dentin.

between the cement and root canal dentin which is required for optimal adhesion, as a result this allows chemical bonding or micromechanical interlocking.

There are several studies on the effects of various variables, such as the different types of cement, intracanal medicaments (2, 3), placement techniques of cement, and irrigation regimens (21) on the bond strength of CSC. However, there has been limited research focus on the effect of chelating agents on the bond strength of CSC. Based on this information, the effects of 17% EDTA, 7% MA, and 10% CA on bond strength of ProRoot MTA and ERRM were examined in the present study. Both null hypotheses of the study were rejected because both EDTA and MA increased the bond strength values of endodontic cements as compared to CA. In addition, ERRM was found to have higher bond strength values than ProRoot MTA.

There are several methods to test the bond strength (22). In this study, push-out bond strength test was used. This is a commonly used test to measure the bond strength in the root canal (22). Goracci et al. reported that the push-out test better reflects the clinical conditions of the fracture pattern than microshear or microtensile methods, and is more reliable than other tests (22). Not only were there numerous failures in the preparation of the samples in the microtensile test, the observed data in such tests were distributed over a wide range. On the contrary, the method used in our study allows testing of regional differences and reduces premature failure rates as compared to other tests (19).

Irrigation of root canals with chelating agents such as EDTA, MA or CA is recommended to effectively remove the smear layer, (1, 10, 13, 15). However, MA has been shown to be more biocompatible than EDTA(23), with a better smear layer-removal ability in sclerotic root canals (10). MA at a concentration of 7% was used in this study since higher concentrations may cause damage to intertubular dentin as reported previously (24). CA at a recommended concentration of 10% was used in this study. The decalcifying action of 10% CA was found to be double or more than that of 1% CA (15).

The results of the present study can be attributed to various factors. The first is the region where the discs were obtained. In the present study, dentin discs were obtained from the coronal third of the root canal. This is consistent with the study by Ballal et al. (10), in which the authors reported that one minute application of 7% MA was more effective than 17% EDTA in removing the smear layer in the apical third of the root canal system, but not in the middle and coronal third. In addition, no significant difference was found to exist between MA and EDTA with respect to the degree of microhardness as reported by Ballal et al. (25). In contrast, Ulusoy and Gorgul reported that MA had a higher reduction in dentine microhardness as compared to EDTA (26). In our study, the push-out bond strength was found to be significantly less for CA than for EDTA and MA ($p < 0.05$). This is consistent with a previous finding that CA was less effective than EDTA in removal of the smear layer (27).

Secondly, the results of the present study can also be attributed to the irrigation procedure employed in our study. There is no definite protocol of the type or concentration of chelating agents. However, different irrigation solutions have been shown to affect the adhesion of materials to dentine surfaces as a result of the effect on dentinal walls which includes alteration of surface energy or wetting ability of dentinal walls. Consistent with this study, Ballal et al. (10) have reported the decreased surface tension of 17% EDTA compared to 7% MA, which may be a possible explanation for the higher bond strength of tested CSC in our study. In addition, while EDTA has been shown to cause complete demineralization of the root canal dentine, MA and CA generate mineral gradients (10). One reason for the lower bond strength values of CA groups may be because the decalcifying capacity of CA is time-dependent. Lopez et al. (28) reported that the amount of Ca²⁺ extracted in the CA and EDTA solutions increased with longer immersion time. Consistent with the findings of the present study, Ballal et al. reported that MA is highly acidic and has a better demineralizing effect (25).

