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REVIEW

The Effect of Minimally Invasive Treatments on Enamel Microhardness and Resistance to Further Demineralization

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Research Article

ABSTRACT

Objectives: This study compared the microhardness of inactive proximal lesions after different remineralizing treatments, and investigated the resistance of treated lesions to a further demineralization challenge.
Materials and Methods: In this in-vitro study, 30 human molars with inactive proximal lesions were selected and randomly divided into three groups of 10. In group 1, the lesions were treated with a resin infiltrant (Icon). In group 2, the surface was conditioned by an Er:YAG laser before resin infiltration. The specimens in group 3 were remineralized by the bioactive glass. The specimens were kept in artificial saliva for 1 week and then immersed in a demineralization solution for 8 weeks. Microhardness was measured at baseline (T0), after remineralization (T1), and after exposure to the demineralization (T2) solution. Hardness values were compared between the treatment intervals in each group. The alterations in microhardness after the treatment and the demineralization challenge (ΔVHN) were calculated and compared among the groups.
Results: In all groups, microhardness after demineralization was significantly lower than other intervals, but no significant difference was found between the T0 and T1 values (P>0.05). The alterations in microhardness between T1 and T0 (ΔVHN$_{T1-T0}$) and between T2 and T1 (ΔVHN$_{T2-T1}$) were not significantly different among the groups.
Conclusions: Pretreatment by Er:YAG laser before resin infiltration was more effective than other treatments in enhancing microhardness and protecting the tooth against the acidic challenge. However, the difference between groups did not reach statistical significance, implying the need for further studies to achieve more conclusive results.
Keywords: Bioactive glass, proximal caries, Er:YAG laser, remineralization, resin infiltration .

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Introduction

Restorative therapy for an interproximal lesion usually involves the removal of the marginal ridge, which inevitably compromises the strength of the residual tooth structure.[1] Therefore, special attention should be paid to conservative treatment of early proximal lesions to preserve tooth integrity. Even easily detectable proximal caries in radiography may be associated with an intact enamel surface[2], making non-invasive or minimally-invasive treatments the ideal treatment options. The non-invasive therapy has been defined as reducing caries activity and remineralizing the enamel lesion through oral hygiene instructions, diet education, and the application of remineralization agents such as fluoride or casein phosphopeptide amorphous calcium phosphate.[3,4] On the other hand, minimally invasive therapy involves mechanical blocking or sealing of the lesion via the placement of resin-based sealants or infiltrates.[5] It is believed that the application of minimally invasive therapy for early proximal caries is more sensible in patients with high caries risk, due to the greater progression speed of infection in these cases, as compared to the normal population.

Resin infiltration is a minimally invasive treatment for initial dental caries.[6] This technique is particularly recommended to postpone the need for restoration when caries have been extended into the inner half of enamel or the outer one-third of dentin.[7] It is believed that the pores within the enamel and dentin caries could be filled by the resin infiltrant, thus enhancing tooth strength and preventing lesion progression. Several systematic reviews have shown that resin infiltration or surface sealing has a better therapeutic effect on arresting lesion progression compared to non-invasive treatments.[7-11]

Lasers can be employed for numerous procedures in dentistry including caries prevention and treatment. The erbium family lasers including erbium:yttrium–aluminum–garnet (Er:YAG) and erbium, chromium: yttrium–scandium–gallium–garnet (Er,Cr:YSGG) are mainly absorbed by water and hydroxyapatite, making them applicable for hard tissue treatment.[12,13,14] Erbium lasers have been employed for the surface conditioning of enamel and dentin before restorative treatments[15,16] or cleaning and sterilizing enamel fissures before sealant therapy.[17] It is

also possible to use erbium lasers for the pretreatment of initial caries lesions before resin infiltration therapy to create a rough and micro-fractured pattern on the surface, which possibly enhances resin penetration.

Bioactive glass (BAG) is a relatively new and biocompatible agent that has been applied in many healthcare fields.[5] The first bioactive glass introduced in 1969 was a sodium, calcium, and phosphorus silicate glass.[5] Currently, there are different types of bioactive glasses, such as silicate-based glass and phosphate-based glass.[5] It has been demonstrated that the bioactive glass could be effective in preventing and arresting dental caries through different mechanisms including the antibacterial effect on cariogenic bacteria, inhibition of tooth demineralization, and promotion of remineralization.[5] It is assumed that bioactive glasses are dissolved after being implanted in the human body and the accumulation of dissolved particles not only alters the chemical composition and pH of the environment but also creates a layer of hydroxyapatite or hydroxycarbonate apatite (HCA) on the surface, which enhances remineralization.[18]

There are few studies concerning the comparison of resin infiltration and bioactive glass for the treatment of arrested proximal lesions.[19] In addition, no study evaluated the effect of pretreatment by Er:YAG laser on the results of resin infiltration. Therefore, the present study was conducted to compare the microhardness of inactive proximal lesions treated by resin infiltration, Er:YAG laser conditioning + resin infiltration, and bioactive glass, and investigate the resistance of treated lesions to a further demineralization challenge. The null hypothesis of this study was that there is no difference in microhardness and resistance to demineralization of proximal lesions treated by different remineralization methods.

Materials and Methods

Specimen preparation

This in-vitro study was conducted with the permission of the Ethics Committee of Mashhad University of Medical Sciences. Thirty human maxillary or mandibular molars with inactive proximal caries lesions were selected. The sample size was estimated as n=9, according to the data extracted from Torres *et al.*[20], using an alpha significance level of 0.05 and a beta of 0.9. The sample size was then increased to 10 teeth per group.

The Nyvad Criteria (visual-tactile clinical examination)[21], was used to detect inactive white spot lesions. Accordingly, lesions with matt and rough enamel were classified as active, whereas those with a glossy and smooth appearance were classified as inactive lesions, and selected for experimentation. The teeth were cleaned of any residual tissue with a water slurry of pumice and brush and then were kept in a 0.1% thymol solution at room temperature.

The teeth were cut in the buccolingual direction by a low-speed water-cooled diamond saw parallel to the proximal surface of the tooth. In this way, thirty disks containing carious proximal surfaces were created. The disks were then mounted in epoxy resin with the WSL

surface parallel to the ground. The proximal surface was then polished with 800- and 1200-grit silicon carbide sandpapers (AsiaSayesh, Iran) to create a "window" containing WSL for further treatments.

Treatment procedures

The specimens were randomly assigned into three groups of 10 each; then underwent the following treatments:

Group 1 (resin infiltration): In this group, a resin infiltrant (Icon, DMG, Hamburg, Germany) was applied to proximal lesions according to the manufacturer's instructions. The dental proximal surface was etched with hydrochloric acid 15% for 120 seconds, then washed and dried for 30 seconds. After that, ethanol 95% (Icon-Dry) was applied for 30 seconds to dehydrate the surface and air dried. The lesion was coated by the Icon-infiltrant twice, the first one for 180 seconds, and the second for 60 seconds.[1] After each application, the infiltrant was light-cured for 40 seconds from different directions. Finally, the specimens were polished for 20 seconds using 4000- grit, aluminum oxide abrasive papers.

