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scores of the groups was statistically significant under a predefined effect. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

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Journal Article in Print

1. Burrow MF, Tagami J, Negishi T. Early tensile bone strengths of several enamel and dentin bonding systems. J Dent Res 1994; 74: 522-8.

Journal Article Electronic Publication Ahead of Print

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

Book

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

Chapter in a book

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

Thesis

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

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

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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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Original research

The effects of morus nigra on the alveolar bone loss in experimentally-induced periodontitis

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Purpose
The aim of this study is to evalute the anti-inflammatory effects of morus migra on experimentally-induced periodontitis in rats.

Materials and Methods
Twenty-four Wistar-albino rats were randomly divided into three groups: control group (C, n=8), experimental periodontitis (PER, n=8), experimental periodontitis and treated with Morus nigra (MN+PER, n=8) (50 mg/kg per day for 21 days). After 21 days, the rats were sacrificed, and alveolar bones were evaluated histopathologically and histometrically analyzed to obtain level of alveolar bone loss. The detection of RANKL and OPG were immunohistochemically performed. Serum and tissue levels of MMP-8 and MMP-13 were also analyzed.

Results
Morus nigra treatment decreased tissue MMP-8 and MMP-13 levels and there were significant differences in the case of tissue levels of MMP-8 and MMP-13 between groups PER and MN+PER (p=0.035, p=0.041). There were no significant differences among all the groups serum levels of MMP-8 and MMP-13 (p=0.067, p=0.082). In the histometric evaluation, alveolar bone loss was greater in the PER group compared to C and MN groups (p=0.035). Immuno-histochemical staining of RANKL activities were found significantly lower (p=0.037) and OPG activities were found significantly higher in MN+PER group when compared to PER group (p=0.021).

Conclusion
The present study reveals that systemic administration of Morus nigra significantly inhibited the regional alveolar bone resorption and contributes to periodontal healing in the rat experimental-periodontitis models.

Keywords: *Experimental periodontitis; cytokines; MMP-8; MMP-13; morus nigra*

Introduction

Periodontal diseases are chronic inflammatory disorders that affect periodontal attachments and alveolar bone around the teeth (1). The main etiological factors for the initiation and progression of periodontitis are; genetic predisposition, environmental factors and a dysbiotic microbiota with an excessive host response (2). A microbial biofilm layer starts periodontal disorders by alerting immune system with periodontopathogens and plays a significant role in the advancement of this diseases (3). Osteoclastogenic mediators, matrix metalloproteinases (MMPs), and inflammatory cytokines are released from immune system cells during periodontal inflammation. These factors also improve the association between the receptor activation of nuclear factor κB (RANK) and its ligand (RANKL) (4). An excessive host response is dependent on periodontopathogens that cause tissue degradation due to complicated associations between periodontopathogens and the host's defense system (4).

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MMPs are proteolytic enzymes that are responsible for tissue remodeling and destruction of the extracellular matrix (ECM) (5). Various hormones and pro-inflammatory cytokines such as interleukin (IL) 8, IL-1β, and tumor necrosis factor (TNF)-α, which are released during the inflammatory process, cause the release of MMPs from inflammatory cells (5). Metalloproteinases are classified into five subgroups: collagenases, gelatinases, stromelysins, membrane types, and others. Previous studies related to MMPs have suggested that MMP-8 and MMP-13 are the major mediators of collagenases and are effective in the destruction of type I, II, and III collagen (6). MMP-8 is an important factor of degradation in inflammatory disorders and associated with periodontal diseases. MMP-13 have significant role in various aspects of bone metabolism such as resorption and remodeling (7). Furthermore, MMP-13 is one of the most dominant MMPs in resorption areas in bone tissue (6). Previous studies related to MMP-8 and MMP-13 suggest that these are indicators of the destruction of tissue in periodontal diseases (5). Most of the studies have suggested that MMP-13 and MMP-8 are released in higher levels in patients with periodontal diseases than healthy individuals (6). RANKL, a member of the TNF superfamily, is a crucial factor in bone resorption. RANKL expression has been detected in osteoblastic, stromal, and activated B- and T cells (4). RANKL stimulates osteoclast differentiation and bone resorption (1). IL-1β and TNF-α lead to periodontal tissue destruction and alveolar bone resorption via decreases in osteoprotegerin (OPG). OPG has an important role in inhibiting bone resorption (8). Bone loss occurs as a result of an increased RANKL/OPG ratio, and this ratio is increased at the sites of active periodontal disease and related to the disease severity (9). Previous studies have suggested that RANKL levels in gingival crevicular fluid (GCF) are increased in individuals with periodontitis (1, 4).

Morus nigra (Urticales Moraceae), commonly known as the black mulberry, possesses many characteristics including anxiolytic, sedative, diuretic, analgesic and hypotensive properties. It is also used in the treatment of various disorders including inflammatory diseases (10) pharyngitis, toothache, snake bites, antidote to action poisoning (11). The berries, bark, and leaves of M. nigra are used for various ailments. The berries inhibit inflammation and hemorrhage, the leaves are an antidote to poisoning, and the bark is used for odontalgia (12). The anti-inflammatory and analgesic activities of M. nigra have been shown in several experimental models (10). M. nigra is also used to make traditional Turkish foods such as mulberry pekmez, mulberry pestil, and mulberry kome. In addition, the fruit are eaten fresh and made into natural dyes, marmalades, liquors, and juices (13).

Based on the beneficial properties of M. nigra, we suggest that it may decrease both inflammation in periodontal tissue and alveolar bone loss rate in periodontal disorders. To the best of our knowledge, there are limited number of investigations focused on the effect of M. nigra on periodontal tissues (10-13). Therefore, current research was planned to analyze the possible therapeutic effects of systemic delivery of M. nigra extract on alveolar bone resorption by examining RANKL, OPG, MMP-8, and MMP-13 levels in both periodontal tissues and serum in ligature induced periodontitis models.

Materials and Methods

Experimental design

All experimental procedures and animal care were performed in accordance with the protocol approved by the Animal Experimental Ethics Committee of the Fırat University, Elazığ, Turkey (No: 2012-013). The rats used in this study were maintained in accordance with the Declaration of Helsinki. The sample size (n=8) was determined with a power calculation to provide 80% power to recognize significant differences among groups with a 95% confidence interval (a=0.05), considering the means and standard deviations of the alveolar bone in the furcation area of the study by Saglam et al. (1) and MMPs (14).Twenty-four 12-week old male Wistar rats (weight: 220±10 g) were divided randomly into three groups as follows: control (C), experimental periodontitis (PER) with no treatment, and experimental periodontitis treated with M. nigra (MN+PER). Pairs of rats were placed in wire cages and maintained on a 12:12-hour light-dark cycle with an ambient room temperature of 23±2 °C. Rats were fed with standard rat pellets and tap water ad libitum.

For experimental periodontitis induction, xylazine hydrochloride (Rompun, Bayer, Germany; 10 mg/kg) and ketamine hydrochloride (Ketalar, Bayer, Germany; 40 mg/kg) were used to provide anesthesia. 3-0 sterile silk sutures were ligatured two sides of the mandibular first molars of the rats in the PER and MN+PER groups for 21 days in a submarginal position to induce microbial dental plaque accumulation and inflammation, according to previous studies (15). The MN+PER group received M. nigra with an intragastric dose of 50 mg/kg/day until their sacrifice at 21st day (11).

Preparation of the M. nigra extract

Fresh fruits of M. nigra were collected from Elazig, Sivrice, Turkey, during its fruit season between 15 August and 15 September 2015. M. nigra extract was prepared from fresh fruits. We homogenized 200 grams of fruit in 200 ml of water and the homogenization was then filtered through cheese cloth. The filtrate was boiled for 10 minutes. The final solution was dissolved in distilled water at 50 mg/kg concentrations for the experiment. After obtaining the extract, it was stored at -20 °C until tested (11).

Blood and tissue sampling

After 21 days, xylazine hydrochloride (Rompun, Bayer, Germany; 10 mg/kg) and ketamine hydrochloride (Ketalar, Bayer, Germany; 40 mg/kg) were used for anesthesia. After the cardiac blood samples were collected from the heart, the animals were sacrificed by guillotine method. Mandibula samples were removed from the heads. The mandibles were divided into two equal pieces (16). The right sides were forwarded to the histology laboratory for histological evaluation and 10% neutral-buffered formaldehyde solution was used for fixation. The left sides of the mandibula were sent to the biochemistry laboratory for biochemical analysis. The blood samples were centrifuged at 3500 xg for 15 minutes to obtain serum. Sterile polypropylene tubes were used to save

serum samples and kept at -80°C until being analyzed for MMP-8 and MMP-13 (1).

Biochemical measurement of serum and tissue parameters

In all groups, periodontal soft tissues from the left side of the mandibula were carefully retained for biochemical analysis. A buffer solution (4.5 ml) was added into 0.5 g of the periodontal soft tissue. After 15 minutes homogenization, the mixtures were filtered and centrifuged at 3500 rpm using a refrigerated centrifuge at 4 °C. The tissue and serum samples were used for analysis of MMP-8 and MMP-13 levels by rat enzyme linked immunosorbent assay (Rel assay -ELISA, Gaziantep, Turkey) kits (4).

Histopathological and histometric evaluation

Alveolar bone samples were removed from the mandibles of rats for histological analysis. These samples were then fixed for 72 hours in 10% formalin and washed with phosphate buffered saline (PBS) (P4417, Sigma-Aldrich, St. Louis, Missouri, USA). Then, the tissues were immersed in 10% ethylenediaminetetraacetic acid (EDTA) for decalcification for approximately 60 days. At the end of this decalcification process, routine paraffin techniques were applied. Tissues that were embedded in paraffin blocks were cut into 5–6-µm thick sections. The prepared slides were then stained with hematoxylin and eosin (H&E). Finally, the stained slides were interpreted and imaged under a light microscope (Olympus, CH BI45 T S, Japan)(1).

Histometric evaluation was done according to the method explained by Lucinda et al.. Three points were considered as that is, the apex of the distal/mesial root (A), the alveolar bone on the distal/mesial area of the tooth (B), and the top of the distal/mesial cusp (C) (Figure 1) (17). The distances between AB and AC points were calculated to determine the periodontal bone level with this formula: AB/AC ×100 (15). All measurements were performed by a single examiner (E.E.) and a mean value was defined for each tooth.

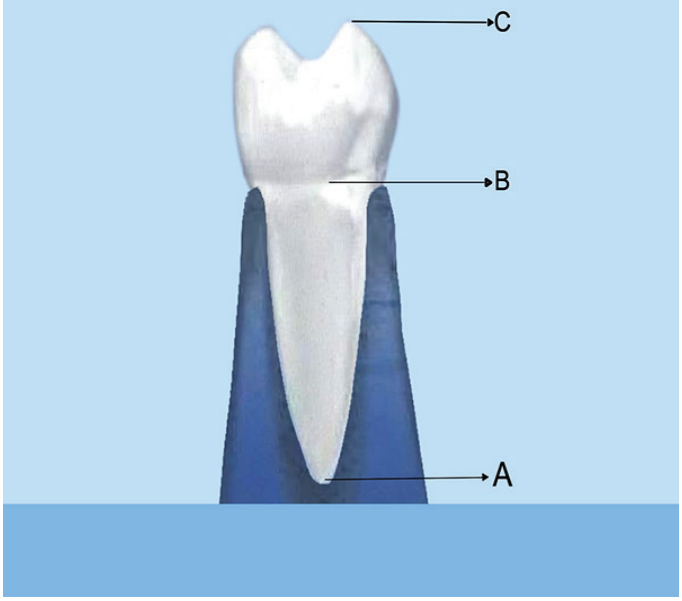

Figure 1. *Periodontal bone loss measurement: A, root apex; B, crest bone; C, tip of the cusp of the first molar.*

Immunohistochemical evaluation

An avidin-biotin-peroxidase complex procedure was applied to determine RANKL and OPG immunoreactivities in rat alveolar bone tissue. The 5–6-µm thickness tissue slides with poly L lysine were prepared. Deparaffinized and rehydrated tissues were then boiled in a citrate buffer at a pH of 6.5 in a microwave oven (750 W) for 7+5 minutes and cooled for about 20 minutes at room temperature. After washing with PBS for 3×5 minutes, the tissues were incubated in hydrogen peroxide (Hydrogen Peroxide Block, Thermo Fisher Scientific, TA-125-HP, Fremont, California, USA) for 5 minutes to obstruct endogenous peroxidase activity and then washed with PBS for 3×5 minutes. Ultra V Block (Ultra V Block, TA-125-UB, Fremont, California, USA) was enforced for 5 minutes in order to avoid background staining. Tissues were incubated in 1/200 diluted primary antibodies (RANKL (sc-9073) and OPG (sc-11383) Rabbit Polyclonal IgG Antibodies, Santa Cruz Biotechnology, Santa Cruz, California, USA) in a humid environment for 60 minutes at room temperature, they were then washed with PBS for 3×5 minutes. The tissues were then incubated with a secondary antibody (Biotinylated Goat Anti-Polyvalent, Thermo Fisher Scientific, TP–060-BN, Fremont, California, USA) in a humid environment for 30 minutes at room temperature. After that, tissues were washed with PBS for 3×5 minutes and incubated with streptavidin peroxidase (Streptavidin Peroxidase, Thermo Fisher Scientific, TS-060-HR, Fremont, California, USA) in a humid environment for 30 minutes at room temperature. Then, 3 amino 9-ethylcarbazol (AEC) solution (Large Volume AEC Substrate System (RTU), Thermo Fisher Scientific, TA-060-HA, Fremont, California, USA) was dropped into the tissues and the tissues were examined by light microscopy. The reaction was completed when the video signal was received. Counterstaining textures of tissues were provided with Mayer's hematoxylin staining. The stained slides were then closed with a proper closing solution (Large Volume Vision Mount, Thermo Fisher Scientific, TR-125-UG, Fremont, California, USA). The slides were imaged under a light microscope (Olympus, CH-BI45-T-S, Japan) The immunohistochemical histoscore was created on the basis of immunoreactivity prevalence (0.1: < 25%, 0.4: 26-50%, 0.6: 51-75%, 0.9: 76-100 %) and severity (0: no, +0.5: very little, +1: little, +2: medium, +3: severe) (Histoscore = prevalence × severity) (18).

Statistical analysis

SPSS 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY, USA) software was used for statistical analysis. One-way analysis of variance (ANOVA) and Tukey's HSD post hoc tests were performed. Data was expressed as mean ± standard deviation (SD). The confidence interval was set to 95% and p values less than 0.05 were considered statistically significant.

Results

Biochemical results

The mean percentage values of MMP-8 and MMP-13 in tissue are shown in Table 1. The MMP-8 and MMP-13 levels were higher in the PER group tissue samples than in the

Table 1. *The tissue levels of MMP-8 and MMP-13. Values are expressed as means±standard deviations. The diffent letters in the same row indicate significant differences among the study groups (MMP: matrix metalloproteinase, PER: periodontitis, MN+PER: periodontitis and morus nigra treatment).*

	CONTROL	PER	MN+PER
MMP-8	0.50 ±0.16a	0.75±0.10b	0.19±0.20c
MMP-13	0.36±0.21a	0.74±0.23b	0.40±0.17c

Table 2. *The serum levels of MMP-8 and MMP-13. Values are expressed as means±standard deviations (MMP: matrix metalloproteinase, PER: periodontitis, MN+PER: periodontitis and morus nigra treatment).*

	CONTROL	PER	MN+PER
MMP-8	0.76±0.26	0.81±0.15	0.75± 0.09
MMP-13	0.98±0.15	0.99±0.08	0.90±0.04

Table 3: *The level of RANK-L and OPG*

	CONTROL	PER	PER+MN
RANK-L	0.817 ± 0.22^{a}	$2.15\pm0.64^{a,b}$	1.25 ± 0.48^{b}
OPG	1.68 ± 0.64^{c}	$0.70\pm0.41^{c,d}$	1.58 ± 0.67^{d}

Values are expressed as means±standard deviation. Letters (a, b, c or d) in the same row indicate significant differences between groups (n=8); p<0.05. For statistical analysis, differences between groups were tested by analysis of variance followed by the Tukey post hoc test.

fibers, and the destruction of cementoenamel attachments were detected (Figure2). However, in the MN+PER group, there were decreases in both the resorption of alveolar bone and the destruction of cementoenamel attachments and periodontal ligaments (Figure 2).

In the histometric evaluation, alveolar bone loss was greater in the PER group compared to C and MN groups (p=0.035; Figure 3). There were no difference between C and MN+PER groups (p=0.084).

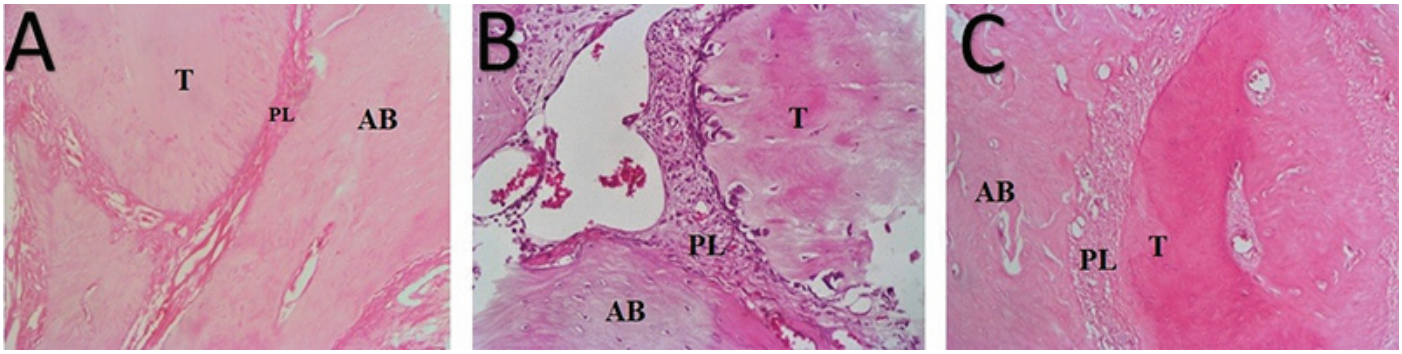

Figure 2. *Histopathological findings on the alveolar bone (H&E staining 100x magnification). Control group (A); showing normal periodontium, periodontitis group (B); showing intense inflammatory cell infiltrate, dilated blood vessels, and osteoclasts in their Howship's lacunae with multiple resorption foci, periodontitis group treated with morus nigra (C); showing moderate inflammatory cell infiltrate in periodontal ligament and osteoclasts in their Howship's lacunae with multiple resorption foci (AB: alveolar bone, T: tooth, PL: periodontal ligament).*

control group and were significantly lower in the MN+PER group compared with the PER group (p =0.035, p= 0.041). In contrast, there were no significant differences in the serum levels of MMP-8 and MMP-13 between all groups (p=0.067, p=0.082) (Table 2). According to these results, while M. nigra treatment significantly reduced MMP 8 and MMP-13 levels in gingival tissue (p=0.035, p=0.041), no significant differences were detected between the C and MN+PER groups (p = 0.075).

Histopathological, histometric and immunohistochemical results

In the histolopathological evaluation, normal histological structures, connective tissue, and fiber organization were observed in sections of the control group (Figure 2). In the sections of the PER group, resorption of alveolar bone, degradation of periodontal ligaments, disorganization of

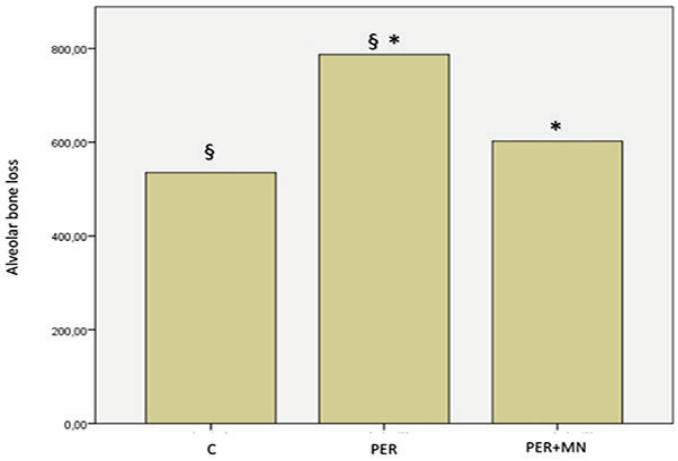

Figure 3. *Graphical representation of the mean alveolar bone loss in the study groups (C: control group, PER: experimental periodontitis, MN+PER: experimental periodontitis group treated with morus nigra).*

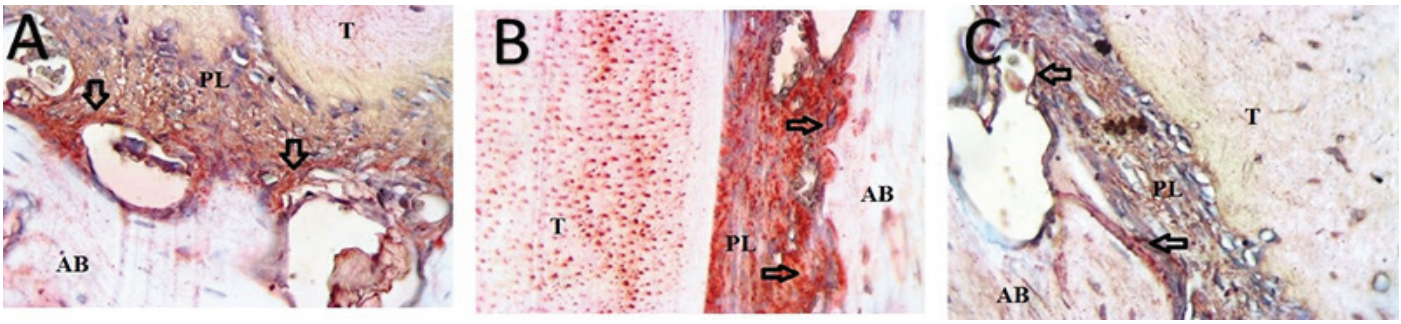

Figure 4. *RANKL expressions in the study groups. (A) control group at 200x magnification, (B) periodontitis group at 200x magnification, (C) peridontitis group treated with morus nigra at 200x magnification (AB: alveolar bone, T: tooth, PL: periodontal ligament).*

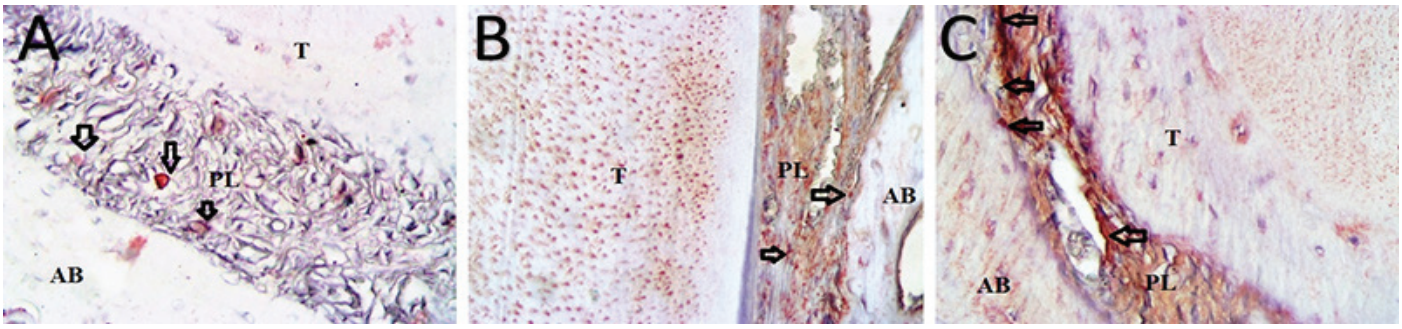

Figure 5. *OPG expressions in the study groups. (A) control group at 200x magnification, (B) periodontitis group at 200x magnification, (C) peridontitis group treated with morus nigra at 200x magnification (AB: alveolar bone, T: tooth, PL: periodontal ligament).*

These results show that the levels of RANKL were higher in PER group when compared to the control group (Figure 4) (Table 3). These findings also present that the administration of M. nigra markedly decreased the level of anti-RANKL–positive cells in comparison with the PER group (p =0.037) (Figure 4). In addition, the immunoreactivity level of OPG decreased in the PER group when compared with the MN+PER group (Figure 5) (Table 3). Also significant differences were observed between the PER and MN+PER groups (p=0.021). However, the immunoreactivity levels of OPG were similar in the C and MN+PER groups (Figure 5) (p = 0.085).