In this study, irrespective of the chelating agents tested, ERRM was found to have higher bond strength values than ProRoot MTA ($p < 0.05$). One of the reasons may be due to the physical and chemical properties of cements that were tested. The presence of zirconium oxide improved certain physical properties of bioceramics. The composition and particle size of existing cements affect the interaction between cement and root canal dentin (21). ERRM has a smaller particle size than MTA. In addition, ERRM can form chemical bonds with root canal dentin walls, thus creating a robust connection. It was argued that the bioceramic cements when reacted with moisture, form hydroxyapatite that may chemically bond to the tooth structure (29). This may result in a 2% expansion because of the setting reaction, thus adapting better to the root canals. Because of crystal growth in dentin tubules, the effect of dentinal bridge formation can be strengthened, thereby increasing micromechanical involvement. Furthermore, the bond strength of ERRM may be higher than that of ProRoot MTA owing to the particle structure and hydrophilic properties of ERRM (30). Inconsistent with the present study, Shokohienad et al. (31) reported that bond strength of MTA and ERRM paste was significantly lower in samples stored in conditions with an acidic pH; however, the push-out bond strength of the ERRM putty was not influenced by acidity. However, while the samples were kept in an acidic medium for 4 days in their study, the total contact with the root surface of the chelating agents tested was limited to five minutes in our study, consistent with the recommended clinical use.

One limitation of the present study was that it was an in vitro study. Thus, it was not possible to fully reflect the oral environment (occlusal stresses, blood-saliva contamination, etc.). Therefore, further in vivo studies are needed to investigate the actual bond strength of the tested materials.

Conclusion

Within the limitations, it may be concluded that the use of chelating agents increased the push-out bond strength of CSC. Both of EDTA and MA increased the bond strength of CSC when compared to CA. ERRM had higher bond strength values than ProRoot MTA.

Türkçe Öz: *Proroot MTA ve endosequence kök tamir materyalinin bağlanma dayanımı üzerine farklı şelasyon ajanlarının etkisinin incelenmesi. Amaç: Bu çalışmada, ProRoot MTA ve Endosequence Kök Tamir Materyalinin (ERRM) push-out bağlanma dayanımı üzerine %17 etilendiamin tetraasetik asit (EDTA), % 7 maleik asit (MA) ve %10 sitrik asitin (SA) etkilerinin değerlendirilmesi amaçlandı. Gereç ve Yöntem: Standardize edilmiş olgunlaşmamış bir diş modelini elde etmek için seksen adet tek köklü yeni çekilmiş insan dişleri prepare edildi. Test edilen şelasyon ajanlarına göre örnekler rastgele üç deney grubuna ayrıldı: Grup 1; (% 17 EDTA), Grup 2; (% 7 MA), Grup 3; (% 10 SA) ve Grup 4; (Pozitif Kontrol) (n = 20). Her bir ana grup daha sonra iki alt gruba ayrıldı: Grup A; (ProRoot MTA) ve Grup B; ERRM (n = 10). İrrigasyon ve simanların yerleştirilmesinden sonra, dişler 37°C'de ve %100 nemli ortamda bir hafta boyunca saklandı. Toplam 240 dentin diski (diş başına üç disk) alındı ve push-out bağlanma testi yapıldı. Elde edilen veriler, iki yönlü varyans analizi ve Tukey's post hoc t-testi kullanılarak analiz edildi ($p=0.05$). Bulgular: Hem test edilen şelasyon ajanlarının hem de kalsiyum silikat bazlı simanların türü, push-out bağlanma dayanımı değerleri ile anlamlı derecede ilişkili bulundu ($p<0.05$). Push-out bağlanma dayanımı, EDTA veya MA'e kıyasla SA için anlamlı olarak daha az görüldü ($p<0.05$). ERRM, ProRoot MTA'dan daha yüksek bağlanma dayanımı değerlerine sahipti ($p < 0.05$). Sonuç: Şelasyon ajanlarının kullanımı kalsiyum silikat simanlarının bağlanma dayanımını artırmıştır. Test edilen şelasyon ajanlarından bağımsız olarak, ERRM, ProRoot MTA'ya göre daha yüksek bağlanma dayanımı değerlerine sahip bulundu. Anahtar kelimeler: kalsiyum silikat siman; şelasyon ajanları; endosequence kök kanal tamir materyali; mineral trioksit agregat; bağlanma dayanımı*

Ethics Committee Approval: Ethical approval was obtained from the Health Ethics Committee of the University of Cumhuriyet, Sivas, Turkey (ID: 2016-12/08).

Informed Consent: Informed consent was obtained from the participants.