Group 2 (Er:YAG laser conditioning + resin infiltration): In this group, Er:YAG laser (KaVo Key Laser 3, KaVo Co., Biberach, Germany) was applied for surface conditioning before resin infiltration. The laser emitted photons at a wavelength of 2.94 μm and was set at the energy of 50 mJ, and frequency of 10 HZ, using air/water spray.[13] The tip was positioned at a distance of 1 mm from the enamel surface (focused mode) and the window was irradiated through scanning movements for 10 seconds. The resin infiltration was then performed similarly to that explained in group 1.

Group 3 (bioactive glass): In this group, the bioactive glass was applied on the surface of the samples. The glass was prepared by mixing a tenth of a gram of Bioglass 45S5 (Nikceram, Iran; containing 24.5 wt% Na2O, 24.4 wt% CaO, 6 wt% P2O, and 5.45 wt% SiO2) with 0.2 ml of phosphoric acid 50%, for one minute, creating a substance with the consistency of the dough. The phosphoric acid-bioactive glass gel was applied on the samples by a micro-brush; then covered by a bonding layer (Clearfil SE Bond, Kuraray Medical, Tokyo, Japan), and cured for 20 seconds.

The treated specimens in all groups were stored in artificial saliva for one week. The artificial saliva used in this experiment contained 4.3 g xylitol, 1 g sodium carboxymethylcellulose, 0.1 g potassium chloride, 40 mg potassium phosphate, 1 mg potassium thiocyanate, 5 mg calcium chloride, and 100 g distilled deionized water.[22]

After 1-week storage in artificial saliva, the treated samples were immersed in a demineralization solution for 8 weeks. This solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2Po_4, and 50 mM acetic acid, with PH adjusted at 4.8. Each sample was individually placed in the demineralization solution and the solution was changed weekly.

Microhardness assessment

A Vickers microhardness tester (model MH3, Koopa Pazhoohesh, Iran) was employed to measure the microhardness of the specimens at baseline (T0), after the

treatment and 1-week storage in artificial saliva (T1) and after exposure to the demineralization solution (T2). Two indentations were made, 100 μm apart, at the center of the treatment window using a load of 100 g applied for 10 seconds, and the mean value was recorded as the Vickers hardness number (VHN) for that specimen. Hardness values were compared between the treatment intervals in each group. Furthermore, the alterations in microhardness after the remineralizing treatment (ΔVHN_{T1-T0}) and the demineralization challenge (ΔVHN_{T2-T1}) were calculated and compared among the groups.

Statistical analysis

The normality of the data was evaluated using the Shapiro-Wilk test, which revealed that only ΔVHN values followed the normal distribution (P > 0.05). The intragroup comparisons of hardness values were made by the Friedman test, followed by Dunn's test for pairwise comparisons. One-way analysis of variance (ANOVA) was run to detect any significant difference in ΔVHN values among the three groups. The statistical analysis was performed through SPSS software (version 16.0; SPSS In., Chicago, Il), and the significance level was set at P<0.05.

Results

Table 1 presents the mean and standard deviation (SD) of microhardness values at baseline (T0), after various surface treatments (T1), and after exposure to the demineralization solution (T2) in the study groups. Microhardness increased after treatments and decreased following exposure to the acidic challenge. Friedman test displayed a significant alteration in VHN values throughout the experiment in all the study groups (P<0.005). Further analysis by the Dunn test revealed that microhardness after demineralization (T2) was significantly lower than the T0 and T1 values, but no significant difference was found in VHN between the T0 and T1 time points in any of the treatment groups.

Table 2 presents the alterations in the Vickers hardness number after the remineralizing treatment (ΔVHN_{T1-T0}) and the demineralization challenge (ΔVHN_{T2-T1}). The greatest enhancement in microhardness after the remineralization treatment belonged to the Er:YAG laser conditioning + resin infiltration group and the lowest to the bioactive glass group. Following exposure to the demineralization solution, the greatest loss in microhardness was observed in the resin infiltration group and the lowest was found in the specimens treated by Er:YAG laser + resin infiltration. The statistical analysis failed to reveal a significant difference either in ΔVHN_{T1-T0} or in ΔVHN_{T2-T1} among the study groups (P >0.05; Table 2)

Discussion

The present study compared the effects of resin infiltration, Er:YAG laser conditioning + resin infiltration, and bioactive glass for remineralization of arrested caries lesions. Hardness testing is a simple and accurate method to detect changes in the mineral content of teeth and has been widely used in the literature.[23,24] Based on the findings of this study, pretreatment of enamel caries by Er:YAG laser followed by resin infiltration caused the highest increase in microhardness and created the greatest resistance against the demineralization attack. However, the difference between groups was not statistically significant, possibly due to the small sample size and great variations in the hardness values. So the null hypothesis of this study was accepted, implying that there is no difference in microhardness and resistance to demineralization of proximal lesions treated by resin infiltration, Er:YAG laser conditioning + resin infiltration, or bioactive glass.

During Resin infiltration, a low-viscosity resin fills the pores within the demineralized enamel to hamper further caries progression.[6] Resin infiltration also strengthens the enamel structure and thus prevents cavitation.[21] Icon is a methacrylate-based resin containing TEG-DMA, Bis-GMA, initiators, and solvents. The presence of TEG-DMA leads to the high elasticity of the resin[25], whereas Bis-GMA reduces polymerization shrinkage and increases lesion hardness due to its greater molecular weight. Adding ethanol as the solvent improves the permeability of the resin by increasing the penetration coefficient.[26] In the present study, the infiltrant was applied twice; because repeated resin application is assumed to enhance lesion microhardness and provide beneficial effects on demineralization resistance.[25] It should be noted that proximal infiltrated lesions do not withstand chewing forces.[25] Therefore, an excessively high microhardness is not required at the proximal surface, but resin infiltration should provide sufficient microhardness to restore the proximal contact, provide resistance against proximal attrition and abrasion, and prevent lesion cavitation.[26] In the present study, resin infiltration did not show a significant superiority compared to the other techniques for enhancing remineralization and improving the resistance of treated lesions to acidic attack. Paris *et al.*[27] also reported that some demineralization can still occur after exposure to a new acidic challenge in lesions infiltrated with Icon. They attributed this finding to the incomplete inclusion of some resin minerals in the lesion or the occurrence of resin shrinkage during light curing, which leads to leakage and thus reduction in acid resistance of the substrate.[21]

The caries prevention effects of lasers have been demonstrated in previous studies and explained through different mechanisms. The laser absorption in tooth tissue can cause physical and chemical changes through the oxidation of organic components, conversion of acid phosphate to pyrophosphates, and reduction of carbonate content.[13,29] The caries-preventive effects of erbium lasers may be related to the induction of physical and structural changes in enamel and dentin and also to the creation of a rough and etched surface, which could increase the absorption and penetration of mineral agents.[30] Laser etching combined with conventional etching has also revealed satisfactory results in enhancing

bond strength to the tooth structure.[31-34] In the present study, pretreatment with Er:YAG laser before resin infiltration lead to higher microhardness and greater resistance to demineralization than resin infiltration alone, although the difference between groups failed to achieve statistical significance. It can be assumed that the formation of micro-cracks by Er:YAG laser can increase the penetration of infiltrant within the pores of demineralized enamel, or enhance the bond strength of infiltrant to mineral tissues. However, further studies with larger sample sizes are warranted to accept or reject this hypothesis.