Discussion

The current study establishes the therapeutic effects of M.nigra on alveolar bone loss in rats with experimental periodontitis. Our findings show that MMP-8 and MMP-13 levels were significantly lower in the MN+PER group (p=0.035, p=0.041). Further, in the MN+PER group, alveolar bone resorption decreases and the levels of RANKL were higher in PER group. Furthermore, the immunoreactivity level of OPG decreased in the PER group.

M. Nigras importance has been shown in worldwide for over 40 years by experimental studies (12). M. nigra has many characteristics that include analgesic, diuretic, antitussive, sedative, anxiolytic, and hypotensive properties. Furthermore, it has been used in the treatment of various disorders (pharyngitis, toothache, snake bites), including inflammatory diseases (10). Inflammation is a complex of defensive reactions to remove the irritating stimuli from the organism with various pathophysiological stimuli to repair tissue. Periodontitis is also a chronic inflammatory disease connected with the destruction of tissue (19) . In the current research, we revealed the therapeutic effects of M. nigra extract on alveolar bone loss, RANKL, OPG expression, and MMP-8 and MMP-13 levels in both tissue and serum in a rat periodontitis model. The present study is the first to demonstrate the effects of M. nigra on these variables in an experimental periodontitis model.

Periodontitis is an inflammatory disorder characterized with degradation of extra- cellular matrix (ECM), connective and bone tissues. MMP-8 and MMP-13 including collagenases are able to degradate all components of ECM (24). MMP-8, the main interstitial collagenase, destroys the ECM in periodontitis. We can also accept that MMP-8 is the main form of collagenase in chronic periodontal diseases (20). In the current research, the highest tissue MMP-8 levels were detected in the PER group. There was a significant difference between the PER and MN+PER groups. However, serum MMP-8 levels were similar in all groups. These results may explain how periodontal diseases commonly cause local inflammation. Morus nigra, includes germanicol, betulinic acid and β sitosterol (10). The anti-inflammatory properties of betulinic acid were demonstrated with the experimental models in mice (21). β Sitosterol was found to possess potent anti-inflammatory activity, similar to that of hydrocortisone when administered intraperitoneally (22). For these properties, findings of this study suggest that M. nigra extract may reduce MMP-8 expression by its anti-inflammatory properties. The anti-inflammatory properties of M. nigra extract have also been shown in the literature (10, 23). In addition, most studies have shown that the levels of MMPs are higher with periodontitis compared with gingivitis or the levels observed in healthy people (24, 25). Similar to our results, previous studies have suggested that MMP-8 levels are higher in periodontitis sites than in healthy control sites (25).

MMP-13 has significant role in the pathological processes of periodontitis, rheumatoid arthritis, and osteoarthritis (26). The level of collagenase is increased with different disorders characterized by the destruction of bone and non-mineralized connective tissues, such as with periodontitis (26). The current study investigated the therapeutic effects of M. nigra in experimental periodontitis. Our findings suggest that M. nigra can significantly reduce only tissue MMP-13 levels and not serum levels in the MN+PER group in experimental periodontitis. Serum findings were not different between groups, it may be related that experimental periodontitis was only in the lower molar region. There was no severe inflammatory response to affect the level of inflammatory mediators in the serum. Furthermore, we observed that these results may be attributed to the inhibitory effects of M. nigra on MMP-13 expression. Previous studies have suggested that pro-inflammatory cytokines are found in increased levels in periodontitis sites and can stimulate MMP-13 expression (6, 27). Zelova et al. (19) reported that compounds of M. Nigra (prenylated flavonoids, kuwanon, morusinol) inhibite the dominant role of TNF-α and IL-1β in the pathogenesis of inflammation. However, we found no studies which have investigated the effect of M. nigra on MMP-13 levels in ligature-induced rat periodontitis. Previous studies have also observed that alveolar bone resorption is affected by the level of MMP 13 expression (6, 26). Hence, the therapeutic effects of M. nigra on alveolar bone may partially contribute to the decrease in MMP-13 levels (28).

OPG and RANKL can be found in different tissues and fluids such as serum, saliva, gingival crevicular fluid (GCF), and gingival tissue. These findings can aid in identifying the periodontal disease severity (29). Garlet et al. (30) reported that the levels of both RANKL and OPG expressions were increased in gingival tissues (aggressive priodontitis [AP] and chronic periodontitis [CP] tissues) compared to healthy tissues. In the present research, we also examined RANKL and OPG levels to assess periodontal bone level in ligature induced periodontitis. Bone formation and resorption are related to RANK-RANKL and OPG. OPG binds to RANKL and inhibits the RANK-RANKL connection and osteoclastic bone resorption (8). The ratio RANKL/OPG levels plays an important role bone resorption in periodontitis (1). This ratio is increased at the sites of active periodontal disease and is also related to the disease severity (29).

In the current study, we detected a significant effect of M. nigra on RANKL and OPG levels. The RANKL level was lower and the OPG level was higher in the MN+PER group compared to the PER group. RANKL is a member of the TNF ligand super-family which is responsible for osteoclast activation and increased of bone loss (4). For these reasons, we observed that treatment of M. Nigra can change these events by regulating RANKL expression via decreasing the release of pro-inflammatuar molecules with support the bone remodeling. This finding may be related to the anti-inflammatory effect of M. nigra. These results suggest that M. nigra also has antiresorptive effects on alveolar bone. Many studies have reported that the GCF levels of RANKL are increased patients with periodontal diseases. In addition, additional host response modulation therapies could be benefical in decreasing the RANKL/OPG ratio for periodontal disease treatment (4). Most studies have reported that the ligature-induced experimental periodontitis model increases RANKL activity (1, 29). The limitations of this study may be the only administration of M. nigra systemically in a single dose. The number of groups could be increased and the effect of the M. nigra on periodontitis at different doses could be evaluated.

Conclusion

This study demonstrates that M. nigra treatment significantly decrease MMP-8 and MMP-13 levels in periodontal tissue through its anti-inflammatory properties. Our results also shows that M. nigra inhibits alveolar bone resorption by suppressing the expression of RANKL and OPG. However, the therapeutic effects of the M. nigra should be explored in further studies.

Türkçe Öz: *Morus nigra'nın deneysel olarak oluşturulmuş periodontiste alveolar kemik kaybı üzerine etkisi. Amaç: Bu çalışmanın amacı, Morus nigra'nın ratlarda deneysel olarak oluşturulmuş periodontitis üzerindeki anti-enflamatuar etkisini değerlendirmektir. Gereç ve Yöntem: Yirmi dört Wistar-albino rat; kontrol (K, n = 8), deneysel periodontitis (PER, n = 8), deneysel periodontitis ve Morus nigra (MN + PER, n = 8) (21 gün boyunca günde 50 mg / kg) olmak üzere üç gruba ayrıldı. 21 gün sonra, ratlar sakrifiye edildi ve alveoler kemikleri histopatolojik olarak değerlendirildi ve alveolar kemik kaybı seviyesini belirlemek için histometrik analizler kullanıldı. RANKL ve OPG düzeyi immünohistokimyasal olarak tespit edildi. Ayrıca Serum ve doku MMP-8 ve MMP-13 seviyeleri de analiz edildi. Bulgular: Morus nigra tedavisinin doku MMP-8 ve MMP-13 seviyesini azalttığı ve MMP-8 ve MMP-13 doku seviyelerinde PER ve MN + PER grupları arasında anlamlı fark olduğu tespit edilmiştir (p = 0.035, p = 0.041). Buna karşın, tüm gruplar arasında MMP-8 ve MMP-13 serum seviyeleri konusunda anlamlı bir fark yoktur (p = 0.067, p = 0.082). Histometrik değerlendirmede, alveoler kemik kaybı PER grubunda K ve MN gruplarına göre daha yüksektir (p = 0.035). RANKL aktivitesi PER grubuna kıyasla MN + PER grubunda anlamlı olarak daha düşük bulunmuştur (p = 0.037) ve OPG aktivitesi ise, MN + PER grubunda anlamlı olarak daha yüksek tespit edilmiştir (p = 0.021). Sonuç: Bu çalışma Morus nigra'nın sistemik uygulamasının bölgesel alveoler kemik rezorpsiyonunu anlamlı şekilde inhibe ettiğini ve deneysel-periodontitis oluşturulan rat modellerinde periodontal iyileşmeye katkıda bulunduğunu ortaya koymaktadır. Anahtar Kelimeler: Deneysel periodontitis; sitokin; MMP-8; MMP-13; morus nigra*

Ethics Committee Approval: The study protocol was approved by the Animal Experimental Ethics Committee of the Fırat University, Elazığ, Turkey (No: 2012-013).

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Author contributions: TTY and CAK designed the study. GO, TK, NK and EE participated in generating the data for the study. SD, ND, NK and EE participated in gathering the data for the study. GO, TK and ND participated in the analysis of the data. TTY and AB wrote the majority of the original draft of the paper. TTY and SD participated in writing the paper. All authors approved the final version of this paper.

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Original research

Preservation of keratinized gingiva around dental implants using a diode laser when uncovering implants for second stage surgery

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Purpose

The aim of the present study was to assess if a 940-nm diode laser or a traditional scalpel approach is more effective in minimizing patient comfort and postoperative sequelae, preserving peri-implant keratinized mucosa, and in enhancing impression quality after uncovering dental implants.

Materials and methods

We designed a prospective, split mouth, single blinded, randomi¬zed controlled trial with patients who needed uncovering of dental implants. Our analysis included 388 implants in 73 patients. Split mouth technique was used to compare two approaches for uncovering implants: laser study group vs. scalpel control group. Patients were evaluated for intra- and post-operative pain and bleeding. At 1, 2, and 3 weeks post procedure patients in both groups were rechecked for postoperative sequelae, keratinized mucosal thickness quality and quantity, and accuracy of the implant emergence profile.

Results

The laser study group showed that there was a reduction in postoperative pain and bleeding that was statistically significant in comparison to the control group. There were also statistically significant differences in gingival color and presence or absence of soft tissue edema and in the gingival was emergency profile between the laser and scalpel sides at 1, 2, and 3 weeks' post-procedure. Statistically significant differences ($p< 0.001$) were observed between the two groups in the criteria of ideal or satisfactory soft tissue projection in the gingival emergency profile, which indicates that impressions can be taken immediately or within 1 week after laser surgery.

Conclusion

Uncovering dental implants using a diode laser operating at the wavelength of 940 ±10 nm, and a power output of 0.4-10 W is recommended for preparing an accurate implant emergence profile. Laser treatment can also effectively preserve keratinized mucosa around implants in comparison to the conventional scalpel technique.

Keywords: *Dental implants; diode laser; emergence profile; scalpel approach, keratinized gingiva*

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Introduction

Appropriate dental implant planning and treatment should consider factors for long-term success, including appropriate implant dimensions (vertical, sagittal and transverse), bone quality, healthy soft tissues around the implant shoulder, and non-mobile keratinized gingiva (1). In consideration of these factors, modern approaches in the field of dentistry include performing more conservative surgical procedures, reducing the length of rehabilitation time, and promoting quicker osseointegration. Furthermore, scientific interest in the field of postoperative care has

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intensified in terms of tissues healing more rapidly and patient compliance improving (2).

Different techniques can be used for second stage implant surgery, such as uncovering the implant with either a scalpel, tissue punch, or laser (3). Identification of a patient's periodontal biotype is fundamental to optimal planning of therapeutic management in implantology (4). The sufficiency of the attached gingiva (AG) around fixed restorations is the key factor in choosing a technique for uncovering implants (5). When the attached gingiva around implants is sufficiently thick, the implants can be uncovered without subepithelial connective tissue grafts (SCTG) or free gingival grafts (FGG) (6-8).

The laser technique has comparable results to the traditional scalpel technique, but with less time, micro-invasiveness, and improved patient compliance (3, 9). Furthermore, there is a reduced emotional effect with laser treatment, since laser-assisted surgery is not considered a particularly invasive surgery (9). Using lasers also decreases intra-operative bleeding, therefore the use of anesthetic and vasoconstrictor agents can be reduced (2). Additionally, secondary infections can be avoided postoperatively as lasers have antiseptic properties. The bio-modulating property of lasers further improves the healing process (10-13). Many clinical trials have confirmed that diode lasers reduce pain during surgery and ensure better wound healing, less swelling, edema and scarring, and better coagulation (14-16).

Soft tissue vaporization with the use of diode lasers has been widely described in the literature (17). Lasers play a significant role in soft tissue vaporization and in the decontamination of infected implant surfaces (18). However, there is a risk that the bone can be overheated bone on application of these devices during the surgery (19). The risk of thermal injuries to the bone are relatively high due to the direct bone-implant contact. In the implant neck area there is a particular composition of soft tissue where blood flow is reduced, allowing for a higher risk of thermal injury to the bone via the implant. Eriksson et al. (20,21) found in several studies, permanent changes were made to the bone structure by increasing the temperature of bone tissue by 10°C for 60 s. It can only be assumed therefore, that the ideal and safe tissue temperature gradient (ΔTa) should be below 10°C.

Here we performed a clinical trial to test our hypothesis that patient comfort would improve with 940 nm diode laser compared to the traditional scalpel approach for uncovering dental implants. The aim of this study was to perform a prospective, split mouth clinical analysis to determine if a 940-nm diode laser is more effective at preserving the keratinized mucosa surrounding an implant compared to the traditional scalpel approach. The null hypothesis tested in this research is that no difference can be observed between the two protocols.

Materials and Methods

Study design

In this prospective, split mouth, single blinded, randomi¬zed controlled trial, the study sample was composed of patients presenting to the Iraqi specialized dental implant and cosmetics center in Baghdad, Iraq, from April 2014 to May 2015. The required approval for the study was obtained from the Laser Institute Committee, and we followed the Declaration of Helsinki guidelines. A total of 73 patients, 47 (64.38 %) men and 25 (34.24 %) women, aged 18 - 68 years (mean 31.62 ± 14.76 years) at the time of operation were enrolled in the study and received a total of 388 implants (taper, external hexagon connection; BioHorizons, Laser-Lok, USA). Patients underwent at least more than one dental implant in the left and right mandibular or maxillary regions and sufficient dimensions of keratinized mucosa around the implants, and 56 (76,71%) patients had a history of partial edentulism, 9 (12.32%) had complete edentulism in the upper or lower jaws, and 6 (8.21%) had either a full upper or a lower edentulous jaw. The procedure was explained, and informed consent was obtained from all patients. As this study used a "split-mouth method", one site was assigned for uncovering dental implant using a 940 nm diode laser (study group) and the contralateral site was assigned for uncovering the dental implant using the ordinary surgical scalpel method (control group). The study and control sites were assigned in the same patient to avoid bias from individual variations. Patients were sequentially numbered from 1 to 73. The treatment modality (laser exposure vs scalpel exposure) and the operating site (right vs. left) were determined by tossing a coin, where 'face' was the test site and 'back' was the control site. The treatment modality (laser exposure vs scalpel exposure) sequence was randomly assigned. The laser exposure study group consisted of 194 dental implants; the scalpel exposure control group consisted of 194 dental implants.

Patients were examined and evaluated clinically and radiographically. All patients had osseointegrated dental implants. Preoperative medications were not prescribed in any of the cases. Exclusion criteria were: severe systematic diseases, uncompensated diabetes, or uncontrolled periodontal disease, smoking more than 10 cigarettes daily, failed dental implant, inflammation or peri-implant inflammation, failed examination appointments, radiolucent line around the dental implant, exposed dental implant, and parafunctional habits that could impact osseointegration. All patients had good oral hygiene and sufficient bone volume to allow dental implant insertion. Inclusion criteria were: delayed loading dental implant, delayed insertion dental implant, two stage protocol of dental implant, and healthy keratinized gingival tissues. At the examination appointments, all patients had panoramic radiographs taken. Demographic and clinical data, for example, patient age, gender, clinical presentation, past surgical history, and medical history were noted. This study includes the following parameters: 1) gingival health, 2) mucosal thickness determined using a periodontal probe with a rubber stopper, 3) oral hygiene, 4) bone quality, and 5) the duration of surgical operation of each group. Regarding bone quality, all implant sites were classified as bone type II (implant bone site with a thick layer of compact bone surrounding a core of dense trabecular bone) or bone type III (implant bone site with a thin layer of compact bone surrounding a core of dense trabecular bone) according to Lekholm and Zarb (22).

Surgical procedures

Patients were treated with dental implants that were at least 12 mm in length. All implants were placed by the same

operator (RA) according to the manufacturer's protocol using labially or buccally based flaps. All of the surgeries were carried out without intercurrences, and post-operative follow up was favorable for all patients. All 73 patients were radiographed immediately after surgery and at 3 months and 6 months' follow-up. After osseointegration, the second stage surgery was performed for each patient and the environmental preparations in both groups were the same.

In the scalpel group (control), 194 dental implants were exposed using the scalpel to create a circular incision smaller than the size of the dental implant and using topical application of 20% benzocaine; if pain was greater than 45 mm according to VAS, anesthesia should be administered. After a 3-week interval the contralateral implant was uncovered using the laser (study group). The 3-week interval was scheduled to evade postoperative outcome parameters of one procedure influencing the other.

In the experimental (laser) group, 194 implants were uncovered by using the 940 nm laser diode after tip initiation to make a small opening. This opening was widened until the cover was completely exposed. Subsequently, the tissue over the implant was ablated until the opening allowed for the removal of the screw. A diode laser (commercial trade mark epic Biolase, Irvine, CA, USA) was used, emitting a wavelength of 940 ±10 nm, and a power output of 0.4-10 W in a pulse duration of 100 microseconds, and a pulse interval of 200 microseconds, with a duty cycle of 33%. The optic fiber was 300 µm with a length of 9 mm, average power 0.9 W, and a continuous emission CP1 (Comfort Pulse) mode. The aiming beam was a visible laser diode, max 1 mW, 625 - 670 nm, continuous or intermittent. Laser opening of the implant was conducted without any kind of anesthesia. If pain was greater than 45 mm according to VAS, anesthesia was administered. The cover screw was unscrewed, and the gingival healing abutment was placed inside the implant according to the size and shape of the implant used, in both groups.

Clinical parameters

We used a single qualified experienced blinded operator to compare the parameters of both techniques. He was not involved in the surgeries themselves. Each assessment was repeated three times. The mucosal thickness was determined by the depth of penetration of the probe from the external surface of the mucosa to the point where bony resistance could be felt. The stopper was then adjusted, and the depth of penetration/thickness was measured in millimeters on a geometric scale/ruler. The measurements were done at three points on the crest of the edentulous ridge, namely mesial, mid and distal mucosa in the buccal site. All patients in both groups were assessed at baseline (before uncovering the implant) and then postoperatively at 1, 2, and 3 weeks. The duration of surgery was counted in minutes. The need for local anesthesia was determined and if the pain was greater than 54 mm (mild pain), anesthesia was administered. Intra- and post-operative bleeding were determined by the World Health Organization (WHO) bleeding scale (23) which defined it as: grade 0: no bleeding, grade 1: petechial bleeding, grade 2: mild blood loss (clinically significant), grade 3: gross blood loss, requires transfusion, grade 4: debilitating blood loss, retinal, or cerebral associated with fatality.

In the follow up period, gingival bleeding was postoperatively evaluated at 1, 2, and 3 weeks by visual bleeding from the emergency profile noticed by opening of the gingival former defined as bleeding during probing (using periodontal probe), according to the following modified criteria (modified by the author):grade 0:no bleeding grade 1:one bleeding point, grade 2 :several isolated bleeding points or a small blood area, grade 3:cavity filled with blood soon after probing, grade 4: profuse bleeding when probing, blood spread outside the cavity.

The pain level was evaluated using a 170-mm Heft-Parker visual analog scale (VAS; Figure 1). Each pa¬tient had the VAS explained to them. The VAS was divided into 4 categories: 1) no pain corresponded to 0 mm; 2) mild pain was defined as greater than 0 mm and less than or equal to 54 mm and included the descriptors of faint, weak, and mild pain; 3) moderate pain was defined as greater than 54 mm and less than 114 mm; and 4) severe pain was defined as equal to or greater than 114 mm and in¬cluded the descriptors of strong, intense, and maximum.