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Author contributions: BB, FO and AK participated in designing the study. BB, FO and AK participated in generating the data for the study. BB, FO and AK participated in gathering the data for the study. BB participated in the analysis of the data. BB wrote the majority of the original draft of the paper. BB, FO and AK participated in writing the paper. All authors approved the final version of this paper.

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Effect of different water-to-powder ratios on the dimensional stability and compressive strength of mineral aggregate-based cements

Purpose

The aim of this study was to evaluate the effect of different water-to-powder ratios on the dimensional stability and compressive strength of Portland cement and Mineral Trioxide Aggregate (MTA).

Materials and Methods

Five different volumes of distilled water (0.26; 0.28; 0.30; 0.33 and 0.35 mL) were used for every 1 g of the cements. Twelve samples (12 mm long x 6 mm in diameter) were prepared in Teflon molds. After measuring the initial length, the specimens were stored in distilled water for 24 hours or 30 days. At the end of these time intervals, the specimens were measured again, and the dimensional change was calculated. The same samples used in the previous test were submitted to compression in a universal test machine (1 mm/min⁻¹).





Results

Analysis of the dimensional stability results showed no statistical difference between the cements, proportions and time intervals tested, or between their interactions. After 24 hours, MTA was more resistant than Portland cement ($p < 0.05$). At 30 day-period, both cements had similar, and significantly higher resistance than they did at 24 hours ($p < 0.05$).

Conclusion

The powder/water ratio had no influence on the dimensional stability of cements. Compressive strength of Portland cement was affected at the proportions of 0.30 and 0.35 mL/g.

Keywords: Portland cement; mineral trioxide aggregate; dimensional stability; compressive strength

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Introduction

An ideal retrofilling material must be capable of sealing the pathological communications between root canal system and the surrounding tissues, and the presence of moisture must not interfere in its sealing capacity (1-3). This material should be biocompatible, easy to handle, have adequate radiopacity, be minimally insoluble and dimensionally stable (4-6). In spite of the evolution of cements for endodontic application have presented over the last few years, there is still no material that meets all these requirements (7).

Mineral Trioxide Aggregate (MTA) was developed in the 1990s in Loma Linda, California (4), as a retrofilling cement and perforation sealant, and it was subsequently used in other diverse clinical applications due to its excellent physicochemical and biological characteristics (5,8). Studies

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have shown that MTA can induce mineralized hard tissue deposition (9), in addition to being well tolerated by living tissues (10). It is still outstanding among the other retrofilling cements because it presents good sealing capacity, expands during setting, releases calcium ions and because it is possible to use it in environments with relative moisture (2,11-13).

Conversely, MTA has some inconvenient features such as a long setting time that favors its solubilization and/or disintegration; or could even lead to its displacement from the retrograde cavity; and its sandy consistency, which makes it difficult to inset into the retropreparation and in areas with perforations (14,15).

Despite not being a Food and Drug Administration (FDA) approved commercial product to be used for medical purposes, Portland cement is widely used as an alternative material to MTA in laboratory studies due to its availability and low cost (11). Considering the similar characteristics between MTA and Portland cement, different vehicles and additives have been proposed to be used in association with these cements with the purpose of improving their physicochemical properties (14-16). In addition, the final consistency of both cements is similar, and it is directly related to the water/powder ratio used in the mixture (15). Fridland and Rosado (15) demonstrated the larger the quantity of water used in the manipulation, the greater would be the solubility of MTA.

The manufacturers of MTA recommend a water-to-powder ratio of 3:1, which hinders the cement's manipulation (17,18). Therefore, the ideal water/powder ratio still is a controversial point among researchers, which could be determinant in obtaining a better consistency and make the cement easier to manipulate. Moreover, it may directly influence the physical and mechanical properties of the cement in either a negative or positive manner.

The aim of this study was to evaluate the influence of different powder/water ratios on the dimensional stability and compressive strength of Portland cement and MTA. The null hypothesis tested was that the different water/powder ratios would not interfere in the physical-mechanical properties of the cements.