Table 1. Comparison of microhardness values in the study groups at T0 (baseline), T1 (after treatment application), and T2 (after demineralization)

Group	T0 Mean	T0 SD	T1 Mean	T1 SD	T2 Mean	T2 SD	P-value
Resin infiltration	205.56[Aa]	87.01	223.93[Aa]	110.44	82.57[Ba]	48.03	P<0.001
Er:YAG laser + resin infiltration	169.06[Aa]	117.76	222.86[Aa]	135.66	92.74[Ba]	50.98	P=0.001
Bioactive glass	197.03[Aa]	129.81	200.85[Aa]	125.32	92.5[Ba]	49.41	P=0.003

*The different uppercase superscript letters in the rows indicate statistically significant differences at P<0.05.

Table 2. Mean and standard deviation (SD) of alterations in surface microhardness (ΔVHN) between the treatment stages

Group	ΔVHN_{T1-T0} Mean	SD	ΔVHN_{T2-T1} Mean	SD
Resin infiltration	18.37[a]	111.26	-122.99[a]	83.53
Er:YAG laser + resin infiltration	53.8[a]	82.44	-76.32[a]	76.23
Bioactive glass	3.82[a]	94.46	-104.53[a]	101.50
P value	P=0.503		P=0.497	

*The different uppercase superscript letters in the rows indicate statistically significant differences at P<0.05.

Bioactive glasses have been used in the structure of different products such as bone grafts, scaffolds, coatings of dental implants, and dental desensitizers.[35] One of the most important properties of bioactive glass is its antibacterial activity, which is due to the existence of antibacterial components such as silver, copper, or zinc within the glass structure, and the release of alkaline ions (Na+, Ca2+) that raise the pH of the environment.[35-37] The bioactive glass also could be a therapeutic choice for caries management through inhibiting demineralization and promoting remineralization of caries lesions. After placing bioactive glass in the salivary environment, it takes at least 2 hours to complete the bioactive cycle of the substance, resulting in the release of calcium and phosphorus ions from the silicate network.[28] In this study, a layer of bonding agent was applied over the surface of the bioactive glass, and cured. It has been demonstrated that the temporary coating of the glass with a thin layer of bonding agent for 24 h protects calcium and phosphorous ions against being washed out by saliva.[38] The bioactive glass also bonds tightly to materials and tissues and facilitates remineralization through the formation of stable hydroxyapatite crystals on the lesion surface. In the present study, however, the bioactive glass did not show any superiority over other treatments for the remineralization of caries lesions and for preventing further demineralization.

In the present study, neither of the remineralization agents caused a significant increase in the microhardness of inactive proximal lesions, nor increased the enamel resistance to a further acidic attack. These findings are in contrast to most of the previous investigations, which revealed significant enhancement in the mineral content of teeth exposed to a variety of remineralizing products.[39-44] This controversy may be attributed to the small sample size in the study groups. The use of arrested caries lesions which are usually high in mineral content may also contribute to achieving insignificant results in this study. Furthermore, the study design did not include an untreated control group, and this may be considered a limitation of the present investigation. Further studies with larger sample sizes are warranted to assess the efficacy of resin infiltration (with or without laser pretreatment) and bioactive glass on remineralization of carious lesions in clinical conditions.

Conclusions

1- The greatest enhancement in microhardness after the remineralization treatment belonged to the Er:YAG laser conditioning + resin infiltration group and the lowest to the Bioactive glass group.

2- Following exposure to the demineralization solution, the greatest loss in microhardness was observed in the resin infiltration group and the lowest was found in the specimens treated by Er:YAG laser + resin infiltration.

3- Although pretreatment by Er:YAG laser before resin infiltration was more effective than other treatments in enhancing microhardness and preventing the loss of mineral content, the difference between groups did not reach statistical significance; implying the need for further studies to achieve more conclusive results.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

940325). The results presented in this work have been taken from a student thesis (thesis number 602).

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Oral Health Related Quality of Life in first time complete denture wearers Using Balanced, Monoplane and Group function Occlusion

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Research Article

ABSTRACT

Background: Oral Health Impact profile is one of the most common methods to assess the Oral Health Related Quality of Life (OHRQoL) of the patients. The aim of the study was to assess the oral health-related quality of life of patients using different occlusal schemes ie., balanced occlusion, monoplane occlusion, and group function occlusion.

Methods: In this study, a total of 66 edentulous participants were included and divided into 3 groups, balanced occlusion, monoplane occlusion, and group function occlusion. Each group consisted of 22 participants selected using simple random sampling. OHIP-14 scores were assessed before complete denture treatment and at 6 months' follow-up.

Results: When comparison of mean OHIP – 14 among the study population across gender before and after denture insertion was done the results were statistically significant (p <0.05). The difference between the follow-up scores and the scores before a denture when subjected to statistical analysis was significant for all three groups. When one-way ANOVA was done between the groups the data was statistically significant (p <0.05).

Conclusions: The study conclusively showed that the OHRQoL of the patient improved significantly with complete denture prosthesis at 6 months follow-up. The difference between the three groups was also statistically significant and patients with any of the three aforementioned occlusal schemes showed improvement in their OHRQoL. The patient's scores improved the most with conventional group function occlusion, followed by monoplane and the lowest improvement was in the bilateral balanced group.

Keywords: Oral Health Related Quality of Life, OHIP-14, Complete denture, occlusion, occlusal scheme.

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Introduction

The oral health of an individual has a profound impact on their social and psychological well-being.[1] Loss of teeth can occur due to various reasons of which periodontal disease and dental caries are the most important reasons. Various systemic conditions such as diabetes mellitus can significantly predispose the individual for periodontal damage and poor periodontal parameters are often associated with patients with systemic disorders.[2] The prevalence of edentulous patients in developing nations has been estimated at 11.7 % with India and Mexico having over 16% of their elderly population who are edentulous.[3]

The Oral Health Impact Profile (OHIP) is one of the most commonly and widely used methods to assess the Oral Health Related Quality of Life (OHRQoL) of an individual. The initial OHIP was a 49-question form and this was too long and cumbersome to fill a modification to this was introduced which constitute 14 questions. This OHIP-14 was introduced to assess psychological, functional, and social disability about the oral health of a patient. This OHIP-14 is quite easy for geriatric patients to fill and avoids biased tiredness in geriatric patients.[4-6]

The OHIP-14 has been validated extensively in various languages for use on geriatric patients. There are few studies now that are available that prove that a complete dental prosthesis can help the patient improve on the OHRQoL.[4-6] But, the most important thing that is lacking in the literature is if a particular type of occlusal scheme influences the quality of life of the patients. To overcome this shortfall this study was planned to assess the impact that various occlusal schemes had on OHRQoL. To get a better assessment of the impact on quality of life, a 6-month follow-up using the OHIP-14 was also done as part of this study. The aim of the study was to assess the oral health-related quality of life of patients using different occlusal schemes ie., balanced occlusion, monoplane occlusion, and group function occlusion. The null hypothesis for the study was that there is no difference in the OHIP scores between various occlusal schemes before and after completed denture treatment.