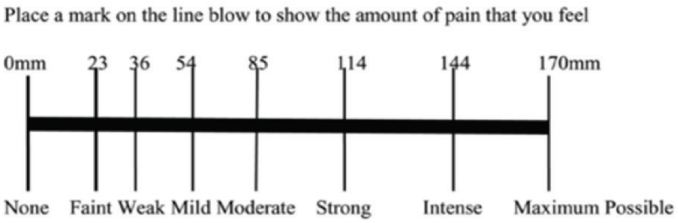

Figure 1. *Heft-Parker visual analog scale (VAS) used for the pain assessment. The millimeter demarcations are not shown on the patient's VAS.*

In the post-operative follow-up, the emergency profile was evaluated by gingival color and the presence or absence of edematous soft tissue and the need to correct this. Gingival color was evaluated using the following modified gingival index (modified by the author): grade 0: normal gingiva color, grade 1: slight change in color, slight edema, grade 2: moderate redness and glazing, grade 3: marked redness. The time for impression taking was determined if there was an ideal or satisfactory soft tissue projection of the gingival emergency profile. If inadequate projection of the soft tissue was observed, impression were delayed until there was evidence of satisfactory projections.

Statistical analysis

Statistical analysis was performed using SPSS13.0 (Statistical Package for Social Sciences, Chicago, IL, USA). The paired t test was used to analyze variables predicting changes between the both groups. The categorical variables were compared with either the Chi-square or Fisher's exact tests. The confidence interval was set to 95% and $p<0.05$ was considered statistically significant.

Results

In this study, the mean follow-up period was three weeks after uncovering the dental implant. Neither serious complications nor complaints were reported after surgery. None of the patients showed any adverse reactions to laser

treatment. All 73 patients completed the follow-up visits, and therefore a total of 388 implants were analyzed in this study.

There were no significant differences in soft tissue thickness on the mesial, mid and distal sites upon uncovering the implant in the scalpel and laser groups (Table 1) at the time of uncovering the dental implant. However, there was statistically significant difference at 1, 2, and 3 weeks after uncovering the dental implant, as shown in Tables 2, 3 and 4.

The average duration of surgery was 1.14 ± 1.30 minutes in the scalpel group versus 2.35 ± 0.97 minutes in the laser group ($p<0.05$). (Table 1). There was significantly less postoperative pain, which led to a decreased need for local anesthesia, in the laser group (2 cases only) compared to the scalpel group (58 cases). In the scalpel group, the mean pain value during uncovering the dental implant was 78.42 ± 11.62 mm (Table 1). In the laser group, the mean pain value was 31.66 ± 9.74. No severe pain was reported in either group during

Table 1. *The operative assessment in both groups (*p< 0.05; †p< 0.01; ‡p< 0.001; VAS: visual analogue scale, SD: standard deviation).*

Criteria	Scalpel group	Laser group	p value
Mucosal thicknesses at the time of uncovering the dental implant (mm)			
Mesial mucosa	1.64±0.47	1.72±0.39	0.81
Mid mucosa	1.23±0.56	1.20±.61	0.73
Distal mucosa	1.57±0.73	1.66±0.69	0.91
Mean of surgical duration procedure in (min)	1.14±1.30	2.35±0.97	0.012*
Cases Need for infiltration local anesthesia	58(29.82%)	2 (2.73 %)	0.001†
Intra and post-operative bleeding			
Grade 0	00(00)	181(93.2)	0.000‡
Grade1	28(14,43)	13(6,70)	0.017*
Grade 2	166(85.56)	00(000)	0.000‡
Pain (VAS)			
No pain	00(00)	19 (26.02)	0.000‡
Weak	15(20.54)	52 (71.23)	0.001†
Moderate	58(79.45)	2 (2.73)	0.000‡
Sever	00(00)	00 (00)	NA
Mean VAS values (mm)	78.42±11.62	31.66±9.74	0.000‡

Table 2. *Postoperative assessment at 1 week in both groups (*p< 0.05; †p< 0.01; ‡p0.001; SD:standard deviation).*

Variable	Index	Scalpel group	Laser group	p
Mucosal thicknesses (mm)	Mesial mucosa	1.93±0.62	1.77±0.36	0.000‡
	Mid mucosa	1.59±0.77	1.30±.68	0.001†
	Distal mucosa	1.84 ±0.90	1.69±0.53	0.007†
Bleeding	Visual bleeding	19(9.79)	00(00)	0.000‡
	Bleeding on probing			
	Grade 0	00(00)	140(72.16)	0.000‡
	Grade 1	00(00)	31(15.97)	0.000‡
	Grade 2	19(9.79)	17(8.76)	0.000‡
	Grade 3	147(75.77)	06(3.09)	0.000‡
	Grade 4	28(14.43)	00(00)	0.000‡
Gingival color (modified Gingival index)	Grade 0	00(00)	113 (58.24)	0.000‡
	Grade 1	30(15.46)	63(32.47)	0.004†
	Grade 2	99(51.03)	18(9.27)	0.000‡
	Grade 3	65 (33.5)	00(00)	0.000‡
Soft tissue edema in the gingival emergency profile	Present	147(75.77)	38(19.58)	0.000‡
	Absence	47(24.22)	156(80.41)	
Secondary correction	Need	NA	NA	NA
	No need	NA	NA	
Time for impression taking	Ideal or satisfactory soft tissue projection	00(00)	166(85.56)	0.000‡
	Inadequate projection of the soft tissue	194 (100)	28 (14.43)	0.000‡

the procedure. Neither group experienced any postoperative pain at 1, 2, and 3 weeks.

Intra- and post-operative bleeding was significantly different in the laser group compared to the scalpel group. No patient in the laser group experienced Grade 2 WHO bleeding; no patient in either group experienced Grade 3 or 4 WHO bleeding (Table 1). We also found a significant difference in postoperative gingival bleeding (p<0.001) at 1, 2, and 3 weeks, as shown in Tables 2, 3 and 4.

We further observed significant postoperative differences

Table 3. *Postoperative assessment at 2 weeks in both groups (*p< 0.05; †p< 0.01; ‡p< 0.001; SD, standard deviation).*

Criteria	Index	Scalpel group	Laser group	p
Mucosal thicknesses (mm)	Mesial mucosa	1.76±0.53	1.72±0.39	0.000‡
	Mid mucosa	1.48±0.49	1.20±.61	0.000‡
	Distal mucosa	1.69±0.77	1.66±0.69	0.000‡
Bleeding	Visual bleeding	00(00)	00(00)	NA
	Bleeding on probing			
	Grade 0	43(22.16)	186 (95.87)	0.000‡
	Grade 1	121 (62.37)	08(4.12)	0.000‡
	Grade 2	14(7.21)	00(00)	0.000‡
	Grade 3	10(0.51)	00(00)	0.000‡
	Grade 4	6(3.09)	00(00)	0.000‡
Gingival color Modified Gingival index	Grade 0	86(44.32)	177(91.23)	0.001†
	Grade 1	29(14.94)	14(7.21)	0.001†
	Grade 2	48(24.74)	3(1.54)	0.000‡
	Grade 3	31(15.97)	00 (00)	0.000‡
Soft tissue edema in the gingival emergency profile	Present	104(53.6)	6(3.09)	0.000‡
	Absence	90(46.39)	188(96.9)	
Secondary correction	Need	NA	NA	NA
	No need	NA	NA	
Time for impression taking	Ideal or satisfactory soft tissue projection	112(57.73)	187(96.39)	0.000‡
	Inadequate projection of the soft tissue	82 (42.26)	7(3.60)	0.000‡

Table 4. *Postoperative assessment at 3 weeks in both groups (*p< 0.05; †p< 0.01; ‡p< 0.001; SD: standard deviation).*

Criteria	Index	Scalpel group	Laser group	p
Mucosal thicknesses (mm)	Mesial mucosa	1.55±0.67	1.70±0.41	0.000‡
	Mid mucosa	1.19±0.62	1.18±.61	0.001†
	Distal mucosa	1.42±0.90	1.63±0.55	0.007†
Bleeding	Visual bleeding	00(00)	00(00)	NA
	Bleeding on probing			
	Grade 0	118(60.82)	194 (100)	0.000‡
	Grade 1	59(30.41)	00(00)	0.000‡
	Grade 2	9(4.63)	00(00)	0.000‡
	Grade 3	6(3.09)	00(00)	0.000‡
	Grade 4	02(1.03)	00(00)	0.000‡
Gingival color Modified Gingival index	Grade 0	115(59.27)	186(95.87)	0.001†
	Grade 1	19(9.79)	8(4.12)	0.001†
	Grade 2	21(10.82)	00(00)	0.000‡
	Grade 3	7(3.60)	00 (00)	0.000‡
Soft tissue edema in the gingival emergency profile	Present	23(11.85)	00(00)	0.000‡
	Absence	171(88.14)	194(100)	
Secondary correction	Need	23(11.85)	00(00)	0.000‡
	No need	171(88.14)	194(100)	
Time for impression taking	Ideal or satisfactory soft tissue projection	148(76.28)	194(100)	0.005†
	Inadequate projection of the soft tissue	46(23.81)	00(00)	0.000‡

at 1, 2, and 3 weeks in gingival color and the presence or absence of soft tissue edema in the gingival emergency profile between laser and control groups. There was marked edema and secondary correction procedure was required (at the postoperative week 3 only) in the control group. A highly statistically significant difference ($p<0.001$) was observed between the two groups for the criteria of the ideal or satisfactory soft tissue projection in the gingival emergency profile which give the great possibility of the impressions taken immediately or in 1st week after the laser surgery. (Tables 2, 3 and 4).

Discussion

There is a very limited amount of literature on the effect of diode laser on preservation of keratinized mucosa around dental implants. The purpose of this clinical trial was to determine if the use of a 940 nm diode laser improves keratinized mucosal thickness quality and patient comfort compared to routine surgical method by the scalpel.

Our hypothesis stated that patient comfort would improve with 940 nm diode laser. The aims of the study were to compare intra and postoperative pain, bleeding, and keratinized mucosal thickness quality and quantity between the study and control groups. The results of this study confirmed our hypothesis.

We previously published data on the effectiveness of a 940 nm Diode Laser during second-stage dental implant surgery (24). The current study involved a larger group of patients with a different parameter of evaluation in the same center. Our data are very interesting in that this null hypothesis was proven when we looked at the data per site of the dental implant and side of the study group. The study group showed that there were statistically significant decreases in the incidence and severity of intra- and post-operative pain and bleeding, improved quality and quantity of keratinized mucosa, and reduced duration of prosthetic treatment after uncovering the dental implant.

Theoretically, the increase in thermal energy caused by the laser beam can spread from the point of impact to the surrounding areas and this may cause postoperative edema and pain (2,3). However, other studies indicated that the diode laser activity on the implant surface neither damages structure nor excessively increases temperature (25)

Matys and Dominiak (26) confirmed that laser ablation permits removal of the soft tissue one layer at a time and allows the emergence profile to be modeled similarly, as in the case of healing screws. These observations are in line with our findings, such that the diode laser resulted in good keratinized mucosal quality and quantity and reduced tissue contraction which allowed for precise modeling of the gum line, easy correction of gingival hypertrophy, and accurate uncovering of the implant by 940 nm diode Laser ablation. Furthermore, the laser provided uniform ablation, prevented scarring, and caused minimal post-surgical complication, all of which ensured faster wound healing (27).

It should be noted that Kissa et al., (28) reported a controversy regarding the association between an adequate amount of keratinized tissue and the peri-implant health following laser treatment. However, our current study using 940 nm diode laser ablation increase the amount of keratinized tissue around the dental implants and reduced gingival inflammation.

In accordance with previous studies (25,26,29), the present study also reported an insignificant difference with regards to duration of surgical procedure postoperative pain, healing time, and implant success with the laser diode compared to the scalpel. However, the laser diode resulted in good pain tolerance with only a topical anesthetic during the surgery, as shown by the VAS tests and decreased fear of pain, the latter of which is a primary reason some patients avoid visits to dental surgery facilities.

All the above reduce the duration of prosthetic treatment after the implantation depending on that the fact that the success of both dental implant and prosthetic treatment is depends on establishing a stable soft-tissue barrier to protect the underlying osseous structures and to guarantee a peri-implant gingival aesthetics over time (30).

The present study demonstrates the 940 nm diode laser is advantageous over traditional scalpel use during prosthetic phase of implant surgeries. The quality of the impression was accurate enough in all of cases to prepare a prosthetic reconstruction much earlier than the control group, thus reducing treatment time.

The main limitations of this study, aside that double blinding was not possible, are the limited sample, which may result in restricted power, and the fact that the postoperative clinical assessment was single-blinded. To obtain more meaningful results, future randomized studies should use different detailed radiographical and histological evaluation methods to add more valuable findings and analyze all variables that can influence uncovering dental implants using 940 nm diode laser and conventional scalpel technique.

Conclusion

This study suggests that a 940 nm diode laser is a desirable surgical option to reduce complications (pain and bleeding) when uncovering dental implants. This technique can be utilized for preparing an accurate implant emergence profile, as the laser provides an effective approach for preserving the keratinized mucosa around the implants.

Türkçe Öz: *İkinci Aşama Cerrahisinde Diode laser kullanılarak İmplant çevresindeki Keratinize Yumuşak Dokunun Korunması. Amaç: Bu çalışmanın amacı, hasta şikayetinin ve postoperatif olumsuzlukların azaltılmasında, peri-implant keratinize mukozanın korunmasında ve implantlar açıldıktan sonra ölçü kalitesinin artırılmasında, 940nm diode laser ve geleneksel bistüri kullanımının karşılaştırmalı olarak incelenmesidir. Gereç ve Yöntem: Bu çalışmada dental implantlarının üzeri açılması gereken hastalarda ileriye dönük, split-mouth, tek kör, randomize kontrollü klinik araştırma planlanmıştır. Araştırmamıza 73 hastada 388 implant dahil edilmiştir. Split-mouth çalışma yöntemi ağzın bir tarafında laser ile diğer tarafında geleneksel bistüri kullanılarak implantların üzeri açılmıştır. Hastalar cerrahi işlem sırasında ve sonrasında ağrı ve kanama açısından değerlendirilmiştir.Cerrahi işlemden 1,2 ve 3 hafta sora hastalar post-operatif şikayetler, keratinize mukoza kalınlığı kalitesi ve miktarı ve implant çıkış profili kesinliği konularında değerlendirilmiştir. Bulgular: Laser grubunda kontrol grubuna göre istatistiksel olarak anlamlı düzeyde daha fazla kanama ve ağrı az görülmüştür. Ayrıca gruplar arasında 1., 2. Ve 3. Haftalarda anlamlı düzeyde mukoza rengi, yumuşak doku ödemi olup olmaması ve çıkış profilinde fark vardı. İmplant çıkış profilinde iki grup arasında istatistiksel olarak anlamlı ($p<0.001$) fark vardı. Bu da laser ile işlem yapıldıktan 1 hafta sonra ölçü alınabileceğini gösteriyordu. Sonuç: Dental implantların üzerinin 940±10 nm*

dalgaboyu ve 0.4-10W gücünde çalışan diode laser ile açılması implant çıkış profili kesinliği için tavsiye edilebilir. Laser tedavisi ayrıca geleneksel bistüri kullanımına göre keratinize yumuşak dokuyu korumakta etkilidir. Anahtar Kelimeler: Dental implantlar; Diode laser; Çıkış profili; Büstirü yaklaşımı; Keratinize dişeti

Ethics Committee Approval: The study protocol was approved by the Laser Institute Committee.

Informed Consent: The informed consents were provided by the participants.

Peer-review: Externally peer-reviewed.

Author contributions: RA designed the study, generated and gathered the data, wrote and approved the final version of the study.

Conflict of Interest: The author had no conflict of interest to declare.

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Original research

Postoperative morbidity in pediatric patients following dental treatment under general anesthesia

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Purpose
The aims of this study were to investigate post-operative complications in pediatric patients 24 and 72 hours after general anesthesia (GA) and to identify any associations between dental procedures and complications.

Materials and Methods
One hundred and thirty three healthy pediatric patients who had undergone dental treatment under GA (age range: two to nine years) were included in this study. The project was designed as a prospective, observational study supported by a questionnaire that collected data on children's post-operative complaints. Preoperative data were obtained from patients' files and included age, gender, medical condition and admission type (inpatient or outpatient) variables. The post-operative complaints were assessed either by phone contact or by face-to-face interviews using a questionnaire 24 and 72 hours after treatment.

Results
69.9% of children reported one or more complaints after 24 hours and 35.3% after 72 hours. Coughing and pain (27.1%), inability to eat (24.8%), psychological changes (24.1%) and a sore throat (21.1%) were the most common complaints during the first day. After 72 hours, the severity and rate of the complications decreased significantly ($p < 0.05$).

Conclusion
Post-operative complaints following dental treatment under GA tended to be of mild severity and were mainly limited to the first day after the procedure.

Keywords: *Children; dental care; general anesthesia; complication; postoperative*

Introduction

Dental caries, which are the most prevalent chronic disease among children worldwide (1), may have life-long effects on oral health status (2, 3). Childhood caries cause pain, eating and swallowing problems, and missed days of school (4). Children with a history of pulpal symptoms also run a greater risk of dental anxiety and behavioral problems (5).

Limited co-operation and anxiety make dental treatment of children challenging for clinicians. Behavior management techniques can help gain children's co-operation (6). While most children can be treated in conventional settings, in some cases, routine treatment protocols are insufficient (7-9). In these cases, dental treatment under general anesthesia (GA) must be considered as an option (10). Given the risk of complication and mortality associated with GA, most parents consider it to be a dramatic departure from the traditional, office-based treatment approach for children (11). While death following dental treatment under GA is relatively rare among healthy children, complications are a common problem

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(12). Studies investigating post-operative complications in children after receiving dental treatment under GA have reported rates ranging from practically negligible to more than 90% of patients (6, 13-15).

The aims of this study were to investigate post-operative complications in pediatric patients at 24 hours (Day 1) and 72 hours (Day 3) following GA and to identify any associations between dental procedures and complications. The null hypothesis tested in the present research is that no difference could be found between the first and third days post-operation regarding the frequency and severity of the symptoms.

Materials and Methods

Sample selection

Ethical approval was obtained from the Kırıkkale University Clinical Research Ethics Committee (2010/89). The research was conducted in full accordance with the World Medical Association Declaration of Helsinki. The participants were recruited among children referred to Kırıkkale University's Faculty of Dentistry's Pediatric Dentistry Clinic due to their uncooperative behavior during dental examinations or their young age. The children had been identified as candidates for dental treatment under GA. Children with complex medical problems, such as Downs' syndrome, heart disease, mental retardation and cerebral palsy, were excluded. Only patients who were classified as Physical Status I or II, according to the American Society of Anesthesiologists (ASA), were included in the study. Accordingly, over a one-year period, the family and caregivers of healthy children were invited one by one to participate in the study, until 150 children (age range 2-9) were enrolled. Written consent was obtained from the parents and guardians of all the participants. This was a prospective observational study supported by a pre-formulated questionnaire that collected data on the children's post-operative complaints.

Dental treatment under general anesthesia

Children were prevented from eating and drinking for a six-hour period prior to the administration of GA. No preoperative medication was given to any of the participants. The majority of children (78%) were administered intravenous propofol (2.0 mg/kg body weight); sevoflurane or a combination of sevoflurane and propofol was administered to the remaining children. The anesthesia was maintained with sevoflurane for all patients; rocuronium bromide (0.5 mg/kg body weight) was used as a muscle relaxant; atropine (0.2 mg/kg body weight) and neostigmine (0.05-0.07 mg/kg body weight) were used for recovery. As complications of nasal intubation are more common in young children (16), all patients were orally intubated, and a throat pack was used to prevent the aspiration of the secretions and dental materials. The cardiac functions were monitored with a 3-lead ECG. All patients received intravenous paracetamol (10 mg/kg body weight) intraoperatively as an analgesic. The anesthesia procedures were performed by one anesthesiologist, and dental treatments were completed under GA in a single session by the same pediatric dentist.

Data collection

All data were collected by a single investigator. Preoperative data were obtained from the patient record sheet that included information about patients' age, gender, medical condition and admission type (inpatient or outpatient). Dental data were reported as the mean number of treated teeth and the treatment type. Post-operative complications were assessed either by phone or by face-to-face interviews using a pre-formulated questionnaire after 24 hours (Day 1) and 72 hours (Day 3) (15).

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences software program (SPSS 15.0, SPSS Inc. Released 2007. SPSS for Windows, Version 15.0. Chicago, SPSS Inc., IL, USA). The normality of the dataset was checked with the Shapiro-Wilks test. As the data were not normally distributed, the non-parametric Mann-Whitney U test was used to identify any significant differences between two scale variables after 24 and 72 hours. The chi-square test was used to examine the differences between frequency and severity of complications following dental procedures with GA after 24 and 72 hours. Fisher's exact test was used in place of the chi-square test when the expected values were less than five in the cell distribution. The confidence interval was set to 95%, and p values less than 0.05 were considered statistically significant.

Results

Of the 150 patients initially enrolled in the present study, 17 patients were excluded because their parent or guardian could not be reached post-operatively or because they refused to complete the questionnaire. The demographic characteristics of the remaining 133 patients are presented in Table 1. The mean age was 4.3 (± 1.4) years. All patients were discharged after the procedure. The mean overall anesthesia time for GA procedures was 69.61 min (range: 10 to 165 min).

The treatment provided under GA is presented in Table 2. The procedures included filling (Dyract Extra, Dentsply, DeTrey Konstanz, Germany) (mean number of teeth treated with fillings per child: 8.1 ± 3.3); endodontic treatment (Metapex, Meta Biomed Co. Ltd, Cheongju, Korea) (mean number of teeth treated endodontically per child: 1.3 ± 1.4); extractions (mean number of teeth treated by extraction per child: 2.6 ± 2.4); and sealant application (Clinpro sealant,

Table 1. *Patient demographic variables and duration of anesthesia.*

Variable		N (%)
Gender	Male	83 (62)
	Female	50 (38)
Age	≤5	104 (78)
	>5	29 (22)
Anesthesia duration (min)	<70	72 (54)
	≥70	61 (46)

Table 2. *Treatment types provided under general anesthesia.*

Type of treatment *	Mean number (± SD)**	Range
Filling	8.1 ± 3.3	1-17
Pulpotomy	0.9±1.1	1-5
Pulpectomy	0.4± 0.9	1-4
Fissure sealant	0.2± 0.8	1-4
Extraction	2.6± 2.4	1-14

*All children received fluoride treatment.
**For those children receiving this type of treatment[3]

3M ESPE, Seefield/Oberbay, Germany) (mean number of teeth treated with sealant per child: 0.2 ± 0.8). In addition, all patients received fluoride varnish (Duraphat Varnish, Colgate-Palmolive, NSW, Australia), which was applied topically. Table 3 shows the frequency of complications on the first (24 hours) and the third (72 hours) days post-operation. While 69.9% of children had one or more complaint after 24 hours, the rate decreased to 35.3% after 72 hours. The most common complaints after 24 hours were coughing (27.1%), dental pain (27.1%), inability to eat (24.8%), psychological changes (24.1%) and a sore throat (21.1%), whereas the most common complaints after 72 hours were psychological changes (14.3%), coughing (16.5%) and inability to eat (9.0%). The severity and frequency of complications after 24 and 72 hours post-operation are presented in Table 4 and Table 5. Statistical analysis indicated a significant reduction in the severity of complaints from day 1 to day 3. In addition, the complaint of an inability to eat was found to be significantly related to the number of teeth extracted ($p < 0.05$). There was no significant relationship between post-operative dental pain and treatment type or number of teeth treated under GA. Also, no significant relationship was found between post-operative dental bleeding and the number of teeth extracted.