Materials and Methods

Specimen preparation

The cements tested in this study were the following: White Portland Cement (Irajazinho, Votoratin, São Paulo, SP, Brazil) (WPC) and White MTA (Angelus Soluções Odontológicas, Londrina, PR, Brazil - Lot nº 21584) (WMTA). As WPC has no radiopacifying agent, bismuth oxide was added to its formula at 20% by weight, the same percentage found in MTA.

WMTA and WPC were mixed using the water/powder (WP) ratio of 0.26, 0.28, 0.30, 0.33, and 0.35 mL of distilled water, establishing the following experimental groups: G1 - WMTA+0.26, G2 - WMTA+0.28, G3 - WMTA+0.30, G4 - WMTA+0.33, G5 - WMTA+0.35, G6 - WPC+0.26, G7 - WPC+0.28, G8 - WPC+0.30, G9 - WPC+0.33, and G10 - WPC+0.35 (n=6). Each sample contained 1.00 g of WMTA or WPC powder measured on an analytic balance. Using a micro-pipette, the appropriate amount of distilled water described above was added to each sample to achieve the proper WP ratio. The cements were hand-mixed on a nonabsorbent pad in a

standardized fashion, totaling 120 specimens. Sixty specimens were used to perform the tests (dimensional stability and compressive strength) at the 24-hour period, and the other sixty specimens were used at the 30-day-period.

Dimensional stability

For each group, 12 cylindrical samples were obtained, measuring 12 mm high by 6 mm in diameter, in accordance with the Specification No. 57 of the American Dental Association (ADA) (19). For this purpose, Teflon molds were placed on a glass slide measuring 1 mm thick by 25 mm wide and 75 mm long, covered with a strip of cellophane paper. After this, the molds were filled with the manipulated cements, so that a slight excess of material could be verified at their upper extremity. After filling, another glass slide, also covered with a strip of cellophane paper was placed over the top surface of the mold. The set was kept firmly united by means of a C-shaped clip. After the elapse of 5 minutes from the time of starting the mixture, the set was transferred to an oven at 37 ± 1 °C, with relative humidity of 95%. After 24 hours, the set was removed from the oven and the sample surfaces were smoothed with water abrasive paper #600 (3M, Sumaré, SP, Brazil), under abundant cooling with distilled water. On conclusion of this stage, the samples of each group were removed from their molds, their lengths measured with a digital pachymeter (Digimes, São Paulo, SP, Brazil), and the measurements were recorded. Right after this, the samples were placed in individual receptacles containing 30 mL of distilled and deionized water, identified by sample group and number, and were kept in the oven at 37 ± 1 °C, for 24 hours or 30 days. Afterwards, the samples were removed from the receptacles, the excess water was removed with the aid of absorbent paper, and a new length measurement was made.

The dimensional change was calculated using the following formula: $[(C_{24 \text{ hours or } 30 \text{ days}} - C) \times 100/C]$, where $C_{24 \text{ hours or } 30 \text{ days}}$ is the sample lengths after elapse of 24 hours or 30 days, and C is the initial sample length (16). The dimensional change of the groups was established by means of the arithmetic mean of 6 repetitions performed. The variables considered for analysis were: cement (WMTA and WPC); time intervals (24 hours and 30 days) and WP ratios (0.26; 0.28; 0.30; 0.33 e 0.35 mL).

Compressive strength

The compressive strength of samples was determined by the method recommended by Standard Specification 6039:1981 of the British Standards Institution (BSI) (20).

The same samples used in the previous test were used, considering the same variables. After each time interval, they were removed from the receptacles, the excess water was removed with the aid of an absorbent paper towel, and the compressive strength was determined in a Universal test machine (Instron, Model 1334, Instron Corp., Canton, MA, USA) at a speed of 1 mm/min^{-1} . The maximum load necessary to fracture each sample was obtained and recorded. The compressive strength was calculated in megapascal (MPa) according to the following formula: $C = 4P / \pi D^2$, where "P" represented the maximum load recorded by the machine in Newtons (N) and "D" the diameter of the sample in millimeters (mm).

Statistical analysis

After verifying the normality of the sample (Shapiro-Wilk test), the values obtained in the dimensional stability and compressive strength tests were statistically compared with three-way analysis of variance (ANOVA), and the Tukey test ($p < 0.05$). The statistical analysis was performed using the Graphpad Prism 4.0 Software program (GraphPad Software, La Jolla, CA, USA).