Materials and Methods

Ethical clearance was obtained from Institute's ethical clearance committee. (ABSM Ethics Committee,

ABSM/EC52/2020 on 10th December 2020) In this study, a total of 66 edentulous participants were included. The sample size was calculated based on a 5% level of significance, 80% power, and an effect size of 0.8 The sample size of 66 was calculated using the G* Power sample size calculator. The patients were allocated using simple random sampling into one of the three occlusal schemes.

A total of 240 completely edentulous patients were examined for this study and only 126 patients were eligible for the study. Only 66 patients were willing to give written consent and hence 66 participants were included in this study [Figure-1]. The participants were divided into 3 groups using simple block randomization. The three groups included the balanced, monoplane, and group function occlusal schemes. The patient population included patients who are completely edentulous and were first-time denture wearers with a period of edentulousness of greater than 6 months. The patients were segregated into one of the 3 occlusal schemes using sealed envelopes. The study participants were blinded to the type of occlusal scheme. The study compared the three occlusal schemes in a group of 22 patients each.

For the study, the patient must be completely edentulous with high well-rounded ridges, with an age range of 40 to 80 years, with an inter-arch distance of at least 20 mm, and must be willing to give written consent. Patients who had a history of neuromuscular disorders suffered from uncontrolled diabetes mellitus or systemic hypertension were excluded from the study. A total of 240 completely edentulous patients were screened for this study and only 66 fit the inclusion and exclusion criteria hence, these patients were included in the study. The participants were given an OHIP-14 questionnaire prior to beginning of complete denture fabrication. The questionnaire was devised as a five-point Likert scale ranging from very likely to never [Figure-2]. The same questionnaire was given to the patient again 6 months after completion of treatment and the data were tabulated.

The complete denture fabrication was performed by initially making a primary impression using irreversible hydrocolloid material and a stock metal tray. Following this casts were poured with Type III gypsum product (Kalabhai, India) and the base for these casts was made using Type II gypsum product (Kalabhai, India). Specially customized trays were made on each of these arched with self-cured acrylic resin. Border molding was done using a green stick impression compound and a wash impression was made using zinc oxide eugenol impression paste (Dental product of India (DPI), Mumbai, India). The impression was beaded and boxed and poured with type III gypsum product (Kalabhai, India). Heat cured acrylic denture base was made to support the maxillary and mandibular occlusal rims. Following this, maxillomandibular jaw relation was recorded and the casts were mounted.

Figure 1. Patient selection flow chart

Figure 2. Questionnaire for the study

Teeth setting was done for each type of occlusal scheme and try-in of waxed-up dentures was done. In a Balanced occlusal scheme bilateral balance was achieved with a minimum of 4 workings, 3 non-working contacts on each side, and at least 4 anterior and 4 posterior teeth contact during protrusion. For the group function occlusal scheme, the teeth were set in maximum intercuspation at patients centric. Furthermore, for the monoplane occlusion the teeth used were zero-degree teeth, and set up was done in the patient's centric position. Once the patient was satisfied with the try-in, the denture bases were sealed to the casts, flasked, dewaxed, packed with heat-cured acrylic resin (AcryPolR, Ruthinium Group, New Delhi, India) and cured using a long curing cycle. Following curing the flasks were bench cooled and lab remounting was done. The dentures were trimmed and polished and fit and insertion of the prosthesis were done. Patients were followed up after 24 hours and one week to make any alterations to the denture and relieve areas of sore spots.

Statistical analyses were done using Statistical Package for Social Sciences

(SPSS Inc., Chicago, IL, USA). Statistical methods used were paired Student T-test, Analysis of Variance (ANOVA) and Post-hoc multiple comparison test.

Results

In this study, the Oral Health Impact Profile (OHIP – 14) was assessed among 66 completely edentulous patients.

The mean age of the study population was 62.34±11.04 years. Among the study participants, 44 (66.7%) were male and 22 (33.3%) were female. When a comparison of mean OHIP–14 among the study population across gender before and after denture insertion was done the results were statistically significant (P<0.05). The results showed a significant decrease in both male and female participants. [Table-1] When the pre and post-insertion scores were compared among all the study participants it was noted that there was a significant decrease in OHIP-14 score before and after complete dentures and this data was statistically significant (P <0.05) [Table-2].

When the data was analyzed for participants below and above the age of 60 years the data was statistically significant (P<0.05) [Table-3]. When the data was subjected to pairs t-test for before and after for different types of occlusions, there was a significant reduction in OHIP scores across all three occlusal schemes and this

data was statistically significant [Table-4]. The mean scores of OHIP-14 were compared before and after complete denture prosthesis there was the largest reduction in the scores were noted in the group function group of 15.636 followed by monoplane occlusion with a score reduction of 10.045 and the least difference was noted in the balanced occlusal scheme group with a value of 8.909. The difference between the follow-up scores and the scores before a denture when subjected to statistical analysis was significant for all three groups (P<0.05). When one-way ANOVA was done between the groups the data was statistically significant (P<0.05). When multiple comparisons post-hoc test were made between monoplane occlusion and group function occlusion and between group function and balanced occlusion the data was statistically significant (P<0.05). While the data was not statistically significant when comparison was made between monoplane occlusion and balanced occlusion.

Table 1. The comparison of mean OHIP – 14 among the study population across gender before and after denture insertion.

Gender*	OHIP - 14 (Mean ± SD)*		t - value	Df	p - value
	Pre - Denture	Post -Denture			
Male	37.61 ± 12.93	26.45 ± 7.86	11.34	43	<0.05
Female	41.91 ± 12.71	29.64 ± 9.09	8.96	21	<0.05

*Paired Samples t – test

Table 2. The comparison of mean OHIP -14 among the study participants pre and post denture insertion.

Denture*	N	Mean ± SD*	t- value	Df	p - value
Pre- Insertion	66	39.05 ± 12.90	14.49	65	<0.05
Post- Insertion		27.52 ± 8.36			

*Paired Samples t – test

Table 3. The comparison of mean OHIP – 14 among the study population across age groups, before and after denture insertion.