Table 3. *Frequencies of complications on Days 1 and 3 following pediatric dental procedures with general anesthesia.*

Post-operative complaints	Complaints after 1 day		Complaints after 3 days		p value
	n	(%)	n	(%)	
Dental pain	36	27 .1	7	5 .3	0 .016
Dental bleeding	22	16 .5	3	2 .3	0 .004
Sore throat	28	21 .1	9	6 .8	0 .000
Fever	21	15 .8	5	3 .8	0 .002
Vomiting	13	9 .8	4	3 .0	0 .003
Inability to eat	33	24 .8	12	9 .0	0 .000
Sleepiness	20	15 .0	4	3 .0	0 .011
Drowsiness	23	17 .3	3	2 .3	0 .005
Nausea	16	12 .0	6	4 .5	0 .002
Psychological changes	32	24 .1	22	16 .5	0 .000
Coughing	36	27 .1	19	14 .3	0 .000

Table 4. *Frequency/severity of complications on Day 1 (24 h) following pediatric dental procedures with general anesthesia.*

24 h	None		Mild		Moderate		Severe	
	n	%	n	%	n	%	n	%
Dental pain	97	72 .9	24	18 .0	8	6 .0	4	3 .0
Dental bleeding	111	83 .5	19	14 .3	2	1 .5	1	0 .8
Sore throat	105	78 .9	15	11 .3	8	6 .0	5	3 .8
Fever	112	84 .2	13	9 .8	4	3 .0	4	3 .0
Vomiting	120	90 .2	11	8 .3	2	1 .5	0	0 .0
Inability to eat	100	75 .2	33	24 .8	0	0 .0	0	0 .0
Sleepiness	113	85 .0	20	15 .0	0	0 .0	0	0 .0
Drowsiness	110	82 .7	23	17 .3	0	0 .0	0	0 .0
Nausea	117	88 .0	16	12 .0	0	0 .0	0	0 .0
Psychological changes	101	75 .9	32	24 .1	0	0 .0	0	0 .0
Coughing	97	72 .9	36	27 .1	0	0 .0	0	0 .0

Table 5. *Frequency/severity of the complications on Day 3 (72 h) following pediatric dental procedures with GA.*

72 h	None		Mild		Moderate		Severe	
	n	%	n	%	n	%	n	%
Dental pain	126	94 .7	4	3 .0	2	1 .5	1	0 .8
Dental bleeding	130	97 .7	3	2 .3	0	0 .0	0	0 .0
Sore throat	124	93 .2	7	5 .3	1	0 .8	1	0 .8
Fever	128	96 .2	3	2 .3	1	0 .8	1	0 .8
Vomiting	129	97 .0	3	2 .3	1	0 .8	0	0 .0
Inability to eat	121	91 .0	12	9 .0	0	0 .0	0	0 .0
Sleepiness	129	97 .0	4	3 .0	0	0 .0	0	0 .0
Drowsiness	130	97 .7	3	2 .3	0	0 .0	0	0 .0
Nausea	127	95 .5	6	4 .5	0	0 .0	0	0 .0
Psychological changes	111	83 .5	22	16 .5	0	0 .0	0	0 .0
Coughing	114	85 .7	19	14 .3	0	0 .0	0	0 .0

Discussion

Dental treatment under GA is essential for some pediatric patients who require comprehensive dental care but who cannot be treated with local anesthesia and do not respond to standard behavioral management techniques. This study was carried out to examine the nature, severity and duration of post-operative complications in healthy pediatric patients following comprehensive dental treatment under GA.

More than two-thirds of the patients in the present study reported one or more complaints 24 hours after the GA procedure. The number and severity of complaints decreased significantly after 72 hours. These results were consistent with those of several previous studies (15, 17-19). Farsi et al. (15), Atan et al. (17) and Escanilla-Casal et al. (18) also reported a significant reduction in patients' complaints 72 hours after GA procedures.

About one-third of children in the present study experienced dental pain during the first day after GA. This rate is higher than the rate reported by Enever et al. (13). The difference could be due to the smaller sample size of the earlier study. Farsi et al. (15), Atan et al. (17) and Escanilla-Casal et al. (18) reported higher rates of pain than the present study. This could be explained by the different dental treatments applied to patients. In the present study, both the rate and severity of pain were found to be significantly lower at 72 hours than at 24 hours. This finding is in line with previous reports (15, 17, 18, 20). The present study found no significant relationship between post-operative dental pain and treatment type or number of teeth treated under GA. This is inconsistent with the findings of Farsi et al. (15), who reported a significant, positive correlation between post-operative dental pain and the number of procedures performed.

Sleepiness, drowsiness, nausea and vomiting are types of complications that could be related to GA. Nausea, vomiting and drowsiness have been reported to occur in connection with the use of the anesthetic agents, such as sevoflurane and propofol (21, 22). In the present study, reports of nausea, vomiting and drowsiness during the first day occurred at similar rates, and the majority of complications were classified as "mild" severity. After 72 hours, reported rates of nausea, vomiting and drowsiness were all below 5%. Farsi et al. (15) also reported a reduction in the rate and severity of nausea, vomiting and drowsiness after 72 hours. This may be due to the elimination of drugs from the body. Previous studies found post-operative sleepiness was related to the anesthesia duration (15, 17); however, the present study found no such significant relationship.

In the present study, almost 25% of patients complained about an inability to eat 24 hours post-operation; moreover, this complaint was found to be significantly related to the number of teeth extracted. Similarly, Farsi et al. (15) reported that complaints of a sore throat, coughing, pain and vomiting, as well as an inability to eat, decreased by the third day following GA. The complaint of an inability to eat could be associated with the other complaints mentioned (15).

The nasotracheal intubation is more physically traumatic than the orotracheal one (23). It causes an increase in post-operative problems due to the trauma (23, 24), and these complications of have been reported to be more common in young children (16). Therefore, the orotracheal approach was preferred for all patients. Although the tube's position limited the working space, none of the cases presented with dental or soft tissue injury resulting from the orotracheal intubation. The reported complications of orotracheal intubation include sore throat, laryngeal edema, hoarseness, nerve injury, aspiration of oral or gastric contents, superficial laryngeal ulcers and laryngeal granuloma (25). Among these, we only encountered sore throat.

In the present study, complaints of a sore throat and coughing were reported by more than 20% of patients on the first day. This rate is similar to those reported by Escanilla-Casal et al. (18) and Farsi et al. (15) and could be the result of the traumatic intubation and throat pack used by pediatric dentists to prevent aspiration of secretions and dental materials (15). In the present study, these complaints were found to be significantly lower on the third day compared the first.

Some previous studies have reported post-operative fever (15, 18, 26). Escanilla-Casal et al. (18) reported a 9% rate of

post-operative fever in ASA I patients after treatment under GA, and Farsi et al. (15) reported a rate of 21% among healthy children, which is similar to the present study's rate of 16%. Escanilla-Casal et al. (18) mentioned various factors that could be associated with post-operative fever after GA procedures, including tissue destruction, operating-room temperature, medications and dehydration. Farsi et al. (15) suggested that preoperative fasting and the inability to eat post-operatively could cause dehydration, which could also cause fever in children.

In the present study, psychological changes were reported in 24% of children in the first day post-operation, but only in 14% on the third day. Previous studies reported higher rates of behavioral changes in children who had been anesthetized with sevoflurane than in those with halothane (27, 28). The anesthesia procedures could impact the psychology and behavior of children, especially since children who were treated with GA already had dental fear and were unable to comply with local anesthesia and standard behavior management procedures. It is also important to note that the present study included children between the ages of two and nine, and younger children, especially those under the age of six, might have difficulties accurately expressing their signs and symptoms.

Surprisingly, no relationship was found between post-operative dental bleeding and the number of teeth extracted. This is in contrast to the findings of previous studies (15, 16, 29, 30) and could be related to the reduction in bleeding caused by the vasoconstrictor contained in the local anesthesia, which was used prior to extraction.

Conclusion

Post-operative complaints following dental treatment under GA tended to be of mild severity and were mainly limited to the first day following treatment. Given the low rates of post-operative complications, dental treatment under GA could be considered an important and useful treatment option for children whose lack of compliance makes conventional dental procedures impossible.

Türkçe Öz: *Pediatrik hastalarda genel anestezi altında diş tedavilerini takiben görülen postoperatif morbiditeler. Amaç: Bu çalışmanın amaçları; pediatrik hastalarda genel anestezi (GA) sonrası 24 ve 72. saatlerde postoperatif morbiditeleri araştırmak ve dental prosedürler ve morbiditeler arasındaki bir ilişki olup olmadığını tanımlamaktır. Gereç ve Yöntem: Genel anestezi altında diş tedavisi yapılan 133 sağlıklı çocuk hasta (yaş aralığı 2-9) çalışmaya dahil edilmiştir. Bu çalışma, bir anket yardımıyla çocukların postoperatif şikayetleri hakkında veri toplanarak prospektif, gözlemsel bir çalışma olarak tasarlanmıştır. Preoperatif veriler hasta kayıtlarından elde edilerek, hastanın yaşı, cinsiyeti, medikal durumu ve kabul türü (ayakta/yatan hasta) kaydedilmiştir. Postoperatif morbiditeler telefonla veya yüz yüze görüşmelerle, 24 ve 72 saat sonra bir anket yardımıyla kaydedilerek değerlendirilmiştir. Bulgular: Çocukların % 69.9'unun 24 saat sonunda bir veya daha fazla şikayeti olduğu, 72 saat sonunda ise bu oranın sadece % 35.3 olduğu öğrenilmiştir. Birinci gün en sık görülen şikayetler; öksürük ve ağrı (% 27.1), yemek yemede güçlük (% 24.8), psikolojik değişiklikler (% 24.1) ve boğaz ağrısı (% 21.1) olarak kaydedilmiştir. 72 saat sonunda morbidite şiddeti ve oranlarında anlamlı azalma gözlenmiştir (p<0.05). Sonuçlar: GA altında diş tedavilerini takiben postoperatif şikayetler hafif şiddette olma eğilimindedir ve çoğunlukla tedaviyi takip eden ilk gün ile sınırlı kalmaktadır. Anahtar Kelimeler: Çocuk; diş tedavisi; genel anestezi; morbidite, postoperative*

Ethics Committee Approval: The ethical approval was obtained from the Kırıkkale University Clinical Research Ethics Committee (2010/89).

Informed Consent: Patients' parents provided the informed consents.

Peer-review: Externally peer-reviewed.

Author contributions: AAO and ISS designed the study. MEA participated in generating the data and gathering the data for the study. MEA and AAO participated in the analysis of the data. MEA wrote the majority of the original draft of the paper. MES and ISS participated in writing the paper. All authors approved the final version of this paper.

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Original research

Clinical evaluation of a self-adhering flowable composite as occlusal restorative material in primary molars: one-year results

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Purpose

The aim of this study was to evaluate and compare the 1 year clinical performances of a self-adhering flowable composite and a commercially available self-etch adhesive/composite system in occlusal restorations of primary second molars.

Patients and Methods

Thirty-one patients (10 male, 21 female) were recruited into the study. A total of 62 occlusal cavities were restored with either a universal composite or a self-adhering flowable composite according to manufacturers' instructions. The restorations were clinically evaluated 1 month after placement as baseline, and after 3, 6 months and 1 year post-operatively using modified USPHS criteria by two operators.

Results

Lack of retention was not observed in any of the restorations. With respect to color match, marginal adaptation, secondary caries and surface texture, no significant differences were found between two restorative materials tested after 1 year. None of the restorations had marginal discoloration and anatomic form loss on the 1 year follow-up. Restorations did not exhibit post-operative sensitivity at any evaluation period.

Conclusion

The clinical assessment of self-adhering flowable composite exhibited good clinical results with predominating alpha scores after 1 year. Advantage of the application convenience for children is promising for self-adhered flowable composite materials in pediatric use.

Keywords: *Self-adhesive; restorative; primary; children; dentition; caries*

Introduction

Dental caries among children continues to be a major public health problem throughout the world. Preservation of primary teeth is important for the maintenance of arch length, maintenance and improvement of physical appearance, maintenance of healthy oral environment, prevention and relief of pain, functions of chewing and speech (1).

Composite resins are esthetic restorative materials used for anterior and posterior teeth. There are variety of resin products on the market with each having different physical properties and handling characteristics based upon their composition for use in primary dentition. Adhesive systems allow bonding of composite resins to primary and permanent teeth. Practical and time saving restorative materials are convenient for the pediatric practices. Research advancements have mainly aimed on the simplification of the technique while enhancing retention of restorations, minimizing microleakage and reducing sensitivity (2). A further advancement in adhesive dentistry is represented by the recent introduction of a so-called "self-adhering composite resin" (compobond), which combines an all-in-one bonding system and a flowable composite (2). Improving marginal

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adaptation of restorations in relation to their rheological properties is also targeted (2-7). Flowable composites, as compared with conventional hybrid composites, exhibit lower mechanical properties due to their reduced filler content (8,9). However for the restoration of cavities in high load-bearing areas, the use of flowable composites is recommended only for cavity lining (10). Conversely, in the restoration of small-sized cavities, as most of the occlusal forces are resisted by the residual tooth structure, the use of flowable composites as stand-alone materials has been recommended (11). Traditional flowable composite resins require a separate bonding system but this self-adhering flowable composite resin eliminates the need for a separate adhesive application. This presents a practical working condition of treatment for children.

The purpose of this study was to evaluate a self-adhering flowable composite and compare its 12 months clinical performances with a commercially available self-etch adhesive / composite system in occlusal restorations of primary molars. The null hypothesis tested in this study is that no difference could be found between the clinical performances of the composite materials.

Patients and Methods

Study design and ethical approval

This single blind randomized clinical trial was approved by Ethics Committee of the University of Cukurova after written informed consent was obtained from the parents or guardians of all children in the study. The study protocol and informed consent document was approved by the Çukurova University Ethical Committee (April 4, 2014, study approval number 30), a subdivision of Turkish Ministry of Health, works full accordance with the World Medical Association Declaration of Helsinki. Split mouth design was conducted according to the Consolidated Standards of Reporting Trials (CONSORT) (12).

From March 2012 to September 2013, all children scheduled to start the dental treatment in pediatric dental clinic were screened by one instructor and enrolled in this study. The inclusion criteria were; being mentally and physically healthy, having at least two occlusal primary caries lesions on primary molars in a split-mouth design with no clinical or radiographic signs of pulpal or periradicular pathology and pathological wear. All of the primary teeth have their occlusal and proximal contacts.

Exclusion criteria were having one of the following situations; disabilities, pulpitis, non-vital or endodontically treated teeth, profound or chronical periodontitis, deep carious defects(close to pulp, < 1mm distance) or pulp capping, heavy occlusal contacts or history of bruxism, systemic disease or severe medical complications, allergic history concerning methacrylate, rampant caries, xerostomia, lack of compliance and language barriers.

After the clinical and bitewing radiographic examination, convenient sample of 33 healthy children between 4 and 9 years of age were selected. Children were asked for their assent after the parents gave written consents. Split mouth design was applied for the study. The children were randomly assigned either right or left halves of their dentition and were treated with local anesthesia and rubber dam isolation by a pediatric dentist. The side, which restoration method was allocated, was assigned by computer-generated randomisation. The advantage of such a split-mouth design over randomising individual patients was the reduction in interparticipant variability (13). Each child was treated by the same operator to avoid behavioral problems.

Interventions

During the restorative procedure, the operator removed only carious lesions and performed no retention such as undercutting or dovetailing. Occlusal cavities were prepared

Table 1: *Restorative Materials*

Materials	Manufacturer	Composition	Application
Vertise flow	Kerr, Orange, CA, USA	GPDM, Prepolymerized filler, 1-micron barium glass filler, nano-sized colloidal silica, nano-sized Ytterbium fluoride	Apply the first layer of Vertise Flow with moderate pressure for 15-20 seconds, light-cure for 20 seconds. For A3.5 and Universal Opaque, cure for 40 seconds.
			If necessary, build the restoration incrementally with Vertise Flow in 2mm or less thickness, light-cure for 20 seconds. For A3.5 and Universal Opaque, cure for 40 seconds.
Clearfil SE Bond primer	Kuraray Medical Inc, Okayama, Japan	MDP, HEMA, dimethacrylate monomer, water, catalyst	Apply for 20 seconds and dry thoroughly with mild air.
Clearfil SE Bond bond	Kuraray Medical Inc, Okayama, Japan	MDP, HEMA, dimethacrylate monomer, microfiller, catalyst	Apply after application of primer, air-flow gently and light-cure for 10 seconds.
Filtek Z250	3M ESPE, St Paul, MN, ABD	BIS-GMA, UDMA and BIS-EMA. Encore-GMA, UDMA, Encore-EMU, Zirconium/ Silicon 60% (0.01 to 3.5 micrometers)	Place Filtek Z250 Restorative in increments. Light-cure each increment for 40 sec (Reference Light-Cure chart for thickness and cure time.)

Abbreviations: GPDM (glyceroldimethacrylate dihydrogen phosphate), MDP(10-methacryloyloxydecyl dihydrogen phosphate), HEMA (hydroxyethyl methacrylate), BIS-GMA (bisphenol-glycidyl methacrylate), UDMA (urethane dimethacrylate), BIS-EMA (bisphenol-polyethylene glycol dimethacrylate)

2mm to 4mm depth. The dentist prepared the teeth with a 330 bur (KG Sorensen, Sa˜o Paulo, Brazil) in a high speed hand-piece with water coolant. Afterwards a round carbide bur was used at slow speed in dentin. During the dental cavity preparation, if pulp tissue was exposed or the required cavity size was larger than the study design, the teeth were excluded from the study. Eventually 2 patients were excluded.

All 31 patient received two different types of restorative treatment. A total of 62 occlusal cavities were restored with either a self-adhering flowable composite (VF) or a commercially available self-etch adhesive/composite system (CR) according to manufacturers' instructions. Restorative materials were handled and applied in accordance with the manufacturers' instructions (Table 1).

Figure 1 shows primary tooth occlusal restoration with self-adhering flowable composite.

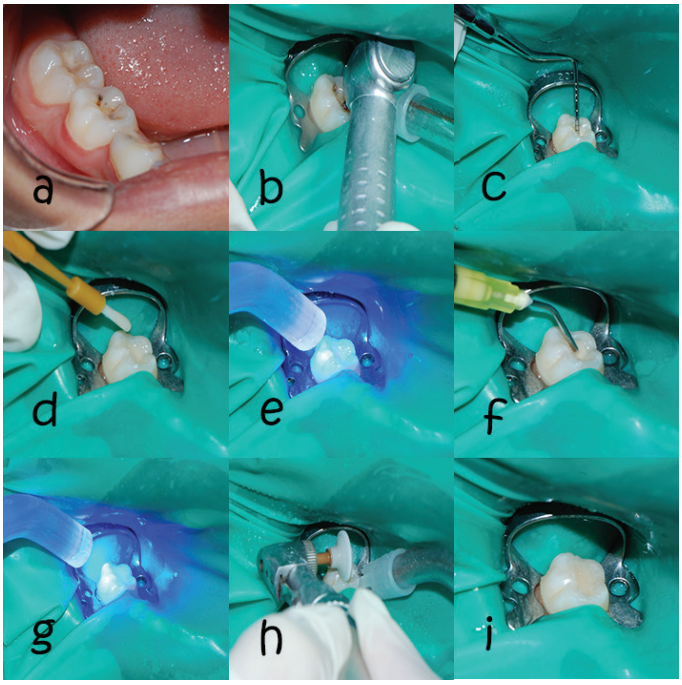

Figure 1. *Cavity preparation and the application of the flowable composite resin. a: preoperative, b: cavity preparation, c: prepared cavity, d: first layer replacement and brushing for 20 seconds, e: 20s polymerization, f: second layer placement, g: 20s polymerization h: finishing and polishing, i: final restoration.*

The occlusal relationship was checked with carbon paper (Accufilm II, Parkell, USA) and adjusted with fine granulation burs. Final finishing and polishing procedures were carried out with fine and ultrafine granulation diamond burs (KG Sorensen, Sa˜o Paulo, Brazil) and finishing was made with a diamond polishing paste (Dentsply, Rio de Janeiro, RJ, Brazil).

Outcomes

The restorations were clinically evaluated 1 month after restorations, and after 3, 6 months and 1 year post-operatively using modified United States Public Health Service (USPHS) criteria by two previously calibrated operators who were different from the treatment applied operator (14). The restorations were re-evaluated by two blind examiners (total weighted kappa (k) between 0.85 and 0.92 for intraexaminer and interexaminer agreements). Each restoration was assessed at baseline placement and at the 1st, 3rd, 6th months and first year with modified USPHS criteria for retention (R), color match (CM), marginal discoloration (MD), secondary caries (SC), wear (W), marginal adaptation (MA) and postoperative sensitivity (PS). The Alfa and Bravo scores were considered clinically acceptable/ successful of the restorative treatment, while, Charlie scores were clinically unacceptable/ unsuccessful restorative treatment and had to be replaced and excluded from the study. All evaluations were carried out with a dental operating light, mouth mirror, dental explorer and dental floss. Figure 2 shows one year follow up pictures of the self-adhesive restorations.

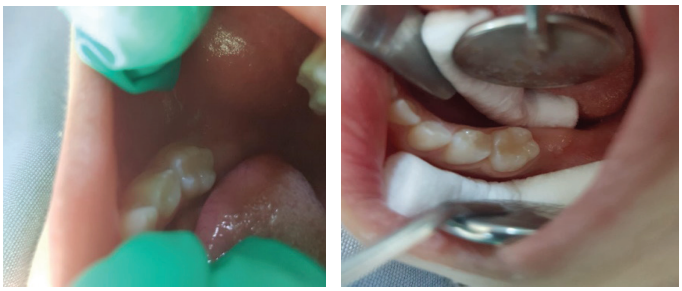

Figure 2. *1 year follow up pictures of the self-adhesive restorations.*

In addition, each patient received professional cleaning of the teeth and neutral topical fluoride application during the dental appointments. All of the procedures were done by the operators involved in the study.