Results

Dimensional stability

Table 1 shows the mean dimensional stability values of each group.

The positive and negative mean values indicate expansion and contraction of the cements, respectively. In general, WPC and WMTA had expansion in 24 hours. However, after 30 days, WPC had contraction in all the WP ratios tested; and WMTA, expansion, with exception of G7 (0.28 mL). In spite of the different behavior between the two cements after 30 days, there was no significant differences among the variables tested, or between their interactions, demonstrating that the dimensional stability between the cements and the WP ratios tested were equivalent in all the situations.

Compressive strength

Table 2 shows the mean compressive strength values of each group.

Both cements tested with the different WP ratios had significant increase in compressive strength over the course of time, with the exception of G6 (0.26 mL), G9 (0.33 mL) and G10 (0.35 mL). WPC and WMTA had similar compressive strength at 30 day-period, however, the mean values of WMTA were significantly higher at

24 hours in comparison with WPC ($p < 0.05$), except for G7 (0.28 mL). The WP ratios in the G3 and G5 significantly diminished the compressive strength of WPC cement in comparison with G8 and G10 (WMTA), at the 24 hour-period ($p < 0.05$).

Discussion

The aim of this study was to evaluate the influence of different WP ratios on the dimensional stability and compressive strength of Portland cement and MTA. According to the results obtained in this study, the null hypothesis tested was partially accepted, since the two cements presented similar dimensional stability, however, the compressive strength of the cements was affected by the WP ratio.

The microstructure of hydraulic cements, such as Portland cement and MTA, is basically formed of pores and channels that serve to diffuse water within the cement mass, guaranteeing its continuous process of hydration until final hardening (21,22). The characteristics of this microstructure may be influenced by diverse factors, such as the pressure used during manipulation of the cement, the method of mixture, and the water/powder ratio, and these factors are difficult to control (15). During manipulation of the cement, for example, the greater the force applied in the cement, the more compacted it will be, and consequently, the less presence of canals that form its microstructure there will be (23). Moreover, the less incorporation of bubbles of air there will be, thus diminishing the presence of porosities (15,23). The absence or diminishment of these structures will make hydration of the cement difficult, compromising its performance (22).

During the setting process of hydraulic cements, the water is not only incorporated into the powder, but it initiates a process in which its molecules bind chemically to diverse phases of these materials (22). Portland cement and MTA set, and become more resistant as time passes, presenting a faster initial hardening, but one that lasts, and becomes

Table 1. Mean values and standard deviation (SD) for dimensional stability (%) of the different groups at the two-time intervals of analysis. There was no significant difference among groups (cements/WP ratios) (3-way ANOVA, the Tukey test, $p < 0.05$). $n=6$.

Cements	WPC					WMTA				
	1 (0.26mL)	2 (0.28mL)	3 (0.30mL)	4 (0.33mL)	5 (0.35mL)	6 (0.26mL)	7 (0.28mL)	8 (0.30mL)	9 (0.33mL)	10 (0.35mL)
24 h	-0.334	0.000	0.048	0.145	0.236	0.048	-0.096	-0.191	0.001	-0.048
	(0.334)	(0.183)	(0.214)	(0.352)	(0.215)	(0.117)	(0.148)	(0.148)	(0.256)	(0.217)
30 days	-0.284	-0.050	-0.328	-0.292	-0.190	0.145	-0.429	0.375	0.967	0.239
	(0.178)	(1.250)	(0.212)	(0.261)	(0.394)	(0.623)	(0.398)	(0.384)	(3.731)	(0.492)

Table 2. Mean values (MPa) and standard deviation (SD) for compression strength of the different groups at the two-time intervals of analysis. Different capital letters in columns and lowercase letters in lines indicate statistically significant difference (3-way ANOVA, the Tukey test, $p < 0.05$). $n=6$.