Age*	OHIP - 14 (Mean ± SD)*		t - value	Df	p - value
	Pre - Denture	Post -Denture			
≤ 60 years	37.84 ± 13.09	26.48 ± 7.82	9.32	30	<0.05
≥ 61 years	40.11 ± 12.82	28.43 ± 8.82	11.03	34	<0.05

*Paired Samples t – test

Table 4: Mean, standard deviation and statistical comparison between before and after OHIP-14 scores among various types of occlusion

Type of Occlusal Scheme	Number of Participants	Before denture (Pre), After denture (post)	Mean	Standard Deviation	Std. Error Mean	P-value
Monoplane Occlusion	22	Pre	32.45	11.505	2.453	<0.05
		Post	22.41	5.114	1.090	
Group function occlusion	22	Pre	50.09	6.240	1.330	<0.05
		Post	34.45	5.804	1.237	
Balanced occlusion	22	Pre	34.59	12.250	2.612	<0.05
		Post	25.68	8.682	1.851	

Discussion

The present study compared the impact of various occlusal schemes on OHIP-14 scores in completely edentulous patients before and after complete denture prosthesis. In the current study two-thirds of the participants were males and one-third were females. This gender distribution is similar to studies done by Shrestha *et al.*[7] while it was contrary to the results of Montero *et al.*[8],

and Postic *et al.*[9] This difference in gender distribution can be attributed to the different geographic gender ratios and profiles in these different studies. The mean age of the present study was 62.34±11.04 years which was similar to results obtained by Montero *et al.*[8], and Shrestha *et al.*[7], while Postic *et al.*[9], reported a slightly higher average age of the participants 72.70± 9.04 years in men and 73.10 ± 6.73 years in women. This can be attributed to the age restriction in this study where patients above the age

of 80 years were not included and hence the average age might have been lower than the Postic *et al.* study.

This study showed that the OHIP-14 scores were higher in participants with age above 60 years and lower for participants below 60 years. This result is contrary to results obtained by Shrestha *et al.*[7] and John *et al.*[10] where participants with increasing age had lower scores. This difference can be attributed to the fact that patients in our study were first-time denture wearers.

In the present study, there was a statistically significant drop in the overall score for the OHIP-14 post denture insertion and this difference was statistically significant. This reduction in the scores posts denture insertion was similar to the results obtained by Kaushik *et al.*[11] Shrestha *et al.*[7] in their study also showed an improvement in OHRQoL in patients after a follow-up period was 8 weeks and their data was statistically significant. Jenei *et al.*[12] in their study on patients with various prosthetic rehabilitation assessed the Hungarian version of the OHIP-49 questionnaire. A subset of patients in this study was completely edentulous and they reported a reduction in the scores of patients to post denture insertion.

In a randomized control trial by Kawai *et al.*[13], it was noted that the bilateral balanced group had improvement in their OHRQoL scores but the difference was statistically not significant. This result is contrary to the results of our study where the difference between pre and post-insertion was statistically significant in the Balanced occlusion group. This difference can be attributed to the use of two different methods to assess OHRQoL in the studies.

The present study also assessed the impact of monoplane occlusion on OHRQoL of the patients and there was a statistically significant difference before and after complete denture prosthesis. The result, when compared to bilateral balanced occlusion, was not significant while it was significant when it was compared to conventional group function occlusion. From our literature search, there were no studies that have compared these three types of occlusions and hence comparison with other studies was not done for the monoplane group. The overall OHRQoL improved for all patients in this study with the maximum improvement in OHIP scores was noted for conventional group function occlusion, followed by monoplane and least improvement in Bilateral balanced occlusion. Although, all three data were statistically significant. The present study showed that there was a difference in OHIP-14 scores between monoplane occlusion, group function occlusion and balanced occlusion. Hence, the null hypothesis was rejected for the study.

Conclusions

The study assessed the impact of the various occlusal scheme on OHIP-14 scores in the patient. The study conclusively showed that the OHRQoL of the patient improved significantly with complete denture prosthesis at 6 months follow-up. The difference between the three groups was also statistically significant and patients with any of the three aforementioned occlusal schemes showed improvement in their OHRQoL. The patient's scores improved the most with conventional group function occlusion, followed by monoplane and the lowest improvement Was in the bilateral balanced occlusion group.

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Cumhuriyet Dental Journal

Water Sorption, Solubility and Color Stability of Different Bulk-Fill Restorative Materials

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Research Article

ABSTRACT

Objectives: The aim of this study was to evaluate the color stability, water sorption and solubility of recent bulk-fill restorative materials.

Materials and Methods Seventy disc shaped specimens (height:1mm, diameter:15mm) were prepared with a micro-hybrid composite resin (Z250; Filtek Z250, 3M ESPE), two resin-based bulk-fill (Filtek One Bulk Fill, 3M ESPE and EBF; Estelite Bulk-Fill Flow, Tokuyama Dental), glass ionomer-based restorative (Equia FF; Equia Forte Fil, GC Dental), self-cure and light-cure alkasite (CN; Cention N, Ivoclar Vivadent) and indirect composite resin (Gradia P; Gradia Plus, GC Dental) (n=10). Baseline color measurements were performed and mass of the samples (m1) were noted. Color measurements were repeated after 24 h and 28 days immersion of the specimens in water. After the 28-days immersion, mass was noted as m2. Specimens were kept in desiccators and final mass (m3) were recorded. The color change (ΔE_{00}) water absorption (WSP) and water solubility (WSL) of the specimens were calculated.

Results: After 28-days immersion, Gradia P showed significantly lower water sorption than EBF and Equia FF groups (p<0.05). Light-cured and self-cured CN groups showed similar sorption with resin composite groups (p>0.05). The lowest solubility was observed in Z250 and Gradia P. The lowest (ΔE_{00}) was observed in self-cured CN group (p<0.05) and Gradia P showed the highest ΔE_{00}.

Conclusions: Light-cured and self-cured CN groups demonstrated similar sorption to the conventional composite resin and indirect composite resin. Self-cured CN demonstrated higher water solubility than composite resins. Restorative materials showed moderately unacceptable color change after 28-day immersion in distilled water.

Keywords: Water Sorption, Solubility, Color Change.

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Introduction

Along with the advances in restorative materials, composite resins have been the material of choice in clinical practice for restoring anterior and posterior teeth. Maintenance of esthetic, mechanical and physical properties over time, that could be the reason for the restoration replacement, is required for the longevity.[1] However, long-term stability of restorative materials is a major concern due to the ambient conditions of oral environment and the detrimental effects on the restorative material structure.[2,3]

Water sorption can produce deleterious effects on the restorative material by leading to swelling, oxidation, hydrolysis, softening and plasticization.[3] Ideally the restorative material should be impermeable and highly resistant to water sorption and solubility.[4] However previous studies reported different extents of water sorption and solubility values for restorative materials varying according to the type and ingredients of the materials.[4-7] Water sorption of resin-based materials occurs through the voids in the structure and binding of water to hydrophilic polymers.[4] For the glass ionomer based restorative materials, as a result of water sorption, calcium and aluminum ions are transported and in fact,

ions removed from the material over time.[6] In addition, water sorption and solubility shortens the life span of the restorations by causing color change.[5,8]

The indirect composite resins have been used for about three decades with higher mechanical and physical properties and reduced polymerization shrinkage in comparison with conventional composite resins.[9] Indirect composite resins require longer polymerization time or additional heat, vacuum and pressure treatments applied based on the initiator system used.[10] Owing to the higher polymerization rates and reduced unreacted monomers, less water sorption and solubility can be expected for the indirect composite resins.[11]

Recently, tooth colored and self-adhesive direct restorative material, Cention N (CN; Ivoclar Vivadent, Schaan, Liechtenstein) was introduced. CN is papered by hand mixing the powder and liquid, and can release calcium, fluoride and hydroxide ions during the service life. In addition, CN can be considered as a basic filling material due to it can be used with or without an adhesive system and can be set self-cure or with light activation.[12] Generally, ease of use of restorative materials is desired and user-friendly materials are preferred by the

clinicians.[13] In addition, CN can be placed within the cavity in bulk likewise the bulk-fill composite resins and restorative glass ionomers. Bulk-fill composite resins were introduced with the purpose of simplifying the restorative procedure and shorten the chair time. Recently, regular and flowable restorative bulk-fill composite resins that not need to a capping layer are available on the market.