Statistical Analysis

The data were processed by SPSS software (12.0, SPSS Inc., Chicago IL, USA). 'Sample size' was calculated 28 at 80% power, while carrying out a two tailed test at 5% significance level. 'Sample size' was calculated by G*Power 3.0.10 (15). The kappa statistic was used to measure interrater reliability. The descriptive statistics; the frequency, the mean, the standard deviation and median were calculated for each group. Normality was analyzed using Shapiro–Wilks test. Modified USPHS results were assessed by Mann–Whitney U-test at significance level of $p<.05$.

Results

Baseline characteristics (patients and teeth)

31 patients attended the 1st, 3rd and 6th months recall and 29 patients attended 1 year recall. Lack of retention was not observed in any of the restorations. Split mouth design avoids residual confounding. The age, gender and bio-characteristic of the two groups were identical. Baseline characteristics of the groups; are also included the modified USPHS criteria's of the treatment day results; are given at the Table 2. The children were between the ages of 4 to 9, mean 6.67.

Baseline data and final outcome

With respect to color match, marginal adaptation, marginal discoloration, secondary caries, postoperative sensitivity no significant differences were found between a self-adhering flowable composite (VF) and a commercially available self-

Table 2: *Baseline characteristics of the groups*

	N	Frequency	Mean/median	
gender				
male	15	%48.4		
female	16	%51.6		
Age				
4	2	%6.5	6.67/7	
5	4	%12.9		
6	7	%22.6		
7	9	%29.0		
8	7	%22.6		
9	2	%6.5		
group				
CR	31	%50		
VF	31	%50		
Modified USPHS criteria				
Retention*/alpha/bravo/charlie*	62	%100	1	Alpha
Color Match*/alpha/bravo/charlie*	62	%100	1	Alpha
Marginal Discoloration*/alpha/bravo/charlie*	62	%100	1	Alpha
Secondary Caries*/alpha/charlie*	62	%100	1	Alpha
Wear*/alpha/charlie*	62	%100	1	Alpha
Retention*/alpha/charlie*	62	%100	1	Alpha
Color Match*/alpha/charlie*	62	%100	1	Alpha

Table 3: *Modified USPHS scores of 1st and 3th month*

	1.month		3. month	
	VF Mean±SD (Median)	**CR Mean±SD (Median)**	**VF Mean±SD (Median)**	**CR Mean±SD (Median)**
Retention	1.00±0.00(1.00)	1.03±0.18(1.00)	1.03±0.18(1.00)	1.03±0.18(1.00)
Color Match	1.03±0.18(1.00)	1.00±0.00(1.00)	1.03±0.18(1.00)	1.00±0.00(1.00)
Marginal Discoloration	1.00±0.00(1.00)	1.00±0.00(1.00)	1.00±0.00(1.00)	1.03±0.18(1.00)
Secondary Caries	1.00±0.00(1.00)	1.00±0.00(1.00)	1.03±0.18(1.00)	1.00±0.00(1.00)
Wear	1.00±0.00(1.00)	1.00±0.00(1.00)	1.00±0.00(1.00)	1.03±0.18(1.00)
Marginal Adaptation	1.00±0.00(1.00)	1.03/ 1.00/ .189	1.00±0.00(1.00)	1.03±0.18(1.00)
Postoperative Sentisivity	1.03±0.18(1.00)	1.03±0.18(1.00)	1.03±0.18(1.00)	1.03±0.18(1.00)
Total	7.06±0.25(7)	7.10±0.31(7)	7.16±0.37(7)	7.6±0.45(7)

Mann whitney U test for grouping materials VF (self adhering flowable composite) and CR (commercially available self-etch adhesive/ composite system) for the 1. and 3. months; $p<0.05$ is statistically significant

etch adhesive/composite system (CR) after 1 month, 3 months, 6 months and 1 year (Figure 2). Table 3 showed the difference between the groups according to results of first and third month USPHS control. A score called 'total' which was the sum of the modified USPHS scores; were added to the tables. At the end of the 3th month 'Total' scores were also not statistically significant between the groups (p=0.765).

Table 4 shows the 6th month and 1 year results of the VF and CR groups, there were no significant differences between the groups for retention, color match, marginal discoloration, secondary caries, wear, marginal adaptation, postoperative sensitivity and total USPHS criteria.

Discussion

This study aimed to evaluate the clinical performance of a new self-adhering flowable composite for 1 year using modified USPHS criteria. Based on the findings of

Table 4: *Modified USPHS scores of 6th month and 1 year results*

	6. month		1. year	
	VF Mean±SD (Median)	CR Mean±SD (Median)	VF Mean±SD (Median)	CR Mean±SD (Median)
Retention	1.03±0.18(1.00)	1.00±0.39(1.00)	1.00±0.00(1.00)	1.10±0.49(1.00)
Color Match	1.03±0.18(1.00)	1.00±0.00(1.00)	1.03±0.18(1.00)	1.00±0.00(1.00)
Marginal Discoloration	1.06±0.35(1.00)	1.03±0.18(1.00)	1.07±0.37(1.00)	1.10±0.41(1.00)
Secondary Caries	1.03±0.18(1.00)	1.03±0.18(1.00)	1.07±0.25(1.00)	1.03±0.18(1.00)
Wear	1.00±0.00(1.00)	1.03±0.18(1.00)	1.00±0.00(1.00)	1.07±0.25(1.00)
Marginal Adaptation	1.03±0.18(1.00)	1.06±0.18(1.00)	1.03±0.18(1.00)	1.07±0.25(1.00)
Postoperative Sensitivity	1.06±0.25(1.00)	1.03±0.18(1.00)	1.07±0.25(1.00)	1.07±0.25(1.00)
Total	7.26±0.77(7)	7.29±0.58(7)	7.28±0.79(7)	7.31±0.60(7)

Mann Whitney U test for grouping materials VF (self adhering flowable composite) and CR (commercially available self-etch adhesive/ composite system) for the 6. month and 1st year; $p<0.05$ is statistically significant.

the present study; clinical assessment of self-adhering flowable composite exhibited acceptable clinical results with predominating alpha scores after 1 year. Incorporation of the bonding agent into a flowable composite holds great potential such as; saving chair time and minimizing handling errors. In this study, a commercially self-etch adhesive and a composite resin was used as control group because of its announced gold standard for in vitro studies and its good clinical performance.

The self-adhering flowable composite holds great potential with respect to saving chair time and minimizing handling errors. The advantages for pediatric dentistry are reducing operative procedures, minimizing the technical sensitivity, simultaneous demineralization and resin infiltration as well as in reducing postoperative complaints like pain (16).

The use of flowable restorative systems in dentistry has increased because of their beneficial properties, such as low viscosity, low modulus elasticity and ease of handling (13). For the restoration of cavities in high load bearing areas, the use of flowable composite resins are recommended only for cavity lining but flowable composites has been proposed for the restoration of small-sized cavities, while the occlusal forces are resisted by the residual tooth structure (2).

Various methods were designed for clinical evaluation of restorations (17,18). Among them, modified United States Public Health Service (USPHS) criteria has been used the most widely with various modified forms to determine the clinical performance of dental restorations (14,19-21). In this study modified USPHS criteria was used which is a long-established method used in clinical trials. In between the two materials there were no difference for the criteria's 'retention', 'color match', 'marginal discoloration', 'secondary caries', 'wear', 'marginal adaptation' and 'postoperative sensitivity' in the first, third, sixth months and the first year.

There were limited studies about self-adhering flowable composite resin. Pacifici et al. (22) had concluded that occlusal cavities, restored with self-adhering flowable composite resin, provided satisfactory sealing ability despite the relatively low bond strength recorded on enamel and dentin. The results of the study by Tuloğlu et al. (23) showed that, the self-adhering flowable composite resin has lower bond strength values than conventional flowable resin composite for both primary and permanent dentin. They suggested that the use of a bonding agent significantly increased the shear bond strength values of self-adhering flowable composite resin to both permanent and primary tooth dentin. Self-adhering flowable composite resin established similar bond strength values as glass ionomer cements on primary dentin (24). Although VF resulted in lower bond strengths values on either dental substrate, better marginal sealing ability was visualized in comparison with all-in-one adhesive systems (2,25). Recent studies showed similar successful results of clinical usage of self-adhesive flowable restorative materials in primary dentition (26,27).

The preservation of primary teeth is important for the management of the developing dentition until normal exfoliation takes place. Restorations of primary teeth are usually performed using composite resin, compomer or glass ionomer and needs to be durable. For the clinical success of composite resin restoration an effective bond between dental materials and tooth substrates is critical (28). Pediatric restorative dentistry is a dynamic area with rapid development of technology and new materials. Among the materials used in the pediatric dental restorations, self-adhering flowable composite resin, with its clinical handling properties and the ability of reducing the time on dental unit, has different advantages during dental treatment.

Conclusion

Clinical assessment of self-adhering flowable composite exhibited good clinical results with predominating alpha scores after 1 year in this study. The findings of this clinical study suggest that self-adhering flowable composite resin can be used successfully in occlusal cavities of primary teeth. The advantage of the application convenience for children is promising for self-adhered flowable composite materials in pediatric use.

Türkçe Öz: *Kendinden adezyonlu akışkan bir kompozitin süt dişlerinde okluzal kavite materyali olarak klinik değerlendirmesi: 1 yıllık sonuçlar. Amaç: Kendinden adezyonlu akışkan kompozitler ayrı bir adeziv olmadan*

diş dokusuna bağlanır ve bond maddesini doğrudan akışkan rezinin içine yerleştirerek restoratif prosedürü kolaylaştırır. Bu çalışmanın amacı, kendinden adezyonlu akıcı bir kompozitin ve konvansiyonel bir kompozit sisteminin klinik performanslarını 12 ay süresince değerlendirmek ve karşılaştırmaktır. Gereç ve Yöntem: Otuz bir hasta (10 erkek, 21 kadın) çalışmaya alındı. Üreticinin talimatlarına göre konvansiyonel bir kompozit veya kendinden adezyonlu bir akışkan kompozit ile toplam 62 okluzal kavite restore edildi. Restorasyonlar işlem sonrası 1 ay ve sonrasında 3, 6 ve 12. aylarda modifiye USPHS kriterleri ile klinik olarak değerlendirildi. Bulgular: Restorasyonların hiçbirinde retansiyon problemi gözlenmedi. Renk uyumu, marjinal adaptasyon, ikincil çürükler ve yüzey dokusu açısından 12 ay sonra test edilen iki restoratif materyal arasında anlamlı bir fark bulunmadı. Restorasyonların hiçbirinde 12 aylık takipte marjinal renk değişikliği ve anatomik form kaybı olmadı. Restorasyonlar, herhangi bir değerlendirme sırasında işlem sonrası hassasiyet göstermedi. Sonuç: Kendinden adezyonlu akıcı kompozitin klinik değerlendirmesi, 12 ay sonra baskın alfa skorları ile iyi sonuçlar vermiştir. Çocuklar için uygulama rahatlığının avantajı, pediatrik kullanımda kendinden adezyonlu akıcı kompozit malzemeler için umut vericidir. Anahtar Kelimeler: Kendinden adezyonlu; restoratif; süt; dişlenme; çocuk; çürük

Ethics Committee Approval: The study protocol and informed consent document was approved by the Çukurova University Ethical Committee (April 4, 2014, study approval number 30).

Informed Consent: Informed consents was provided by the participants' parents.

Peer-review: Externally peer-reviewed.

Author contributions: BAS, IY and MCD designed the study. IY and CD participated in generating the data for the study. BAS and IY participated in gathering the data for the study. IY participated in the analysis of the data. BAS and IY wrote the majority of the original draft of the paper. BAS and IY participated in writing the paper. All authors approved the final version of this paper.

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Original research

The assessment of new bone formation induced by unfocused extracorporeal shock wave therapy applied on pre-surgical phase of distraction osteogenesis

Erman Senel[1], Enes Ozkan[2], Mehmet Cihan Bereket[3], Mehmet Emin Onger[4]

Purpose
This study aims to evaluate the effects of extracorporeal shock wave therapy applied before and/or immediately after the osteotomy on the maturation during the consolidation phase.

Materials and Methods
21 female New Zealand rabbits were used in the study. Subjects were divided randomly into three groups: Control (Distraction without ESWT), A (Distraction +ESWT After Osteotomy), AB (Distraction+ESWT After and Before Osteotomy). ESWT (500 pulses, 5 Hz, 0.19 mJ/mm^2 energy flux density) was applied to group A and group AB after 5, 12 and 19 days after osteotomy and group AB only on days 7,14 and 21 before osteotomy. On the 28th day of the consolidation period, all subjects were sacrificed. Dual-energy x-ray absorptiometry (DEXA) was used to determine bone mineral density (BMD) and bone mineral content (BMC), and stereological methods were used to determine the new bone, connective tissue and neovascularization volumes.

Results
As a result of DEXA examinations made on the 1st and 4th week of consolidation, there was no significant difference between groups regarding BMD and BMC values. According to the results of stereological examination, when the connective tissue and new bone tissue were evaluated, higher values were observed in AB when compared to A, and in AB and A compared to the control group, but the differences are not statistically significant. There was no difference between the groups in terms of neovascularization.

Conclusion
ESWT in these parameters was not positively effective in bone maturation during consolidation when applied before osteotomy or both before and after osteotomy.

Keywords: *Bone regeneration; distraction osteogenesis; dual-energy x-ray absorptiometry; extracorporeal shock wave therapy; organ volume*

Introduction

The reconstruction of congenital deformities and large bone defects due to trauma and cysts/tumors in the oral and maxillofacial region are challenging and requires complicated surgical procedures. Grafting or orthognathic surgery methods are used in the treatment of these deformities. However, these applications cannot be fully trusted in obtaining the most ideal results (1,2). Owing to its many basic advantages, distraction osteogenesis (DO) has been used successfully for many years in the treatment of deformities in the maxillofacial region (3,4). The greatest advantage of DO over other surgical techniques is the simultaneous expansion of the surrounding soft tissue matrix (periost, blood vessels,

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nerve, muscle, mucosa, gingiva, skin, etc.) during bone extension (3). Despite the advances in DO techniques and technology, serious complications can occur because of the long consolidation phase (up to six months) (1,5). Pin-tract infection, fibrous union or non-union, pain, fracture of the distractor, and psychological problems are among these complications (3,6). These complications can be avoided by changing the rhythm of distraction or by stimulating bone regeneration that occurs during consolidation (7).

Many biostimulatory methods (low-density ultrasound, low dose laser therapy, recombinant growth factors, etc.) to induce new bone formation in DO have been the subject of research (4,8-10). Although positive results have been reported in most of the methods used, clinical use is still not accepted (11). Extracorporeal shock wave therapy (ESWT), which is reported to induce new bone formation by increasing osteogenesis and angiogenesis in the bone recovery process, may contribute to the reduction of the treatment period by inducing consolidation-phase bone regeneration in DO (12).

Various mechanisms have been claimed to influence the osteostimulatory effect of ESWT. Many studies have shown that ESWT has this effect by triggering the expression of transcription factors, mediators, and growth hormones (13-15). ESWT has also been reported to induce mesenchymal cell proliferation and differentiation, and to promote osteogenesis by stimulating osteoblasts (12,13,15,16). These effects have been shown to induce open fracture healing, shorten consolidation period in DO, and increase bone mineral density (12,17). In DO, all the biostimulatory methods for accelerating the consolidation period are applied within the consolidation process. To date, no study has yet evaluated the prophylactic application of ESWT in DO prior to osteotomy. Therefore, this study aims to compare the results of ESWT before and after surgical procedure to determine the most appropriate application and to obtain the best bone healing process. The null hypothesis tested in this research is that the ESWT application does not affect the bone healing process.

Materials and Methods

Laboratory animals

Supported by the Project Management Office of Ondokuz Mayıs University with project number PYO.DIS.1904.12.007, this study received ethical approval on November 25, 2012 numbered 2011/65 from the Animal Experiments Local Ethics Committee. The experiment abided by the maintenance and use agreement of experimental animals. A total of 21 female New Zealand rabbits, 6–9 months of age and weighing approximately 2.75 kg, were used in the study. The subjects were kept in separate cages provided with standard food and water support in a 12 h night/day cycle. All animals received the same distractor device and bone extension protocol. After they were identified, the subjects were randomly assigned to three groups: Control (DO without ESWT) (n=7), A (ESWT applied after the latency period) (n=7), AB (ESWT applied before osteotomy and after the latency period) (n=7)

ESWT protocol

The electrohydraulic ESWT device (Orthogold 100, MTS Medical, Konstanz, Germany) and the unfocused applicator (OP155) were used in the study. Surgical lubricant gel was applied to the skin before ESWT application. The application was made by contacting the applicator of the device with the right mandible angle (Figure 1). ESWT with 500 pulse, 5 Hz, and 0.19 mJ /mm^2 energy flow intensity was applied in the AB group in the first, second, and third weeks before osteotomy. For the control group, a placebo application was conducted with the applicator of the device turned off. In the A and AB groups, the same feature of ESWT was applied to the distraction zone at 5, 12, and 19 days after osteotomy. On the 28th day of the consolidation period, all the rabbits were sacrificed with a high dose of sodium pentobarbitone (Pentalyn; IE Ulagay, Istanbul, Turkey).

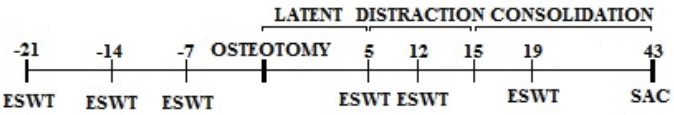

Figure 1. *Timeline of experimental procedures. (SAC: Sacrification) Surgical procedure.*

Surgical procedure

All subjects were starved a day before the surgery. The experimental animals were randomly selected without knowing to which group they belonged. All animals were

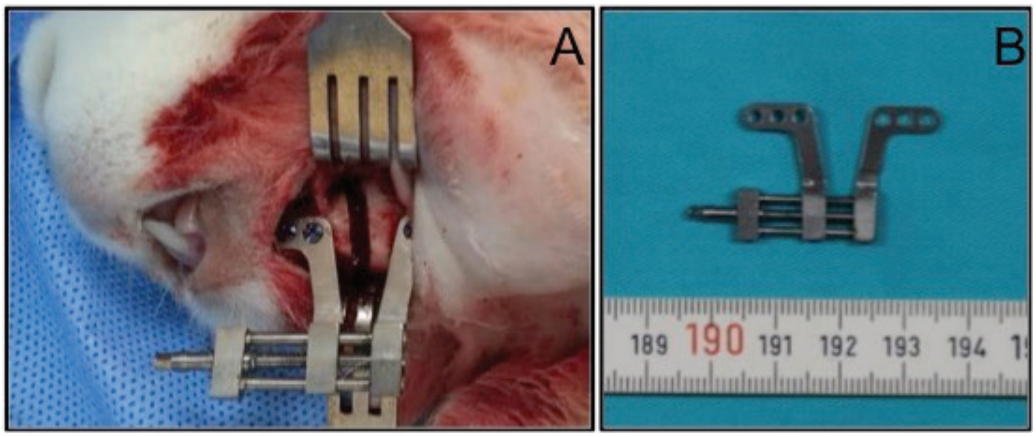

Figure 2. A. *Osteotomy line is posterior to mental foramen. Distractor was adapted with six mini screw.* ***B.*** *The custom made titanium distractor with 6 hole can provide 10 mm extension.*

intramuscularly administered with 50 mg/kg ketamine HCl (Ketalar, Pfizer, Istanbul, Turkey) and 8 mg/kg xylazine HCl (Rompun, Bayer, Istanbul, Turkey) as general anesthesia. About 0.5 ml articaine containing 1:200,000 epinephrine (Ultracain-DS; Hoechst Marion Roussel, Istanbul, Turkey) was applied as local anesthesia to the surgical site. After the mandible ramus area of the rabbits was shaved and aseptic conditions were established with iodine, a 3 cm linear incision was made at the inferior border of the left mandible. The bone surface was uncovered by elevating the full thickness flap. The osteotomy area passing between the first premolar tooth and the mental foramen was determined. Before osteotomy, a titanium distractor (Trimed, Electron Medical, Ankara, Turkey) was adapted parallel to the mandibular border (Figure 2A). Afterward, a bone fracture was performed with a fissure bur under sterile saline irrigation and osteotomes without causing mental nerve damage (Figure 2B). The incision area was closed up in layers with 4/0 suture (Vicryl, Ethicon, Brussels, Belgium). After a 5-day latency period, a distraction protocol was applied for 10 days at a distraction rate of 0.35 mm/12 h.

Postoperative care

For postsurgical pain and infection control, 1 mg/kg Tramadol (Contramal, Abdi İbrahim, Istanbul, Turkey) and 50 mg/kg Cefazolin Sodium (Sefazol, M Nevzat, Istanbul, Turkey) were administered intramuscularly twice a day for 4 days. The animals were given a soft-food diet for a week. The weights and nutritional status of the animals were checked daily by the veterinarian.

DEXA examination

The measurements were conducted with the DEXA scanner Hologic QDR 2000, (Discovery Series, Hologic, Inc., Waltham, Mass., USA) at Ondokuz Mayıs University Faculty of Medicine, Nuclear Medicine Department, on the first and fourth weeks of consolidation under general anesthesia applied intramuscularly with 20 mg/kg ketamine HCL (Ketalar, Pfizer, Istanbul, Turkey) and 5 mg/kg xylazine HCL (Rompun, Bayer, Istanbul, Turkey). The DEXA measurements were taken by the same clinician from the center of the distracted area, without knowing to which group the subjects belonged. The bone mineral density (BMD) and bone mineral content (BMC) values were determined using the small subject program (Figure 3B).

Stereological analysis

The preparations and stereological examinations of the tissue specimens were conducted blindly by a histologist. Soft tissues on the jaws were removed and decalcified for 21 days in formic acid (5%). After the decalcification, the tissues were fixed with formaldehyde (10%) and dehydrated gradually with alcohol. After dehydration, the samples were buried in fresh paraffin. About 7 µm-thick serial sections were obtained from each paraffin block with microtomes (Leica RM 2135; Leica Instruments, Nussloch, Germany). According to systematic random sampling manner, the paraffin blocks obtained from the samples taken from each jaw were sampled at a rate of 1/10. The first section was selected randomly. The selected sections were stained with hematoxylin–eosin and photographed with a color digital camera (Microbrightfield, Williston, VT, USA) using a light microscope (Leica M 4000 B, Germany) in a stereology analysis system (Stereoinvestigator 9.0, Microbrightfield, Williston, VT, USA). The Cavalieri method was applied to the light microscopy images to stereologically evaluate new bone, connective tissue, and neovascularization volumes. Point counting test grids were used to designate areas in sections (Figure 3A). Gundersen and Jensen's formula was applied to determine the point density. The coefficient of error and the coefficient of variation were determined with this formula (18). This grid was randomly positioned on the computer screen. The volume of the distraction area in all the mandibular incisions was determined by the following formula: Volume= t x a/p x ∑p where t is the section thickness; a/p is the area representing each point on the point counting table; and ∑p the total number of points corresponding to the distraction area) (18).