Cements	WPC					WMTA				
	1	2	3	4	5	6	7	8	9	10
Groups	1 (0.26mL)	2 (0.28mL)	3 (0.30mL)	4 (0.33mL)	5 (0.35mL)	6 (0.26mL)	7 (0.28mL)	8 (0.30mL)	9 (0.33mL)	10 (0.35mL)
24 h	38.86 ^{A,a}	52.07 ^{A,a}	34.35 ^{A,a}	45.82 ^{A,a}	36.50 ^{A,a}	60.06 ^{A,b}	40.06 ^{A,a}	52.87 ^{A,b}	56.08 ^{A,b}	62.93 ^{A,b}
	(6.44)	(4.96)	(5.46)	(5.05)	(5.57)	(9.09)	(19.06)	(9.58)	(15.15)	(13.77)
30 days	74.90 ^{B,a}	73.56 ^{B,a}	56.95 ^{B,a}	75.30 ^{B,a}	50.20 ^{B,a}	57.32 ^{A,a}	58.08 ^{B,a}	59.76 ^{B,a}	59.51 ^{A,a}	53.63 ^{A,a}
	(12.33)	(6.22)	(10.94)	(20.70)	(14.95)	(9.89)	(10.99)	(10.38)	(13.12)	(8.16)

slower during the following days (24). The longer the time of hydration, the more organized and rigid will be the crystalline microstructure that can be formed, improving the physicochemical properties of these cements (24).

Numerous studies have demonstrated that the compressive strength of MTA was significantly lower than that of amalgam after 24 hours (4,5). However, after 3 weeks, there was no significant difference between the materials relative to compressive strength (4,5). These results corroborated the findings of the present study, because Portland cement and MTA presented significantly higher compressive strength values at 30 days in comparison with the initial period (24 hours).

The two cements are basically composed of tricalcium silicate (3CaO SiO_2) and dicalcium silicate (2CaO SiO_2) (11), with the addition of bismuth oxide (Bi_2O_3) to give them radiopacity (25). Because the hydration of dicalcium silicate is slower than that of tricalcium silicate, the compressive strength and resistance to displacement attain their maximum values several days after their mixture (26,27).

The two cements compared in the present study presented similar compressive strength values at 30 days, however, MTA showed higher strength values in the initial period of analysis. According to Kao et al. (28), Portland cement has a more delicate microstructure than MTA, due to the different temperatures at which the cements are sintered, leading to the formation of distinct phases and oxides from those found in conventional MTA. Furthermore, the raw materials used in their purification process, guarantee that MTA has advantages in comparison with other mineral aggregate-based cements (28).

Considering the different WP ratios, there was no statistically difference among the experimental groups, except for G3 (0.30 mL) and G5 (0.35 mL), which significantly decrease the strength values of Portland cement in comparison with the WMTA groups at the 24 hour-period. Studies have previously reported that higher ratios of water lead to greater porosity in the final microstructure of the cement, which at first sight would be beneficial, because of network of intercommunicating pores within the cement would allow greater diffusion of water molecules, and consequently, a better and more accentuated hydration during the setting process (15,22). However, Basturk et al. (29) demonstrated a negative correlation between the quantity of pores present and the mechanical strength of mineral aggregate-based cements.

Basturk et al. (30) demonstrated that the increase in the WP ratio from 0.34 mL to 0.40 mL was sufficient to significantly diminish the compressive strength of MTA (30). Shojaee et al. (31) also reported that higher WP ratios (0.40 and 0.50) lead to lower compressive strength of mineral aggregate-based cements. According to Fridland & Rosado (15), a ratio higher than 0.33 mL to 1 g of MTA powder is incapable of producing a sufficiently viscous mass to be manipulated in a clinically adequate manner, making it difficult to insert the cement into the area to be treated. On the other hand, a ratio lower than 0.26 mL did not allow a cement with adequate physicochemical properties to be obtained (15). However, in the present study, these situations were only observed for Portland cement, in which the WP ratios of 0.30 mL and 0.35 mL significantly diminished the strength values of the cement between the time intervals of 24 hours and 30 days.

Considering the dimensional stability, the different WP ratios caused no significant changes in the cements tested.