Previous studies reported similar or lower water sorption and solubility values for the bulk-fill restoratives and higher values for the glass ionomer based restoratives compared to composite resins.[4,6,7] Restricted number of previous studies have evaluated the water sorption and solubility of CN, however results are somewhat conflictive. In addition to the mechanic and physical properties, color stability of the restorative materials is an important esthetic parameter that affect the longevity of the restoration.[14]

Although the color stability and water sorption and solubility of restorative materials has been evaluated in many studies, no published article has yet compared different restorative materials that placed in bulk. Therefore, the aim of this study was to evaluate the color stability, water sorption and solubility of recent bulk-fill restorative materials. The null hypothesis tested were that type of restorative material have no effect on the color stability, water sorption and solubility.

Materials and Methods

A universal micro-hybrid resin composite (Z250; Filtek Z250, 3M ESPE, St Paul, MN, USA), two resin-based bulk-fill restoratives (FOB; Filtek One Bulk Fill, 3M ESPE, St Paul, MN, USA and EBF; Estelite Bulk-Fill Flow, Tokuyama Dental Corporation, Tokyo, Japan), a glass ionomer-based bulk-fill restorative (Equia FF; Equia Forte Fil, GC Dental Products Corp, Tokyo, Japan), a bulk-fill alkasite restorative (CN; Cention N) and an indirect composite resin (Gradia P; Gradia Plus, GC Dental Products Corp, Tokyo, Japan) were tested in the present study. The shades of the materials were A2 except for HB-DA2 was used in Gradia Plus group. The composition and polymerization or setting procedures of the materials are described in Table 1.

Specimen preparation

Assuming a medium effect size (F=0.35) of the difference between the groups, at least 10 samples per group were required with a power of 80% and an alpha error of 5%. Therefore, total of seventy disc-shaped specimens (n=10) were prepared using teflon molds (depth: 1 mm and diameter 15 mm). The tested materials were inserted into the mold that was placed on a microscope slide. After inserting, another microscope slide was placed on the mold and pressed. After the removal of excess material, the resin-based composite materials were polymerized with a Valo LED unit (Ultradent Products Inc., South Jordan, UT, USA) at the standard power of 1000mW/cm2 for 20 s. The chemically activated materials (Equia Forte Fil and Cention N self-cure) were left untouched to set for 2.5 min for Equia Forte Fil and 4 min for Cention N self-cure group. After the setting, the surface coating (Equia Coat, GC) was applied on Equia Forte Fil specimens and light polymerized for 20 s. For the Gradia Plus, initial polymerization was performed using a hand light-curing device (Valo, Ultradent Products Inc.) at the standard power for 20 s. Following, post polymerization was performed in a light-curing oven (Labolight DUO, GC, Tokyo, Japan) for 3 min in full-mode.

Water Sorption and Solubility

After the polymerization or setting of the restorative materials, samples were placed in a glass vacuum desiccator at 37±1 °C, and dried for 22 hours. Then, the samples were transferred to a second desiccator and dried for 2 hours at 23±1 °C. The samples were weighed using a digital assay balance (with an accuracy of 0.01 g). This 24 h cycle was repeated until a constant mass of each sample (not more than ±0.1 mg) was obtained and the baseline mass of the samples (m_1) were noted. Afterwards, the samples were immersed in distilled water at 37 ± 1°C for 28 days, in containers that have separate covered compartment for each sample to avoid contact, and evaporation of the distilled water. The samples were dried with absorbent paper and weighed after 1, 7, and 28 days, and mass value was noted as m_2. After the 28 days, using the same protocol as for m_1, specimens were kept in the desiccators until a constant mass was obtained, and the final mass of the samples (m_3) were noted. The water absorption (W_{SP}) and water solubility (W_{SL}) of the each sample were calculated separately with the following equations:

W_{SP}: $m_2 - m_1 / V$ (mg/mm^3)

W_{SL}: $m_1 - m_3 / V$ (mg/mm^3)

Color Stability

Color measurements were performed using a spectrophotometer (VITA Easyshade, Vita Zahnfabrik, Bad Säckingen, Germany) relative to the standard illumination and against a white background. The measurements were repeated for three times and average L, C, H color parameters of Commission Internationale de l'eclairage (CIEDE2000) system were recorded to calculate the color differences (ΔE_{00}) by the following equation:

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_c S_c}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C} \frac{\Delta H'}{k_H S_H}}$$

The baseline color measurements (T_1) were performed after the specimen preparation. The color measurements were repeated after 24 h (T_2) and 28 days (T_3) immersion of the specimens in the distilled water. The ΔE_{00} values between the T_1-T_2 and T_1-T_3 were calculated separately.

FE-SEM observation

After the 28-day immersion in distilled water, one sample from each group was randomly selected for the observation of surface characteristics. The samples were sputter-coated with Pt-Pd and observed using FE-SEM (Mira 3 XMU, Tescan) at 500x and 3000x magnifications.

Statistical Analysis

Statistical analyses were performed using SPSS version 19 software (SPSS Inc, Chicago, IL, USA). Distribution of data was assessed by the Kolmogorov-Smirnov test and normal distribution of data was confirmed. Color change and water sorbtion values were analyzed separately by repeated measures analysis of variance (ANOVA) and pairwise comparisons were performed with Bonferroni tests (p<0.05). Water solubility data was analyzed using one-way ANOVA and Tukey's HSD post-hoc tests (p<0.05). Pearson's correlation coefficient was used to assess the relationship between water sorption and solubility after 28 d (α= 0.05).

Table 1: Compositions and manufacturer details of the tested restorative materials.