Statistical analysis

The data obtained from the densitometric and stereological evaluations were compared with the one-way ANOVA test by loading it on the SPSS (version 13.0, IL, USA) statistical program in a computer environment. The comparisons between the groups were performed using the one-way analysis of variance (ANOVA) followed by the post-hoc

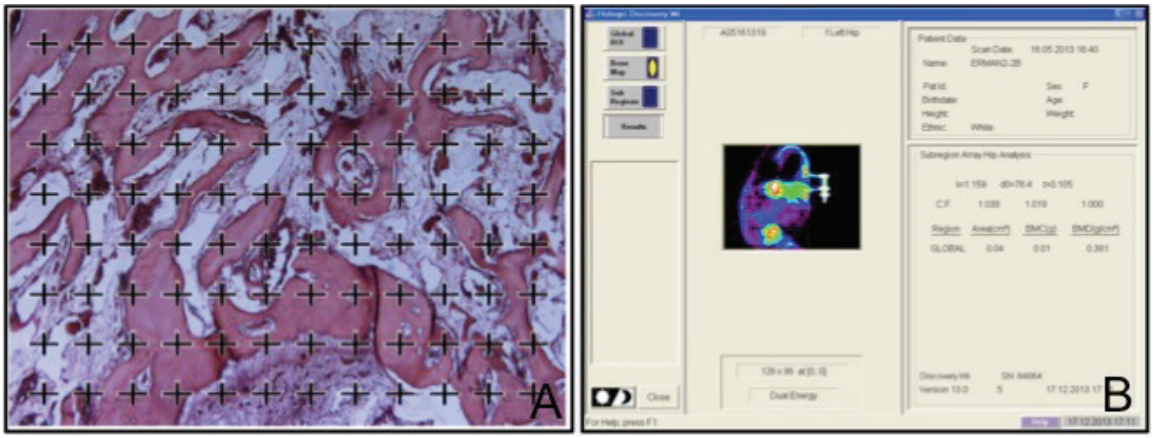

Figure 3. A. *Measurement of tissue volume with point count grid in light microscope images using Cavalieri method.* ***B.*** *The software used for DEXA measurements.*

Tukey's HSD test. p values smaller than 0.05 were considered statistically significant.

Results

Clinical Observations and Animal Condition

One rabbit in group A died in the experimental process because of infection and excessive weight loss. One rabbit in the control group was excluded from the experiment because of the unstable distractor. The remaining animals tolerated the osteotomy and distraction protocols. The distractor remained stable until the end of the experiment. After the DO application, all of the rabbits were observed to have a unilateral crossbite and an extensively lengthened incisor. The high resolution computed tomography (CT) images showed that the new bone formation in the distraction area was healthy in all groups (Figure 4).

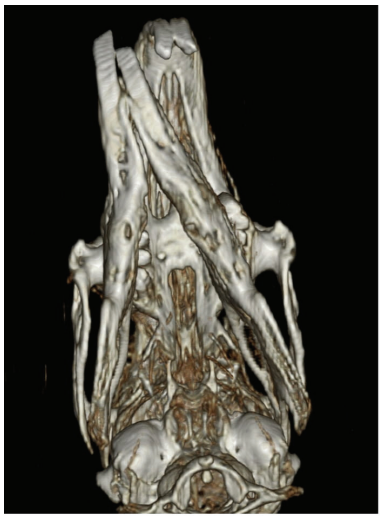

Figure 4. *Three-dimensional (CT) image showing unilateral cross-bite and incisor elongation.*

BMD and BMC

The BMD and BMC values were determined at the end of the first and fourth weeks of the consolidation period in all animals (Table 1). The highest BMD value was found in the control group according to the measurements made at the end of the first week, followed by the A group and the AB group. In the analysis made at the end of the fourth week, the measurement values similar to those of the first week were found. No

Table 1. *BMD and BMC data obtained from DEXA examinations at 1st and 4th week of consolidation (mean±standard deviation, BMD: Bone mineral density, BMC: Bone mineral content)*

		Control	A	AB
First Week	**BMD (g/cm²)**	0.69±0.09	0.62±0.10	0.62±0.08
	BMC (g)	0.022±0.011	0.025±0.005	0.022±0.008
Fourth Week	**BMD (g/cm²)**	0.76±0.04	0.64±0.08	0.64±0.04
	BMC (g)	0.030±0.000	0.027±0.005	0.025±0.005

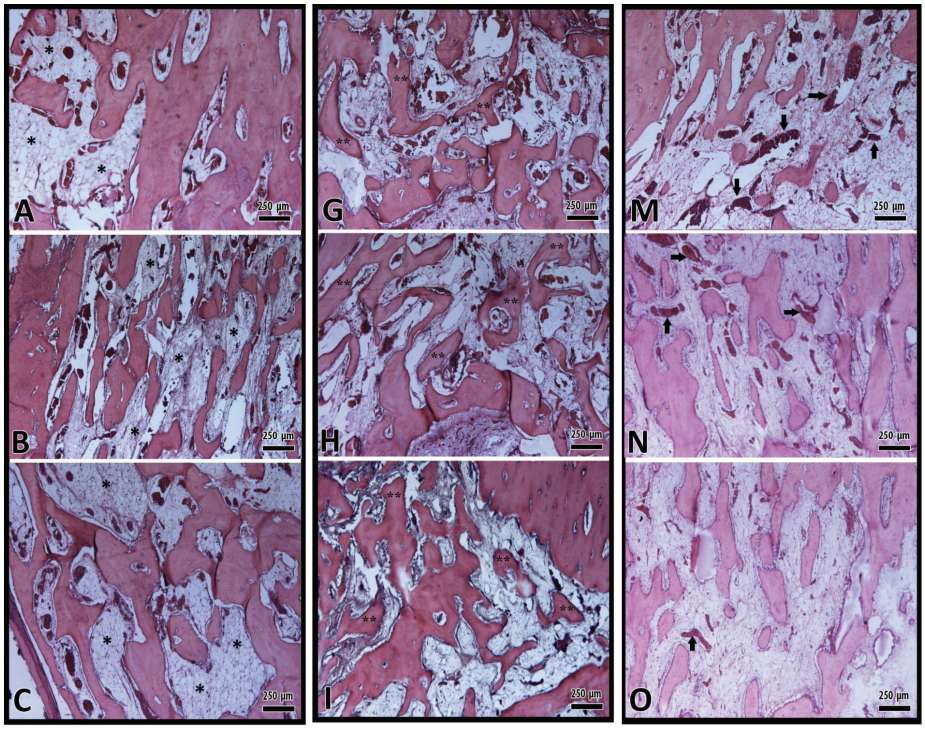

Figure 5. *Histological image showing connective tissue areas of A (Control), B (Group A) and C (Group AB). The connective tissue areas are marked with (*). New bone areas have been shown in G (Control), H (Group A) and I (Group AB) images. New bone areas are marked with (**). New vessel areas have been shown in M (Control), N (Group A) and O (Group AB) images. New vessel areas are marked with black arrow. (original magnification x5, hematoxylin-eosin).*

significant difference was found in the BMD results. Regarding BMC results, the highest value was seen in group A at the end of the first week and in the control group at the end of the fourth week. No statistically significant difference was found in the BMC results. Although the BMD and BMC values increased at the end of the fourth week in comparison with those in the first week, this increase was not statistically significant.

Tissue volumes

As shown in the stereological examination, new bone tissue, connective tissue, and neovascularization volumes were detected in the animals that were sacrificed after the completion of distraction (Figure 5). In comparing the volume of new bone tissue, the highest value was found in the AB group, and the difference was not statistically significant. The lowest new bone volume was observed in the control group. As shown in the results of the connective tissue volumes, the highest volumes were found was in the AB group, followed by the A group and the control group. The differences between the groups were not found to be statistically significant. When the neovascularization volumes of the groups were evaluated, the highest value was found in the control group, and the differences between control and AB and between control and A were statistically significant (Table 2).

Table 2. *Tissue volumes obtained from the stereological examination (mean±standard deviation, different superscript letters indicate significant difference).*

	Control	A	AB
New Bone	0.20±0.02	0.22±0.04	0.24±0.04
Connective Tissue	0.35±0.02	0.39±0.04	0.41±0.11
Neovascularization	0.10±0.02[a]	0.07±0.01[ab]	0.07±0.01[ab]

Discussion

The acceleration of callus maturation, the improvement of the biomechanical properties, and the shortening of the consolidation period in DO have attracted the attention of researchers (17). Using a biostimulatory method that generates signals for the release of growth factors rather than only applying the growth factors in the distraction gap may have a greater strategic advantage (3).

ESWT may shorten the total treatment period by accelerating the callus maturation. In experimental animal models in the literature, ESWT has been reported to increase cell differentiation and neovascularization, thus accelerating the healing of fractures and increasing the amount of callus and cortical bone formation (16,19). In addition, it has been shown to release more osteogenic and angiogenic growth factors, such as VEGF, endothelial nitric oxide synthase, proliferating cell nuclear antigen, and BMP-2 (16). ESWT has been shown to improve bone regeneration when applied with suitable parameters. However, the appropriate parameters for the induction of bone healing have not yet been determined. Not enough studies have been conducted to investigate the different parameters of the effect of ESWT on new bone formation in DO.

The effects of ESWT on DO were examined in two studies: the efficacy of two different energy flow densities in (17) and the effect of different numbers of impulses in (12). In both studies, ESWT was applied at the beginning of the consolidation period. Only Önger et al. (12) performed a second session on the fourth day of the consolidation. Whereas Lai et al. (17) reported that a 500 pulse shock wave therapy with an energy flow density of 0.19 mJ/mm2 is effective in increasing angiogenesis and bone regeneration and in shortening the consolidation process, Önger et al.(12) showed that a 1,000 x2 pulse shock wave therapy at an energy flux density of 0.19 mJ/mm^2 resulted in a higher new bone volume and bone mineral density. Lai et al. (17) also found that shock waves applied at 21 kV and 500 pulses caused necrotic changes. In our study, the parameters reported to have a positive effect on ESWT in distraction were used, and obtaining more effective results would be possible by performing these applications both preoperatively and repeatedly.

Biostimulatory methods are effective in the angiogenesis and proliferative phases of wound healing because cell proliferation and differentiation, as well as the growth factor release, are at their highest during these periods (20). However, ESWT has also been shown to improve cortical and cancellous bone volume and to improve the mechanical properties of the bone in areas that do not undergo surgical procedures. ESWT shows this effect by generating transient bone marrow damage resulting in an anabolic process (21). Moreover, the preoperative application of ESWT to bone sites induces the proliferation of periosteum cambium cells and increases periosteal thickness (22). Thus, ESWT was performed before and after osteotomy in our study. The lack of difference between the AB group and the control group in terms of new bone volume can be due to the stimulation conducted in three sessions, the number of shock waves, or the energy flux density.

DEXA is an important diagnostic method widely used to determine bone mineral density and to predict fracture risk (17,23). The BMD and BMC values of each rabbit were measured using the DEXA method at the end of the first week (early period) and the fourth week (late period) of the consolidation period in our study. No statistically significant difference was found between the pre- and post-distraction applications of ESWT (Group AB) and the post-distraction application (Group A). Therefore, ESWT be sufficiently applied after distraction. Furthermore, the DEXA values in the experimental groups were lower than the values in the control group in the early and late periods. This finding may be due to the use of unfocused applicators (24). The different results obtained from those of Lai and Önger et al. may be due to the increase in the number of sessions, as one or two sessions of treatment were reported to result in higher BMD intensity in the control groups (12,17).

The Cavalieri method in stereology is an effective and easily applied method used to calculate the volume of a tissue or organ. As it enables a three-dimensional evaluation, it reflects the tissue features better than histological evaluations and gives more realistic values (25). In this study, no statistically significant difference was found between the experimental groups and the control group in terms of new bone volume values. These results indicate that the ESWT applied in these parameters is not positively affected. The results of the

stereological examination are consistent with those obtained with DEXA. A temporal and spatial relationship was found between angiogenesis and new bone formation throughout the distraction process (3). The lack of an increase in new bone tissue may be due to the fact that angiogenesis is not induced by ESWT, which provides adequate blood support. In the control group, the neovascularization was found to be significantly higher than that in the experimental groups. Studies in which ESWT induced bone healing reported an increased VEGF and thus neovascularization (16,26). Nonetheless, Özkan et al. reported that unfocused ESWT did not have a positive effect on neovascularization and on the new bone formation in mandibular defect healing (27).

No study has yet examined the efficacy of pre-op applications of ESWT at DO. The answer to the question of what biostimulant method will work best in what phase of distraction is uncertain. However, most researchers reported that biostimulatory methods should be applied during early consolidation (28). The reason for ineffectiveness may be the early application of the ESWT during the healing process. Whereas cartilage tissue is intensely seen in the early stages of distraction, new bone tissue occurs during the consolidation process (29). Freddo et al. reported that the biostimulant method applied during the maturation period caused a further increase in bone hardness and elastic modulus values (30). The other reason for this contradiction between the results of this study and those of other studies may be that ESWT is also applied in the further stages of the consolidation phase of this study. Mature bone may cause more of the shock wave energy to be reflected from the soft–hard tissue boundaries in the later stages of consolidation. Therefore, the stimulation effect of the shock wave will be less in the later stages of consolidation (1).

Conclusion

The ESWT application had no positive effects on the bone maturation during the consolidation phase of DO procedure performed in rats, when applied before osteotomy or both before and after the osteotomy.

Türkçe Öz: *Distraksiyon ssteogenezisi cerrahisinden önce uygulanan odaklanmamış ekstrakorporal şok dalga tedavisinin yeni kemik oluşumuna etkisinin değerlendirilmesi. Amaç: Bu çalışmada osteotomi işleminden önce ve/veya osteotomiden hemen sonra uygulanan ekstrakorporal şok dalga tedavisinin (ESWT) konsolidasyon fazında gözlenen kemik maturasyonuna olan etkisinin incelenmesi amaçlanmıştır. Gereç ve Yöntem: Çalışmada 21 adet dişi New Zealand cinsi tavşan kullanılmıştır. Denekler rastgele olarak 3 gruba ayrılmıştır: Kontrol (Yalnızca Distraksiyon), A Grubu (Osteotomiden sonra ESWT uygulanan), AB (Osteotomiden önce ve sonra ESWT uygulananlar). AB grubuna osteotomiden 5, 12 ve 19 gün sonra ve osteotomiden 7, 14 ve 21 gün önce, A grubuna ise yalnızca osteotomiden 5, 12 ve 19 gün sonra ESWT (500 atım, 5 Hz, 0.19 mJ/mm^2 enerji akış yoğunluğu) uygulaması yapıldı. Konsolidasyon fazının 28. gününde tüm denekler sakrifiye edildi. Kemik mineral içeriğinin ve kemik mineral yoğunluğunun tespit edilmesi amacıyla Dual-energy x-ray absorptiometry (DEXA) kullanıldı. Ayrıca yeni kemik dokusu, bağ dokusu ve yeni damar hacimlerinin tespit edilmesi amacıyla stereolojik methotlar kullanıldı. Bulgular: DEXA incelemesi sonuçlarına göre konsolidasyonun 1. ve 4. haftasında gruplar arasında kemik mineral yoğunluğu ve kemik mineral içeriği açısından anlamlı bir fark görülmemiştir. Stereolojik inceleme sonuçlarına göre ise AB grubunda A grubuna göre, AB ve A grubunda ise kontrol grubuna göre daha yüksek oranda bağ dokusu ve yeni kemik dokusu hacmi görülmüştür. Ancak sonuçlar istatistik olarak anlamlı farklılık göstermemiştir. Yeni damar hacmi açısından ise bir farklılık görülmemiştir. Sonuç: Bu parametreler ile osteotomiden önce ve/veya osteotomiden sonra uygulanan ESWT, konsolidasyon fazında gözlenen kemik matürasyonunda pozitif etkili değildir. Anahtar Kelimeler: Kemik rejenerasyonu; distraksiyon osteogenezisi; dual enerji x-ray absorbsiyometri; ekstrakorporal şok dalga tedavisi; doku hacmi.*

Ethics Committee Approval: This study received ethical approval with the number 2011/65 from the Animal Experiments Local Ethics Committee of OnDokuz Mayıs University.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: ES and MCB designed the study. ES and EO participated in generating the data and gathering the data for the study. MCB and MEO participated in the analysis of the data. EO wrote the majority of the original draft of the paper and participated in writing the paper. All authors approved the final version of this paper.

Conflict of Interest: The author had no conflict of interest to declare.

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Original research

Are age and radiographic features effective on orthodontic alignment of palatally impacted maxillary canines? a retrospective study

Purpose
The purpose of the study was to evaluate the effects of age and radiographic parameters on success of orthodontic alignment of impacted maxillary canines.

Materials and Methods
The retrospective records of 50 patients (mean age 20.44 years) who had impacted maxillary canines in palatal position were included. The patients was requiring surgical exposure and mechanical orthodontic treatment were divided into two groups as adolescent (age≤18; n=24) and adult (age>18; n=26). In both groups, the treatment time and success were evaluated clinically and radiographically.

Results
Fifty patients between the ages of 13 to 42 (12 males and 38 females) with palatally impacted canines were treated with combined surgical-orthodontic approach. Forty-seven teeth (94%) had reacted to surgical exposure and orthodontic alignment within 16 to 36 months with a mean of 24.81 months. Three of the impacted canines (6%) were surgically removed because no movement was observed following 10 months of traction forces.

Conclusion
The distance of the canine tip to the occlusal plane on the lateral cephalometric radiographs have found to be related with the total orthodontic treatment time. Neither the age of the patient nor other clinic and radiographic parameters had influence on the treatment results of alignment of maxillary canines following surgical exposure.

Keywords: *Orthodontic alignment; surgical exposure; impacted maxillary canine; radiographic study; retrospective study*

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Introduction

Maxillary canines (MCs) are the second most common impacted teeth; with an incidence between 0.9% and 2.2% in the general population (1-3). Impaction of MCs have several complications including esthetic and phonetic compromises, loss in arch length, resorption of the adjacent teeth and pain (4, 5). Some clinical symptoms, including the absence of canine bulge in the buccal sulcus, over retained primary cusps, delayed eruption of their permanent tooth and asymmetry of the arch and eruption of the right and left canines, indicate impacted MCs (6).

Treatment opinions could be summarized as no treatment except long-term follow-up by monitoring, autotransplantation of impacted MC, extraction of the tooth followed by orthodontic space closure, prosthetic rehabilitation and surgical exposure followed by orthodontic treatment (7). Exposure is a method of simplifying the eruption of impacted or/

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and malpositioned teeth with orthodontic guiding. It is also the preferred approach for eruption of impacted MCs in medically healthy patients where interceptive measures of dental arch are inappropriate (8). This therapeutic approach is interdisciplinary including orthodontic, periodontal and surgical outcomes.

A grading system has been proposed to determine the severity of the palatal impaction MCs based on the radiographic position. (7). Classification of impacted canines according to radiographic features; the incline of the MC to the midline (α) and long axis of the lateral incisor (β), completion of root formation; have been shown to be predictive factors for the durations of orthodontic traction (9). Four main radiographic predictors believed to correlate with the prognosis for exposure and alignment of impacted canines has been described. These four predictors are angulation of long axis to the midline of the canine, vertical position of the crown from the occlusal plane, anteroposterior position of the root apex relative to the midline, and the degree of overlap of the adjacent incisor by the canine crown tip (7).

The purpose of the retrospective study is to evaluate whether these radiological parameters and patient related factors affect the treatment success and time in surgical alignment of MCs. The null hypothesis tested in this project is that the radiological or patient-related variables do not have any effect on the treatment outcome and duration.

Materials and Methods

Study design

The present research is a retrospective review of 50 patients with impacted MCs surgically treated at Department of Oral and Maxillofacial Surgery between 2007 and 2011. The study protocol was approved by the ethics committee for medical research (2012/522). The clinical notes and panoramic and cephalometric radiographs of the patients whose impacted MCs were surgically exposed and had following orthodontic alignment were enrolled in this study. The presence of single or both palatal impacted MCs requiring surgical exposure and mechanical orthodontic eruption that has adequate records including complete clinical records and treatment notes with pretreatment radiographs were the inclusion criteria of the study. The exclusion criteria were systemic diseases including metabolic and endocrine disorders and not having adequate clinical and radiographic data. The radiographic evaluation and operations were performed by one surgeon.

Orthodontic treatment

The teeth were bonded with 0.022-inch slot pre-adjusted MBTTM system brackets. The extractions were made according to the orthodontic treatment plan. After alignment and leveling, an adequate space was opened for impacted canine and then each impacted canine was exposed surgically by removing the palatal flap. The surgical procedures were performed under local anesthesia in all cases by a maxillofacial surgeon. In case of presence of surrounding bone, it would be removed to expose the enamel surface. A button with a chain or wire was bonded onto the crown of the tooth to apply the orthodontic force. Suturing was performed as original position of tissue allowing the chain or wire extending into the oral cavity. After the healing period of approximately 10 days, orthodontic traction was applied. The orthodontic force was applied with ballista spring or elastic chains. When the tooth was visible, continuous force was applied with light NiTi arch wires to align the teeth. Treatment was finished and the canine was extracted when no response to traction was occurred in 9-10 months.

Study variables

The following clinical data were analyzed: age and gender, classification of occlusion, total orthodontic treatment time, complications including; resorption of the roots of adjacent teeth and secondary surgery due to the button breakage; extraction of canine. The following radiographic data were analyzed: canine angulation (α): the angle between tooth's longitudinal axis and the vertical reference line (midline) (Figure 1) (10), the distance from the canine tip to its target point on the occlusal plane (Figure 2), the rate of root formation, the mesiodistal position of the canine tip in relationship to the adjacent lateral incisor (Figure 3) (11).