According to the results obtained in this study, the positive values indicated expansion of the cements, and negative values, the opposite. After the initial 24 hours had elapsed, both cements presented expansion, a common fact, because the hydration process of cements leads to the diffusion and chemical bonding of the water molecules with the cement particles, forming a semi-solid mass of colloidal silica that continues to expand as time passes (22). After 30 days MTA continued to present expansion, however, Portland cement presented contraction, irrespective of the WP ratio tested. The process of expansion of these cements during their setting time is water-dependent and is directly associated with the cement capacity to absorb water from the medium and the use of water in the formation of hydrates during their manipulation (32). The greater this hydration capacity, the more complete will be the cement hardening, and the more satisfactory will be its properties. Although there were no differences between the dimensional stability values of the cements, the expansion of MTA, even after the initial time interval of analysis may have influenced the compressive strength results, which were significantly higher in the initial 24 hours, in comparison with Portland cement. It is worth pointing out that only for the ratio of 0.28 mL was there no significant difference in the compressive strength values of the cements; the same proportion in which MTA presented contraction, 30 days after its manipulation.

Due to the variety of types and commercial brands of mineral aggregate-based cements existent on the market, in-depth comparisons between the studies conducted up to now, have been difficult to carry out in an appropriate manner. Furthermore, samples of Portland cement may present even greater variations, particularly due to the considerable number of manufacturers, limiting the comparisons between scientific findings. In spite of these difficulties, it is justifiable to emphasize that different WP ratios may affect certain properties of this class of cements, however, others do not appear to present significant changes. Such information is crucial for the clinicians when they use mineral aggregate-based cements in areas that receive forces from the condensation of restorative materials or occlusion, as the compressive strength of this type of material may be affected by WP ratio (30).

Conclusion

Based on the results obtained, and considering the limitations of this study, it may be stated that the different water/powder ratios did not influence the dimensional stability of the cements tested, however, MTA was more resistant to compression than Portland cement in the initial 24 hours. Other variables such as time and manner of manipulation, and methods of application of the cement must be investigated before these results are extrapolated to clinical situations.

Türkçe öz: Farklı toz/su oranlarının mineral agregat esaslı simanların boyutsal stabilite ve basınç dayanımları üzerindeki etkileri. Amaç: Bu çalışmanın amacı, farklı toz/su oranlarının Portland simanı ve mineral trioksit agregat'ın (MTA) boyutsal stabilite ve basınç dayanımları üzerindeki etkilerinin araştırılmasıdır. Gereç ve Yöntem: Simanların 1'er gramı 5 farklı hacimde (0,26; 0,28; 0,30; 0,33; ve 0,35 mL) distile su ile karıştırılmıştır. Teflon kalıplar içinde 12'şer örnek (12 mm yükseklik X 6 mm çap) hazırlanmıştır. Başlangıçtaki yükseklikler ölçüldükten sonra, örnekler 24 saat veya 30 gün distile su içinde saklanmıştır. Bu sürelerin sonunda, örnekler

tekrar ölçülmüş ve boyutsal değişim hesaplanmıştır. Daha sonra Universal test cihazı yardımıyla örneklerin basınç dayanımı ölçülmüştür (1 mm/dak-1). Bulgular: Boyutsal stabilite sonuçları değerlendirildiğinde, simanlar, oranlar, süreler ve bunların interaksiyonları arasında istatistiksel bir fark saptanmamıştır. 24 saat sonunda MTA'nın Portland simanından daha dayanıklı olduğu gözlenmiştir ($p < 0,05$). 30 gün sonunda her iki simanın benzer basınç dayanımı sonuçları verdiği ve 24 saat sonundakine kıyasla anlamlı ölçüde daha dayanıklı oldukları bulunmuştur ($p < 0,05$). Sonuç: Toz/su oranı simanların boyutsal stabilitesini etkilememiştir. 0,30 ve 0,35 mL/g oranlarında karıştırıldığında, Portland simanının basınç dayanımının etkilendiği gözlenmiştir. Anahtar kelimeler: Portland simanı; mineral trioksit agregat; boyutsal stabilite; basınç dayanımı

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