Restorative material	Material category	Composition	Application Procedure	Manufacturer
Filtek Z250 (Z250)	Micro-hybrid Resin Composite	Bis-GMA, Bis-EMA, UDMA, PEGDMA, TEGDMA, Zirconia silica, silica filler: 81.8% (wt)	Apply the resin composite in 2 mm layers and light cure for 20 s	3M ESPE, St Paul, MN, USA
Filtek One Bulk Fill (FOB)	Bulk-Fill Resin Composite	AUDMA, AFM, diurethane-DMA, and 1, 12-dodecane- DMA, ytterbium trifluoride, zirconia/silica 76%(wt)	Apply the resin composite in 2-4 mm layers, and light cure for 20 s	3M ESPE, St Paul, MN, USA
Estelite Bulk Fill Flow (EBF)	Bulk-fill Resin Composite	Bis-GMA, TEGDMA, Bis-MPEPP, mequinol, dibutyl hydroxyl toluene, uv adsorber, silicon oxide, zirconium oxide 70% (wt)	Apply the resin composite in 2-4 mm layers, and light cure for 10 s	Tokuyama Dental Corporation, Tokyo, Japan
Equia Forte Fil (Equia FF)	High-viscosity glass ionomer	Powder: fluoro-alumino-silicate glass, polyacrylic acid powder, pigment Liquid: polyacrylic acid, distilled water, polybasic carboxylic acid	Activate the Equia Forte Fil capsule and place it into a mixer (or an amalgamator), and mix for 10 s. Remove the capsule and load it into the GC capsule applier. Apply the material in the cavity, condensed and sculpted and then left to set for 2.5 minutes. After the setting, the surface coating (Equia Coat, GC) was applied on Equia Forte Fil specimens and light polymerised for 20 s.	GC Dental Products Corp, Tokyo, Japan
Cention N self cure (CN-Self cure)	Alkasite restorative	Liquid-UDMA resin, DCP, an aromatic aliphatic-UDMA and PEG-400 DMA. Powder-Ytterbium trifluoride, isofiller, fluorosilicate glass fillers	Manually mix two measuring spoons of powder and two drops of resin till a smooth consistency. The mixing time should not exceed 60 seconds. Leave the material for 10 minutes from the start of mixing (no light curing)	Ivoclar Vivadent AG, Liechtenstein
CentionN light cure (CN-Light cure)	Alkasite restorative	Liquid-UDMA resin, DCP, an aromatic aliphatic-UDMA and PEG-400 DMA. Powder-Ytterbium trifluoride, isofiller, fluorosilicate glass fillers	Manually mix two measuring spoons of powder and two drops of resin till a smooth consistency. The mixing time should not exceed 60 seconds and light cure for 20 s.	Ivoclar Vivadent AG, Liechtenstein
Gradia Plus (Gradia P)	Indirect Micro-hybrid Resin Composite	Bis-GMA, TEDGMA, UDMA, ceramic filler, Photoinitiators, Stabilisers, Pigments 71%(wt)	Apply the composite and light cure for 20 seconds. After that, a light-curing oven was used for further polymerization of the specimens for 3 minutes.	GC Dental Products Corp, Tokyo, Japan

Bis-GMA: bisphenol A diglycidyl methacrylate; Bis-EMA: bisphenol A diglycidyl methacrylate ethoxylated; TEGDMA: triethylene glycol dimethacrylate, UDMA: urethane dimethacrylate; PEGDMA: poly (ethylene glycol) dimethacrylate; AUDMA: Aromatic urethane dimethacrylate; Bis-MPEPP; Bisphenol A polyethoxy dimethacrylate; AFM: addition- fragmentation monomer; DCP: dicalcium phosphate

Table 2: Water sorption after 7 days and 30 days. and water solubility results

Material	Water sorption 7th days	Water sorption 28th days	Solubility
Z250	1.59±0.49[aA]	4.25±0.78[aB]	0.59±0.13[a]
FOB	3.2±0.74[abA]	6.5±0.74[abB]	0.97±0.49[ab]
Gradia P	2.13±0.67[a]	3.81±0.58[a]	0.59±0.22[a]
EBF	7.04±2.56[bA]	9.6±2.52[bB]	0.94±0.27[ab]
Equia FF	29.68±6.64[cA]	34.16±5.6[cB]	12.74±2.7[d]
CN-light-cure	2.64±0.92[a]	4.28±1.09[a]	2.31±0.86[bc]
CN-self-cure	3.34±0.99[ab]	4.35±0.69[a]	2.75±1.33[c]

*The superscripts lower cases indicate significant differences among restorative materials, the superscript uppercases indicate significant difference between the water sorption of restorative materials in different time periods (p<0.05).

Table 3: Color change for restorative materials.

Material	T1-T2	T1-T3
Z250	1.7±0.3[bcA]	2.8±0.44[abcB]
FOB	1.36±0.18[bA]	2.95±0.29[abcB]
Gradia P	1.35±0.24[bA]	3.29±0.57[cB]
EBF	1.6±0.33[bcA]	3.24±0.47[bcB]
Equia FF	1.93±0.25[cdA]	2.54±0.33[aB]
CN-light-cure	2.12±0.2[dA]	2.61±0.19[abB]
CN-self-cure	0.8±0.4[aA]	2.35±0.68[aB]

*The superscripts lower cases indicate significant differences among restorative materials, the superscript uppercases indicate significant difference between the color change of restorative materials in different time periods (p<0.05).
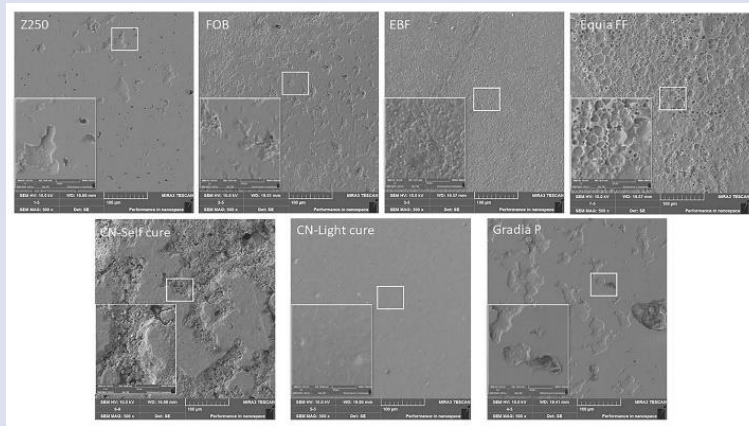
Figure 1. FE-SEM images of the restorative materials after 28-day immersion.

Results

Water Sorption and Solubility

Water sorption after 7 days and 28 days, and water solubility results of the tested restorative material groups are presented in Table 2. The water sorption values of the restorative materials were increased over time. The increase between 7 days to baseline and 28 days to baseline were significant for Z250, FOB, EBF and Equia FF groups (p<0.05).

After 7 days, Equia FF had the significantly highest water sorption values and, EBF showed the highest water sorption among the composite resins (p<0.05). Light-cure and self-cure CN groups demonstrated similar water sorption with Z250, Gradia P and FOB (p>0.05). After 28 days, Equia FF exhibited the highest water sorption values and created a statistically significant difference compared to the other restorative materials tested in the study (p<0.05). The lowest water sorption value was found in Gradia P and was statistically significant from EBF and Equia FF groups (p<0.05). The differences between light-cured and self-cured CN groups and resin composite groups were not significant (p>0.05).

Regarding water solubility, the lowest values were observed in Z250 and Gradia P groups. Equia FF showed more solubility compared to Z250 (p<0.05). No statistically significant difference was found among the composite resin based restorastives (p>0.05).

In addition, a statistically significant and strong positive correlation was found between water solubility and 28-day water sorption (r=0. 905, p<0.001), whereas 28-day water sorption and ΔE_{00} presented a negligible negative correlation (r= -0.164, p>0.05).

Color Stability

The mean color differences (ΔE_{00}) of restorative materials and standard deviations for 24 h and 28 days after polymerization compared to baseline measurements are presented in Table 3. The ΔE_{00} values were significantly higher for all restorative materials after 28 day immersion in distilled water compared to 1 day immersion (p<0.05).

After 24 h, self-cured CN group showed the lowest ΔE_{00} and the difference was significant for all the comparisons (p<0.05). However, light-cured CN group showed the highest ΔE_{00} and the difference was not significant only when compared to Equia FF (p>0.05).