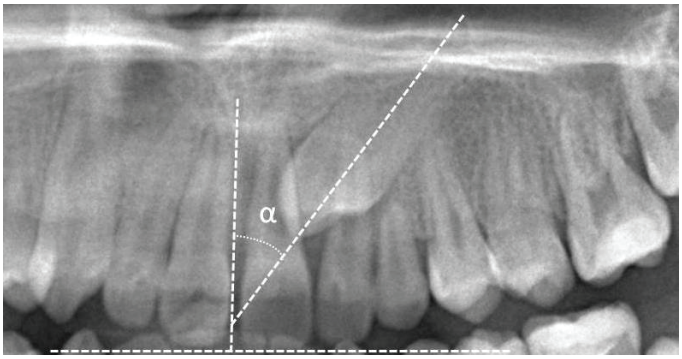

Figure 1. *Determination of canine angulation on panoramic radiograph. The angle between its longitudinal axis and the vertical reference line. Grade 1: 0°–15°; Grade 2: 16°–30°; Grade 3≥ 31°.*

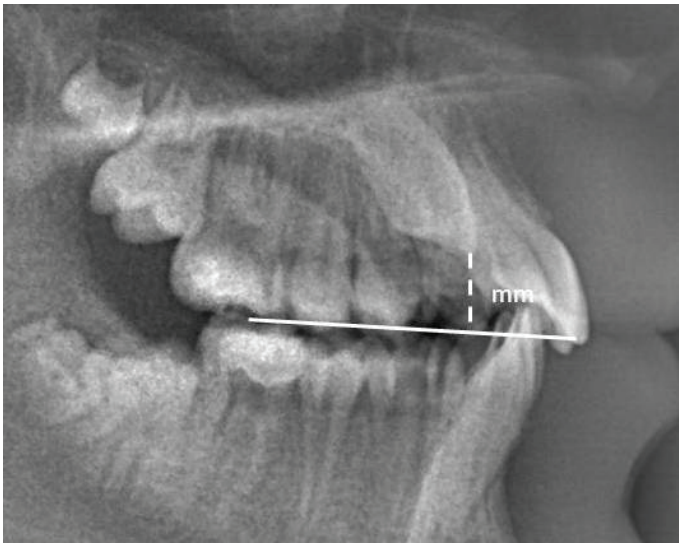

Figure 2. *The measurement of the distance from the canine tip to its target point on the occlusal plane on cephalometric radiography.*

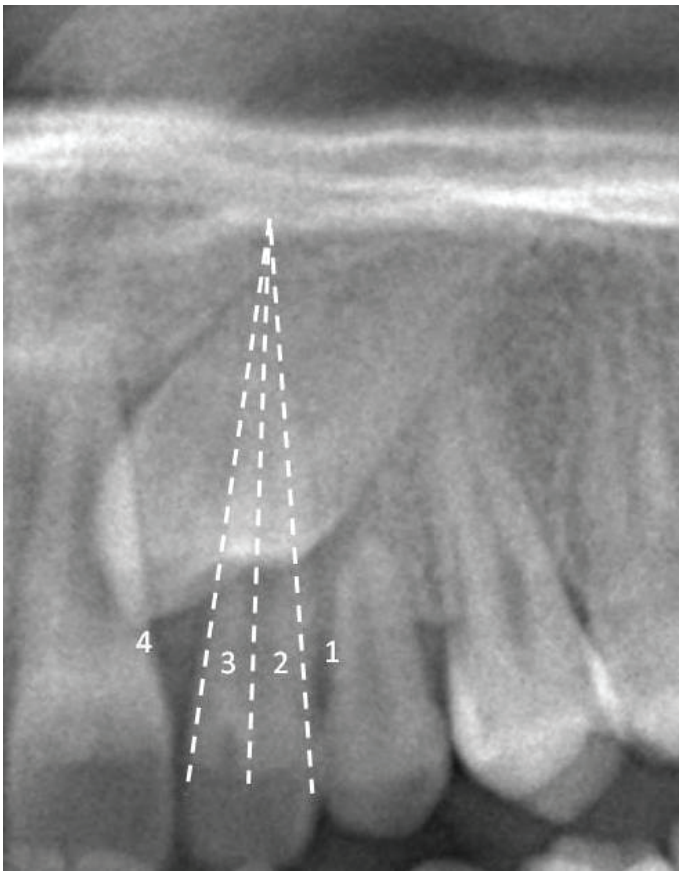

Figure 3. *The determination of mesiodistal position of the canine tip in relationship to the adjacent lateral incisor on panoramic radiography. Grade 1: No horizontal overlap. Grade 2: Less than half the root width. Grade 3: More than half, but less than the whole root width. Grade 4: Complete overlap of root width or more.*

Statistical analysis

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS Inc, Version 13.0, Chicago, IL, USA), the predefined level of statistical significance was *p=0.05*. The statistical analysis of radiographic predictors on treatment duration and success was performed using one way analysis of variance (ANOVA) and Pearson correlation analysis.

Results

The clinical and radiographic data of 50 patients (38 females, 12 males) who had combine surgical and orthodontic treatment for their impacted MCs between 2007 and 2011 were collected. The patients were divided into two groups as adolescent (age≤18; n=24) and adult (age>18; n=26). Thirty-eight of 50 patients were affected by unilateral MC impaction and six patients by bilateral impaction. Twenty-nine of the palatal-impacted MCs were on the left and twenty-one on the right side. However there were no supernumerary teeth or any pathology in any of the cases, twenty-five impermanent canines had retained. The age of the patients when they had surgical experience ranged from 13 to 42 years with a mean of 20±6,3 years. In 6 patients' permanent teeth extraction was needed for the ideal treatment result. The treatment length was ranged from 16 to 36 months with a mean of 24,81±6,3 months.

No irreversible surgical complication was encountered at the pre- and post-operative periods. The most frequently reported post-operative sequel was swelling which was started 24 hours after the surgery and continues 4-5 days postoperatively.

Forty-seven operated MCs were successfully aligned to the dental arch using the closed eruption technique. Three canines were extracted due to the resistance to the traction forces. In three cases, a second surgical operation was needed due to the breakage of the button. Lateral tooth root resorption was occurred in one lateral incisor and was treated with endodontic treatment successfully (Table 1).

Table 1: *Complications seen in the overall treatment period.*

Complications	Percentage
Failed eruption/Extraction of the canine	3 (%6)
Second surgery due to button breakage	3 (%6)
Lateral tooth root resorption	1 (%2)
Total	7 (%14)

No significant relation was found between the adolescent and adult patients by means of duration of orthodontic treatment (*p=0,28*) and occurrence complication (*p=0,26*) (Table 2). The measurement of the distance from the canine tip to its target point on the occlusal plane was found to be correlated positively with the duration of orthodontic treatment (*p=0,304; p=0,01*). No statistical significance was found between the duration of the orthodontic treatment and other radiographic parameters including, canine angulation (*p=0,40*), the mesiodistal position of the canine tip in relation to the neighboring teeth (*p=0,85*), and the rate of root formation (*p=0,16*). No significant relation was found between the complication rate and any of radiologic parameters including, canine inclination (*p=0,55*), the mesiodistal position of the canine tip in relation to the neighboring teeth (*p=0,54*), the rate of root formation (*p=0,34*) and the measurement of the distance from the canine tip to its target point on the occlusal plane (*p=0,36*).

Table 2: *Demographic characteristics and complications that had been recorded in adult and young patients.*

	Age	Male/Female	Duration of canine alignment	Complication without fail	Fail
Adult	24.61 ±6 (n=26)	6/20	23.88±5.96	2	3
Adolescent	15.91±1.7 (n=24)	6/18	25.86 ±6.75	2	0

Discussion

The permanent canine teeth are very important for functional occlusion and esthetics. MCs are the second most frequently impacted tooth due to having a long developmental period, and the most devious path for occlusal plane. (12, 13). Prevalence of MCs impaction ranges from 1.0% to 2.5% (14, 15) where 8.0% to 10.0% (4, 16) of these cases are bilateral. In our study 7% of the cases had bilateral impacted MCs. The duration of treatment to obtain an optimal repositioning of the impacted MCs varies from about 21 to 28 months (17-19). Consistent with the literature the mean treatment duration in our case series was 24,8 months. Patients with impacted MCs seek treatment in a wide range of age group. There have been still controversies in the literature about the success of canine alignment in the adult patients with impacted canines. The literature reveals that; patient's age effects the success of the tooth movement (20). Cappellette et al. (21) reported that canine teeth can be brought into the arch by orthodontic traction following surgical procedure in patients aged 13 to 19 years, however success in elderly patients is lower due to ankylosis risk. Orton et al. (22) claims that the duration of treatments that begin after the end of the pubertal growth period will be longer. Motamedi et al. (7) evaluated 146 impacted canines that undergone surgical exposure and orthodontic alignment and reported that age had no influence on treatment results. However, their study group was consisting 87 cases with a relatively narrow age range of 18 to 24 years. In our study the age range was 13-42 years and we found no correlation between the age and treatment results. We also created two groups as young and adults according to the patients' ages. No differences were found between the young and adult patients by means of the treatment time, complication rate and failure.

In this study, we also aimed to investigate that the position of canine teeth in cephalometric and panoramic radiographs to predict treatment duration and prognosis. The canine angulation was measured with the angle between longitudinal axis and the vertical reference line. Previous studies reported that when the angulations of the impacted canines were increased, the total orthodontic treatment time was also increased (23, 24). The results of our study was contradicted with those past studies. We found no correlation between the canine angulation and the total orthodontic treatment time. It was estimated that the mesiodistal position of the canine tip in relationship to the adjacent lateral incisor is a marker for the treatment success. Motamedi et al. (7) reported that, if the overlap (>half the root) of the adjacent lateral incisor root via the canine crown is increased, it will influence the treatment results negatively. However, we found no significant effect of the canine's mesiodistal position on the treatment results. In this study the only radiologic parameter that was found to be related with the treatment duration, was the distance from the canine tip to its target point on the occlusal plane which was considered as mild correlation. None of other radiological parameters was found to be associated with the success and process of the treatment. Stewart et al. (19) suggested that the distance between the canine tip and occlusal plane might be a factor to estimate the treatment duration. They showed that the distance less than 14 mm exhibits treatment time around 24 months. The result of our study was in compatible with Stewart et al. (19) with an average measurement of the canine tip to its target point on the occlusal plane which was 9,6 mm and the total treatment time of 24,8 months.

Root resorption of the adjacent teeth occurs quite commonly when the incisors are close adjacent to impacted MCs. It was reported that physical proximity (/1 mm) between impacted MCs and adjacent roots is one of the most important predictor associated with root resorption of incisors and the first premolars. The increased risk of root resorption was associated with the eruption motion or migration of impacted MC during the incisors' root development process (25). When evaluated by cone beam computed tomography (CBCT), it was shown that between 27% to 38% of the adjacent laterals and 9% to 23% of adjacent centrals exhibited root resorption associated with the impacted canine tooth (26). Yan et al. (25) was investigated 170 patients with impacted MC and reported an overall prevalence rates of resorption 27%, 18% and 10% at the maxillary lateral incisor, the central incisor, and the first premolar, respectively. In our study root resorption was evaluated by panoramic and periapical radiographs and only 4% of adjacent root resorption were occurred during the treatment period. In our opinion the resorption rate might be found greater if the cases were evaluated with a 3-dimensional CBCT imaging techniques.

Conclusion

The following results may be drawn: [1] no relation was found between young and adult patients by means of treatment duration and prognosis, [2] the canine angulation and classification of mesiodistal position of the canine tip in relation to the adjacent lateral tooth is not related with treatment time and prognosis, [3] the distance of the canine tip to the occlusal plane on the lateral cephalometric radiographs have found to be more related with the total orthodontic treatment time than the radiographic and clinical parameters.

Türkçe Öz: *Palatal gömülü maksiller kanin dişlerin ortodontik sürdürülmesinde yaş ve radyografik özellikler etkili midir? retrospektif bir çalışma. Amaç: Bu çalışmanın amacı, yaş ve radyografik parametrelerin palatal gömülü maksiller kaninlerin ortodontik hizalanmasının başarısına olan etkilerini araştırmaktır. Gereç ve Yöntem: Çalışmaya maksiller gömülü kanini olan 50 hastanın (ortalama yaş: 20.44) retrospektif kayıtları dahil edildi. Cerrahi işlem ve mekanik ortodontik sürdürme gereken palatal gömülü maksiller kaninleri olan hastalar adolesan (yaş 18, n = 24) ve erişkin (yaş = 18; n = 26) olmak üzere iki gruba ayrıldı. Her iki grupta da tedavi süresi ve başarı klinik ve radyografik olarak değerlendirildi. Bulgular: Yaşları 13 ila 42 arasında ve palatal gömülü maksiller kanini olan 50 hasta (12'si erkek 38'i kadın) kombine cerrahi-ortodontik yaklaşımla tedavi edildi. Kırk yedi diş (% 94) cerrahi olarak açığa çıkarılma ve ortodontik hizalamaya 16 ila 36 ay (ortalama 24.81 ay) içinde cevap vermiştir. Gömülü kaninlerden üçü 10 aylık çekme kuvvetini takiben hiçbir hareket gözlenmediğinden cerrahi olarak çıkarılmıştır. Sonuç: Lateral sefalometrik radyografilerdeki kanin dişi tepesinin oklüzal düzleme olan mesafesinin total ortodontik tedavi süresi ile ilişkili olduğu bulunmuştur. Maksiller dişlerin cerrahi olarak açığa çıkarılmasını takiben ortodontik hizalanmasında hastaların yaşı ya da diğer klinik ve radyografik parametler etkili değildir. Anahtar Kelimeler: Ortodontik hizalama; cerrahi açılma; gömülü maksiller kanin; radyografik çalışma; retrospektif çalışma.*

Ethics Committee Approval: The study protocol was approved by the ethics committee for medical research (2012/522).

Informed Consent: Informed consents was provided by the participants' parents.

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Author contributions: SK and BB designed the study. AO, NA and SKa participated in generating the data for the study. AO, NA and SKa 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. SK and AO participated in writing the paper. All authors approved the final version of this paper.

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Original research

Comparison of ISO and ASTM standards in determining the flexural strength of denture base resin

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Purpose
The aim of this study was to compare the differences between the ASTM D790 and ISO 20795.1.2013 standards in evaluating the flexural strength of heat cure poly methyl methacrylate (PMMA) denture base resin.

Materials and Methods
30 heat cure denture base samples were fabricated in accordance to ISO 20795.1.2013 and ASTM D790 Standards. The specimens were finished and stored following the standardized protocol. The flexural strength was determined using universal testing machine at cross head speed of 1.50 mm/min and a span length of 40.00 mm. The mean flexural strength values were calculated in megapascals (MPa), and statistically analyzed.

Results
The mean flexural strength of heat cure PMMA found with ISO and ASTM ranged between 60.492 MPa and 61.470 MPa. There was no significant difference between the two methods.

Conclusion
The quantitative differences existed in the flexural strength of denture base resin between ISO 20795.1.2013 and ASTM 790 protocols but those differences had no statistical and clinical significance.

Keywords: *ISO; ASTM; denture base; poly methyl methacrylate; resin*

Introduction

The flexural strength is the primary mode of evaluation for any additions, reinforcements, modifications and composition changes in denture base materials (1,2). The assessment of acrylic denture bases by bend-testing was first done by National Bureau of Standards in the United States of America. The evolution in the standards and testing equipments has followed from 1930s (3). Sweeney et al. (4), Osborne (5), Souder and Paffenbarger (6) have made significant contributions to the evaluation criteria. The testing techniques such as water cycling machines, continuous loading, and evaluation in dry and moist conditions have evolved over the years (7). Currently, the literature accepts and supports the guidelines of ISO protocol for the evaluation and it is constantly been amended to the needs and requirements. The optimized methodology was adapted and followed by standard organization of all countries. The present guidelines are more structured towards denture base materials applications (8,9).

Numerous studies in the literature evaluated the flexural strength of the various dentures base materials (10-12). However, considerable variations exist in the analysis procedures. Three point bending and four point

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bending tests are commonly employed and few studies have used outdated guidelines (13). The distribution of stress varies between three and four point bending test. The four point bending causes stress distribution whereas three point leads to stress concentration. The difference in standards influence the sample size, shape, analysis procedure and the outcome (2,13,14). Consensus is required in following the particular protocol for dental material analysis (3).

The objective of this study is to evaluate the quantitative differences in the flexural strength in three and four point bending test of heat cure acrylic denture base resin.

Materials and Methods

Sample preparation

The consent and approval for the study was obtained from Institutional review board and ethical committee. The master die for the test samples were prepared in accordance to ISO and ASTM regulation. The dimension of 65mm x 40mm x 5mm die were fabricated for testing ISO 20795.1.2013 samples and 127mm x 12.7mm x 3.2mm die were fabricated for ASTM D790 (8,9). The master die was duplicated with addition silicone impression material (Aquasil soft putty, Dentsply, Germany, Batch no:3162). The duplicated index was used to prepare wax test samples. 30 wax patterns for each group of the above mentioned dimension were prepared for both ISO and ASTM D790 standards. The dimensions of the specimens were verified using a calibrated digital vernier caliper. The wax samples were polymerized by conventional reverse flasking method. Type III gypsum dental stone (Gem stone Mahindra traders Chennai) was used for investing the wax pattern in dental flask. A layer of separating media (cold mold seal) was applied between two investment segments. With the final set of investment stone in the flask, the flask is placed in dewaxing unit to eliminate wax. Any residual wax was manually removed using the hand shower of the same machine. The cavity in the dental flask was used as matrices for the fabrication of heat polymerized acrylic resin specimens. A thin layer of cold mold seal (DPI) was painted over the stone of both flask halves. Heat cure acrylic resin was mixed with monomer in ratio of 3:1 in a porcelain jar. Acrylic resin was packed into the mold space in dough stage. A polythene sheet was placed over the resin and trial closures in the hydraulic press (Hydraulic Press P400, SIRIO Dental SRL) was done to ensure even flow of the resin throughout the mold space. This was repeated until no flash was observed. The flask was then tightened to 100 N using hydraulic press machines and bench curing was done for 20 min. The packed acrylic resin was processed by conventional short polymerization cycle, 70ºC for 90 min and boiled for 1 hour. Once the curing process was finished, the flasks were bench cooled for 30 min. The samples were de-flasked using a wooden mallet and plastic knife. The samples were finished and polished. The test specimens were subjected to grinding with acrylic burs. All the irregularities on the edges were adjusted using conventional acrylic burs by holding the specimen in a low speed dental lathe and followed by fine surface smoothness using 600 grid sand papers. Mechanical polishing performed with pumice slurry and chalk powder in combination of water for 30 s. Group 1 (ISO samples) sample was cut into three equal strips before testing measuring 64mm in length, 10mm in width and 3.3mm in height. The strips were trimmed and polished, all the edges and faces were smoothened and flattened to required size. The dimensions were verified using digital vernier calipers. The test specimens of ISO and ASTM were stored in water at a temperature of 37°C for 50 hours prior to flexural testing (14).

Flexural strength

The flexural strengths of the specimens were determined using a three-point and four-point bending test device in a universal testing machine INSTRON (Autograph universal testing machine, Shimadzu corp, Japan). The ISO specimen (65mm x 40mm x 5mm) were rested on two supports and are loaded by means of a loading nose midway between the supports on the Universal Testing Machine for flexural strength evaluation. Load was applied at the center of the specimen with a cross head speed of 1.50mm/min and a span length of 40.00 mm. The maximum load before fracture was measured. Flexural strength was calculated using the equation (M=3Wl/2bd2). The mean flexural strength of group was calculated, tabulated and the values were statically analyzed (Table 1 and 2)(15).

Table 1: *Descriptive statistics of ISO Vs ASTM test specimen*

Group	N	Mean (MPa)	Standard deviation	Standard deviation error mean
ISO	30	60.492	0.803	0.146
ASTM	30	61.470	1.370	0.250

Table 2: *Independent sample T test*

		T test for equality of mean			
	Sig (2 tailed)	Mean error difference	Standard error difference	95% confidence interval	
				Upper limit	Lower limit
Equal variance assumed	0.001	0.978	.290	0.397	1.558
Equal variance not assumed	0.002	0.978	.290	0.394	1.561

The flexural strength of the ASTM specimen was evaluated by four point bend test. The test specimens (127mm x 12.7mm x 3.2mm) were rested on the cylindrical support arm of the universal testing machine. In order to avoid excessive indentation, or failure due to stress concentration directly under the loading noses, the radii of the loading noses and supports was standardized to 5.0±0.1 mm. The maximum load before fracture was measured. Flexural strength was calculated using the equation S=3PL/4bd2. The mean flexural strength of group was calculated, tabulated and the values were statically analyzed (Table 1 and 2) (3).

Results

The mean flexural strength of was 60.492 MPa and 61.470 MPa for ISO and ASTM specimens. The standard deviation of 0.803 and 1.370 was observed in both ISO and ASTM specimen. The results had 95% confidence interval, 0.310, and 0.529 for both the groups. The distribution was equal and parametric t test was done to analyze the results. The results were statistically significant with P value ≤ 0.001.

Discussion

The flexural strength of heat cure acrylic resin was evaluated in according to ISO 20795.1.2013 and ASTM D 6272 standards (3). These test methods are generally applicable to rigid and semi rigid material. The flexural properties determined by these methods are mostly used for quality control and research (8,9). The study was done to determine the choice and use of appropriate protocol between 3 and 4 point testing protocol.

ASTM is a national organization that is a part of ISO organizations. ISO is an international organization that has representations from all countries including ASTM. ISO establishes documents and updates the standards of testing materials with global consensus from the experts of the associated national organizations. The products thus established are safe, quality and reliable. ISO standards are better valid since it developed and updated to the needs with the opinion of internationally established experts. The initial protocols of ISO had variations in testing procedures. Over the years constant modifications and changes have been made to the needs. Constant efforts have been made to match the testing protocols between the organizations to reduce the duplications of the tests and serve the community better. The standards for the day to determine flexural strength is ISO. Though directions have been issued towards for universal adaptation of latest ISO standards still many literatures employ ANSI or outdated ISO protocols (8,9).

The difference between four and three point bending test exist in specimen size, shape, and thickness, load nose radius, bending momentum, maximum allowable strain and axial stress (3). The test specimen was 65mm x 40mm x 5mm for three-point testing and measurement of 127mm x 12.7mm x 3.2mm for four point bending test testing. In dentistry, the samples for ISO testing were easy to fabricate in regular dental flasks compared to larger specimens of four point bending test. The ASTM samples required larger flasks to fabricate and polymerize PMMA specimen. The variations in samples sizes and protocol do not differentiate the results significantly.

The mean flexural strength of Group ISO is 60.49 MPa and Group ASTM is 61.44 MPa. The results matched the manufacturer and ideal values of flexural strength of denture base materials. The test found no statistical differences between the two methods. But quantitatively ASTM is slightly higher than ISO. Flexural properties in both protocols may vary with in accordance specimen depth, temperature, atmospheric condition and rate of strain. The quantitative variability in this study can be due to the stress distribution. In 4-point bending test the axial stress are uniformly distributed between the loading points compared to 3- point bending test where the maximum axial stress is located immediately under the loading points (9).

The mechanism of stress evaluation can display a minor variation in the strength value. Both the protocols are reliable testing methods. The test sensitivity is less in 4 point compared to 3 point bending test. This makes the 4 point test more ideal for composite and brittle materials. Literatures have determined 10% variations between the ASTM and older ISO protocols (9) . ISO has adapted and modified to the needs of the situations and for dental materials it is more ideal in terms of sample fabrication to mechanical testing (8).

The study evaluated the conventional heat cure specimens without any modifications to the compositions. Further studies are required to determine the influence of testing protocol with changes in composition, reinforcement, composite materials of PMMA and the influence in the testing protocols.

Conclusion

A comparison of the results from three-point and four-point bend tests of denture-base polymers showed no significant statistically and clinical differences in the flexural strength. However, flexural strength values were higher in four-point bending than in three-point bending.

Türkçe Öz: *Protez kaide reçinelerinin bükülme dayanımının belirlenmesinde ISO ve ASTM standartlarının karşılaştırılması. Amaç: Isı ile polimerize olan polimetilmetakrilat (PMMA) protez kaide reçinelerinin bükülme dayanımının belirlenmesinde kullanılan metodoloji standartlarında farklılıklar mevcuttur. ASTM ve farklı ISO standartları, materyalin bükülme dayanımını belirlemek için literatürde uyarlanan protokollerdir. Etkili, kabul edilebilir ve standartlar arası farklılıkları belirlemeye ihtiyaç duyulmaktadır. Gereç ve Yöntem: Bu çalışmanın amacı, protez kaide reçinelerinin bükülme dayanımının değerlendirmek için ideal standardın belirlenmesidir. Amaç, ısı ile polimerize olan PMMA protez kaide reçinelerinin bükülme dayanımlarını ölçmek için kullanılan ASTM D790 ve ISO 20795.1.2013 arasındaki farkları karşılaştırmaktır. 30 adet ısı ile polimerize olan protez kaide örneği ISO 20795.1.2013 ve ASTM D790 standartlarına uygun olarak üretilmiştir. Örnekler rutin olarak kullanılan protocol ile bitirilip muhafaza edilmiştir. Bükülme dayanımı, 1.50 mm / dak yaklaşma hızında ve 40.00 mm bir açıklık uzunluğunda üniversal test makinesi kullanılarak belirlenmiştir. Ortalama bükülme dayanımı değerleri MPa olarak elde edilmiş, tabloya aktarılmış ve student t testi ile istatistiksel olarak analiz edilmiştir. Bulgular: ISO ve ASTM tarafından bulunan ısı ile polimerize olan PMMA'nın ortalama bükülme dayanım değerleri, 60.492 MPa ve 61.470 MPa arasında değişmiştir. İki yöntem arasında anlamlı fark bulunmamıştır. Sonuçlar istatistiksel olarak P ≤ 0.05 anlamlı bulunmamıştır. Sonuç: Isı ile polimerize olan PMMA protez kaide reçinelerinin bükülme dayanım değerlerinde ISO 20795.1.2013 ve ASTM 790 protokolleri arasındaki sayısal farklılıklar mevcuttur. Fakat bu farklılıklar, istatistiksel ve klinik olarak anlamlı değildir. Anahtar kelimeler: ISO; ASTM; protez kaide reçinesi; polimetilmetakrilat.*

Peer-review: Externally peer-reviewed.

Author contributions: NGC, VJ and VS designed the study. NGC and VS participated in generating the data for the study. NGC and VS participated in gathering the data for the study. NGC and VS participated in the analysis of the data. NGC wrote the majority of the original draft of the paper. NGC, VJ and VS participated in writing the paper. All authors approved the final version of this paper.

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Original research

Effect of commonly used irrigants on the colour stabilities of two calcium-silicate based material

Trusha Shah[1], **Kulvinder Singh Banga**[1]

Purpose
The aim of present study was to evaluate the color stability of calcium-silicate based cements (CSC) Mineral Trioxide Aggregate (MTA) and Biodentine™ when exposed to endodontic irrigating solutions 5% Sodium hypochlorite (NaOCl) or 2% Chlorhexidine (CHX).

Materials and Methods
A total of 60 (n=30) cylindrical samples (10 mm diameter, 2 mm height) were prepared by manipulating white MTA Angelus (Angelus, Londrina, PR, Brazil) and Biodentine™ (Septodent, Saint Maur, France) according to manufacturer's instructions. These samples were immersed in 5% sodium hypochlorite (Prime Dental Products Pvt. Ltd., Mumbai, India), 2% chlorhexidine gluconate (Dentochlor, Saronno VA, Italia), or distilled water for 24 hours. Color changes were measured using UV spectrophotometer (UV-1650, Shimadzu, Europe) and the values were tabulated.

Results
A significant difference was observed between group I and II with respect to both parameters A & B (p<0.05). Both the calcium-silicate-based materials exhibited significant discoloration when immersed in NaOCl and CHX. Distilled water did not cause clinically perceptible discoloration of any material.

Conclusion
A significant discoloration was observed with a specific combination of calcium-silicate-based cement and irrigant. Biodentine™ exhibited significant discoloration with CHX whereas, MTA showed more discoloration with NaOCl.

Keywords: *Calcium silicate; chlorhexidine, irrigants; mineral trioxide aggregate; sodium hypochlorite*

Introduction

Over 24 million endodontic procedures are performed worldwide on an annual basis, with up to 5.5% of those procedure involving endodontic apical surgery, perforation repair, and apexification (1). Many materials have been employed for these procedures like; calcium hydroxide, tricalcium phosphate, tetracalcium phosphate, mineral trioxide aggregate (MTA), resin-modified glass ionomer cement, and intermediate restorative material. Calcium-silicate-based materials have gained popularity in recent years due to their various clinical applications. Calcium-silicate-based materials have been proven to be beneficial for various procedures involving pulpal regeneration and hard tissue repair, such as pulp capping, pulpotomy, apexogenesis, apexification, perforation repair, and root-end filling owing to their sealing ability and biocompatibility (2).

MTA is composed of modified Portland cement with added bismuth oxide. MTA is a biomaterial that has been investigated for endodontic applications since the early 1990s (3). MTA is biocompatible and has

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antibacterial properties. It is a bioactive cement originally designed as an endodontic repair and root-end filling material with favorable physical properties and setting characteristics. The indications and clinical applications for MTA have expanded considerably (3,4). Owing to its high alkalinity, it has the ability to induce release of bioactive dentin matrix proteins (5). MTA exhibits good sealing ability, most likely due to a physical bond created by a layer of hydroxyapatite between MTA and dentin (6).

However, MTA has certain drawbacks such as long setting time, difficult handling property and discoloration (4). Biodentine™, a newly developed tricalcium-silicate cement, became commercially available in 2009 to overcome these drawbacks (7). As these calcium-silicate-based materials are similar to MTA in basic composition, they have gained popularity in recent year (8). Biodentine™ has drawn attention in the recent years and has been advocated to be used in various clinical applications that would typically utilize MTA (9). Apart from the various clinical applications, MTA has been reported to cause tooth discoloration when applied in the esthetic zone (10).

Tooth discoloration induced by endodontic materials is a commonly occurring issue (11). Tooth discoloration after endodontic therapy is mainly caused due to blood, necrotic pulp tissue, and endodontic materials penetrating the dentinal tubules (12). There is limited data thus far on color stability of calcium-silicate–based materials. Hence, the current study was devised that aimed to evaluate the color stability of two widely used calcium-silicate based materials (MTA and Biodentine™) when in contact with commonly used irrigating solutions (Sodium hypochlorite and Chlorhexidine). Distilled water served as a negative control for the study.

Materials and Methods

Sample preparation

Two groups of materials were tested in the current study, with 3 subgroups for each material (for irrigant treatment). The materials tested were: wMTA Angelus (Angelus, Londrina, PR, Brazil) and Biodentine™ (Septodent, Saint Maur, France). The irrigant treatment for the above mentioned 2 materials were: 5% sodium hypochlorite (Prime Dental Products Pvt. Ltd., Mumbai, India), 2% chlorhexidine gluconate (Dentochlor, Saronno VA, Italia), and distilled water.

The test materials (wMTA and Biodentine™) were mixed homogeneously following each manufacturer's instructions, and cylindrical specimens were obtained by using moulds of 10mm diameter and 2mm height (Figure 1). The specimens were then stored at 37°C and 100% humidity for the materials to reach their optimal mechanical properties. Following the complete setting of the materials (wMTA: 10 min, and Biodentine™: 10–12 min), the set specimens were immersed for 24 hours; in one of the tree different irrigating solutions (Figure 2). The groups for the current study were as follows: Group I wMTA, and group II Biodentine™ with sub-groups A, B, and C in which the specimens were immersed in 5% NaOCl, 2% CHX, and distilled water, respectively.

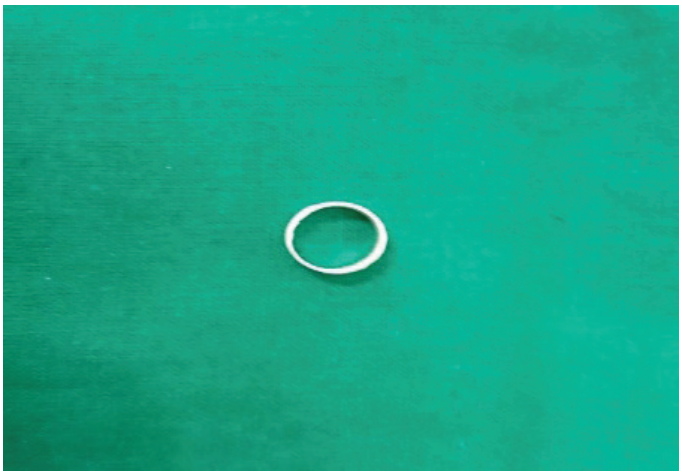

Figure 1. *Mold for sample preparation.*

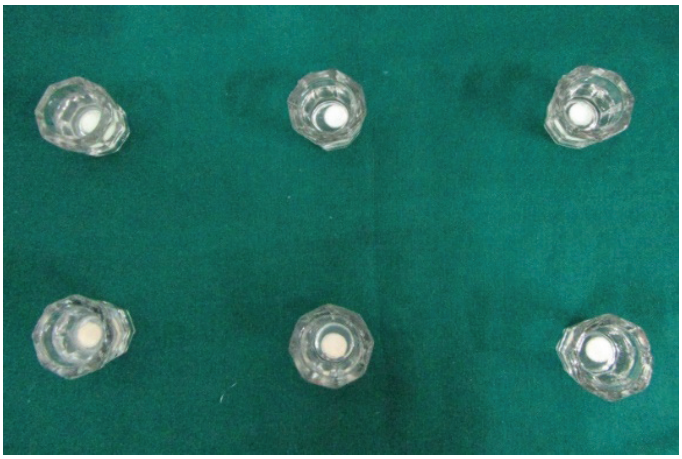

Figure 2. *Specimens immersed in irrigants.*

Spectrophotometric analysis

The specimens were allowed to dry completely before testing them in the UV spectrophotometer (Figure 3). Spectrophotometer (UV-1650, Shimadzu, Europe) was used to measure color under constant laboratory light by the same operator. Spectrophotometric analysis was applied because of the technique's repeatability, objectivity, and sensitivity to small changes in color (13). Images of the samples were taken before and after immersion using a digital camera.

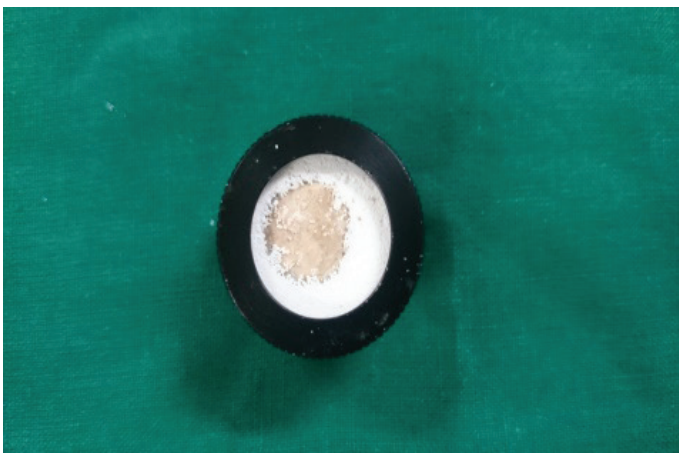

Figure 3. *Specimen preparation before placing in the spectrophotometer.*

Statistical analysis

The data collected was graphically represented as shown. (Figure 4) The data was evaluated with Kruskall Wallis ANOVA by using Statistical package for social sciences (SPSS v 22.0, IBM). The significant effects and interactions were further investigated using Mann Whitney U test for pair wise comparison.

For all the statistical tests, $p<0.05$ was considered to be statistically significant. A significant difference was observed between group I and II with respect to both parameters A & B ($p<0.05$)

Results

The groups tested in the current study exhibited significant color changes. The mean values for each group were calculated and are plotted in Figure 4. Group IA was associated with (0.191667 ± 0.0140119), group IB (0.033667 ± 0.0080208), group IC (0.013000 ± 0.0055678), group IIA (0.100067 ± 0.0090738), group IIB (0.291333 ± 0.0173877), group IIC (0.019 ± 0.0145258). MTA exhibited more discoloration when immersed in Sodium hypochlorite; as compared to Chlorhexidine (p value <0.05). Whereas Biodentine™ exhibited more discoloration when immersed in Chlorhexidine solution (p value <0.05), as compared to Sodium hypochlorite. Distilled water (control group) did not cause clinically perceptible discoloration of any material.

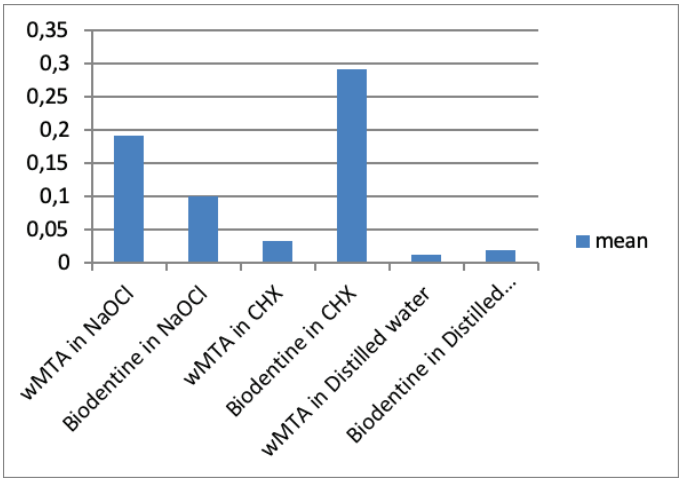

Figure 4. *NaOCL: Sodium hypochlorite, CHX: Chlorhexidine.*

Discussion

This study was performed to provide detailed information regarding the color stability of calcium-silicate-based cements when in contact with common irrigating solutions. Color is one of the most important properties to be observed during dental procedures involving teeth in aesthetic areas. Color changes in dental materials can be measured with specific instruments (14). Visual spectrophotometry is a gold standard method used in dentistry because of the technique's sensitivity to small changes in color, repeatability and objectivity (14).

Calcium-silicate-based cements (CSC), including mineral trioxide aggregate (MTA), are self-setting hydraulic cements (15). The powder of CSC is composed mainly of dicalcium and tricalcium-silicate. After mixing the powder with water, Ca(OH)2 and calcium-silicate hydrate are produced primarily, and the mix forms a sticky colloidal gel (calcium-silicate hydrate gel) that eventually solidifies to a hard structure (16). Calcium-silicate-based cements are used commonly in endodontic procedures involving pulpal regeneration and hard tissue repair, such as pulp capping, pulpotomy, apexogenesis, apexification, perforation repair, and root-end filling (17). The sealing ability and biocompatibility of CSC, in addition to physicochemical interaction with the local environment, are believed to be primary factors contributing to their suitability in the aforementioned clinical situations (18,19).

Mineral trioxide aggregate (MTA) is a biomaterial that has been investigated for endodontic applications since the early 1990s. MTA materials have been demonstrated to be biocompatible endodontic repair materials, with its biocompatible nature strongly suggested by its ability to form hydroxyapatite when exposed to physiologic solutions. MTA possesses biocompatibility, high alkalinity and anti-bacterial properties (20). The initially introduced MTA was grey. Although previous studies have reported frequent discoloration of dentinal tissue with grey MTA (21) Bismuth oxide the radiopacifier present in MTA composition, has been suggested as the chemical compound involved the discoloration verified for this material. To overcome these shortcomings, white MTA was introduced. However, in this study wMTA exhibited significant discoloration with sodium hypochlorite. These results were in accordance with results of previous studies (22,23).

Biodentine™, a new bioactive calcium-silicate-based cement has been introduced in the dental market as a 'dentin substitute' (24). This new biologically active material aids its penetration through opened dentinal tubules to crystallize interlocking with dentin and provide mechanical properties. Biodentine™ has been formulated using MTA-based cement technology and hence; claims improvements of some of the properties such as physical qualities and handling, including its other wide range of applications like endodontic repair and pulp capping in restorative dentistry (25) Biodentine™ contains zirconium oxide as radiopacifier instead of bismuth oxide in MTA. Very few studies have been conducted with respect to material discoloration of Biodentine™.

NaOCl is one of the most commonly used irrigating solutions. NaOCl has a tendency to crystallise and occlude the dentinal tubules; thus, may not be completely removed from the root canals [14]. Chlorhexidine digluconate possesses broad spectrum antimicrobial activity against most endodontic pathogens. As per the results of this current study, chlorhexidine exhibited significant discoloration of Biodentine™. This may be attributed to its property of substantivity, although more research is needed to verify the etiology of discoloration. The results obtained from present study exhibit that CHX and NaOCl cause considerable discoloration. These results are in congruence with previously reported studies (26,27).

Bhavya B et al. (27), attributed the contact of bismuth containing substances to NaOCl for the discoloration in their study. Camilleri J (28) recently reported that contact of wMTA and other bismuth-containing materials with NaOCl produces a change to a darker color because the oxide is converted to bismuth metal in contact with sodium

hypochlorite and oxygen is lost. The mechanism of material discoloration with CHX is explored sparsely thus far. However, CHX has been reported to cause extrinsic discoloration of silicate filling materials and dental tissues at various concentrations by influencing dental pellicle or plaque (29). Also, the property of substantivity exhibited by CHX signifies prolonged interaction of CHX with dental materials. In our study specimens were immersed in irrigation solutions for 24 hours to duplicate prolonged contact of these calcium-silicate-based materials and the irrigating solutions.

However, it is important to note significant discoloration of specific combinations of calcium-silicate cement and irrigating solutions. In accordance with the results of our study, wMTA exhibited significant discoloration with NaOCl, whereas maximum discoloration was observed when Biodentine™ was immersed in CHX.

It was proposed by Camilleri et al. (30), that the discoloration induced by calcium-silicate-based materials can be prevented by the application of a double layer of the dentin bonding agent in the access cavity. Koubi et al. (9), reported that it may be prevented by treating with internal bleaching.

However, the calcium silicate cements in this study were immersed in irrigating solutions for 24 hours, which does not mimic the clinical scenario. Also this study emphasizes upon material discoloration over tooth discoloration. Thus owing to the limitations of this study; more studies are recommended that would mimic clinical conditions pertaining to tooth discoloration.

Conclusion

Calcium-silicate-based cements (wMTA & Biodentine™) showed significant material discoloration when in contact with commonly used irritating solutions (NaOCl & CHX). Thus, in aesthetically critical regions, it becomes imperative to wisely choose the combination of irrigant and calcium-silicate-based cement. In the present study, maximum discoloration was observed when Biodentine™ was immersed in CHX. However, wMTA exhibited significant discoloration with NaOCl. Thus, these combinations must be avoided. Further studies are needed to derive the clinical reflections of this finding to suggest optimal material that fulfils both functional an esthetic criteria.

Türtçe Öz: *Sık kullanılan kanal yıkama çözeltilerinin iki kalsiyum silikat esaslı simanın renk stabilitesi üzerindeki etkileri. Amaç: Bu çalışmanın amacı kalsiyum silikat esaslı simanlar (KSS) olan Mineral Trioksit Agregat (MTA) ve Biodentin'in renk stabiliteleri üzerinde %5'lik sodium hipoklorit (NaOCl) ve %2'lik klorheksidin (CHX) kanal yıkama çözeltilerinin etkilerini incelemektir. Gereç ve Yöntem: Üretici firmaların önerilerine uygun olarak MTA Angelus (Angelus, Londrina, PR, Brezilya) Biodentine™ (Septodent, Saint Maur, Fransa) simanlarından toplam 60 (n=30) adet silindirik şekilli numune (10 mm çapında, 2 mm yüksekliğinde) hazırlanmıştır. Numuneler 24 saat boyunca %5'lik sodium hipoklorit (Prime Dental Products Pvt. Ltd., Mumbai, Hindistan), %2 klorheksidin glukonat (Dentochlor, Saronno VA, İtalya) ya da distile suda bekletilmiştir. Renk değişimleri UV Spektrofotometre cihazı (UV-1650, Shimadzu, Avrupa) ile ölçülmüş ve elde edilen bulgular karşılaştırılmıştır. Bulgular: 1. ve 2. gruplar arasında A ve B parametrelerinde istatistisel olarak anlamlı bir fark olduğu gözlenmiştir ($p<0.05$). Her iki kalsiyum silikat esaslı siman da NaOCl ve CHX çözeltilerinde bekletildiklerinde anlamlı renkleşme göstermişlerdir. Distile su numunelerde klinik olarak fark edilebilir bir renk değişimine sebep olmamıştır. Sonuç: Kalsiyum silikat esaslı simanların tipine göre anlamlı seviyede renk değişimi meydana geldiği izlenmiştir. Biodentine CHX çözeltisi ile belirgin bir renkleşme gösterirken, MTA ise NaOCl çözeltesinde daha fazla renkleşmiştir. Anahtar kelimeler: Kalsiyum silikat; klorheksidin, kanal yıkama çözeltisi; mineral trioksit agregat; sodium hipoklorit*

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: TS and KSB designed the study. KSB participated in generating the data for the study. TS participated in gathering the data for the study. KSB participated in the analysis of the data. TS wrote the majority of the original draft of the paper. TS and KSB participated in writing the paper. All authors approved the final version of this paper.

Conflict of Interest: The author had no conflict of interest to declare.

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Üstün K.
Xiao K.
Yaman D.
Yetkiner E.
Yıldırım H. S.


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