After 28 days, the lowest ΔE_{00} was observed in self-cured CN group and differences between self-cured CN and, EBF and Gradia P were significant (p<0.05). Gradia P showed the highest ΔE_{00} and differences were not significant when compared to other composite resins (p>0.05).

FE-SEM observation

The FE-SEM images of the samples are represented in Figure 1. For the Equia FF, a rough surface with intensive porosities were observed. Composite resin based restorative materials demonstrated shallow porosities and rough surfaces except for EBF, which demonstrated a smoother surface. Similar smooth surface was observed in CN-light cure group whereas CN-self cure group demonstrated distinct irregularities.

Discussion

The color of restorative material should be stable after the placement, and restorative materials should be highly resistant to water sorption and solubility. In the present study, different types of restorative materials that can be placed into the cavities in bulk were evaluated regarding water sorption, solubility and color stability characteristics. According to the results of this study, significant differences in color change, water sorption and solubility among the restorative materials were observed after 28-day immersion in distilled water. Therefore, the null hypothesis were rejected.

Water sorption and solubility of resin based restorative materials is of high importance due to the exerting deleterious effects on material structure and adverse effects on the health.[3] In this study, Equia FF showed higher water sorption and solubility compared to resin based materials in accordance with the previous studies [6, 15] and intensive porosities were observed on the FE-SEM images. These can be considered harmless for the health due to it has been revealed that the aluminum release of glass-ionomer cements is negligible to create health hazard.[16] However, the resin content of surface protector (Equia Forte Coat) that reduce water sorption and solubility of the glass ionomer restorative[15] should be kept in mind.

Hydrophilic properties of the resin matrix and filler content of composite resins have significant influence on the water sorption and solubility chracteristics.[4] In accordance with a previous study[17] that tested the water sorption of flowable bulk-fill restorative materials, in the present study the flowable bulk-fill restorative; EBF, showed the highest water sorption and solubility among composite resins. Despite the similar filler rate of EBF with Gradia P and FOB, triethylene glycol dimethacrylate (TEGDMA) content of EBF could be blamed for the higher water sorption and solubility values. TEGDMA has a lower molecular weight and higher hydrophilicity than bisphenol A-glicidil methacrylate (Bis-GMA) and urethane dimethacriylate (UDMA).[3,8] In addition, heterogenic structure of TEGDMA forms micro-pores between the polymers which contributes the higher water sorption.[8] Tanthanuch et al.[18] evaluated the degradability of bulk-fill composite resins and explained the lowest sorption of FOB by UDMA and ethoxylatedbisphenol-A dimethacrylate (Bis-EMA) content of the material, which absorbs less water than Bis-GMA.[19] In addition, Melo et al.[20] reported similar water sorption for the regular viscosity bulk-fill composite resin and traditional composite resins. In line with previous studies, in the present study FOB showed lower sorption and solubility than the flowable bulk-fill restorative (EBF) and similar values with other resin based and, light-cured and self-cured CN groups.

Indirect composite resins has a longer polymerization time when compared to direct restoratives, thus the higher degree of conversion rate can be expected to result in higher stability against the deterioration process.[10,21] However, in accordance with the results of present study,

Iskender et al.[11] reported similar water sorption for indirect and direct composite resins. As aforementioned, the TEGDMA content of Gradia P could be responsible for the similar water sorption with direct composite resins, in spite of additional polymerization of indirect composite resin.

The most interesting finding of this study was that the light-cured and self-cured CN groups demonstrated similar water sorption to the resin based materials except of EBF, which is presented higher sorption. Cention N includes a hydrophilic liquid monomer (PEG-400 DMA) in content and capable of releasing fluoride, calcium and hydroxide ions. This could be the reason for distinct irregularities observed in CN-self-cure groups on FE-SEM images. However, CN does not contain Bis-GMA, HEMA or TEGDMA. In accordance with our results, de Araújo-Neto et al.[22] reported comparable water sorption for CN with bulk-fill and conventional composite resins. On the other hand, self-cured CN showed higher solubility than composite resins. Resin structure of CN includes mainly low-viscosity monomers and dual-cure initiators, and requires hand mixing. Therefore, more porous resin structure compared to one-paste composite resin sytems[22] can be responsible for the higher solubility.

Color stability of a restorative material is an important esthetic parameter for the long-term evaluation of the restorations. However, all tested restorative materials showed moderately unacceptable ΔE_{00} values (ΔE_{00}; >1.8, ≤3.6) after 28 day immersion in distilled water, according to the classification by Paravina et al.[23] The color change of restorative materials in distilled water was defined as intrinsic discoloration that is considered as the consequence of alteration of the interface between matrix and filler and the resin matrix, oxidation of the resin matrix and unreacted monomers.[24] Therefore, lower ΔE_{00} values of self-cured CN could be explained by the presence of thiocarbamide that improves the color stability of the material, instead of amine. Similar or lower color change was reported for bulk-fill composite resins in a previous study[25] and Barutçugil et al.[14] stated that color change of bulk-fill restoratives is material dependent due to the different contents. Accordingly, in the present study the difference between resin based bul-fill restoratives (EBF and FOB) and conventional composite resin was not significant.

In this study restorative materials were immersed in water for a period of time, however in oral environment, restorations exposed to various types of physical, mechanical and chemical stresses. In addition, restorations exposed to different colorants from beverages and foods within the dynamic cycle of oral hygiene applications. Therefore, these can be considered as the limitations of this study. Further studies incorporating the other intraoral conditions should be performed for recent restorative materials.

Conclusions

Considering the limitations of this study, the following conclusions can be made;

1- Light-cured and self-cured Cention N groups demonstrated similar water sorption to the conventional composite resin, regular bulk-fill restorative and indirect composite resin. Self-cured Cention N demonstrated higher water solubility than all composite resins.

2- Flowable bulk-fill restorative (EBF) showed higher water sorption than the light-cured and self-cured Cention N groups and other composite resins tested. The highest water sorption and solubility was observed in Equia Forte Fil.

3- All tested restorative materials showed moderately unacceptable color change values after 28 day immersion in distilled water.

Conflict of Interest

There are no conflicts of interest.

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Salivary Leptin Levels in Children with Early Childhood Caries – An Interventional Study

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Research Article

ABSTRACT

Objectives: To evaluate the role of salivary leptin, an adipocytokine as a non-invasive inflammatory biomarker in healthy children and in children affected by Early Childhood Caries (ECC).

Materials and Methods A total of 60 children between the age of (3-5) years were selected for the study. The study subjects were assigned into three groups as control (Group 1), mild to moderate ECC (Group 2) and severe ECC (Group 3). Preoperative Saliva samples were collected from all subjects and repeated after 2 months following rehabilitative intervention. Levels of salivary leptin was determined using Enzyme-Linked Immunosorbent Assay (ELISA).

Results: Levels of salivary leptin were significantly associated with severity of ECC. The intragroup comparison of pre and post treatment levels of salivary leptin showed significant reductions in both mild to moderate ECC and severe ECC groups following caries control. Inter group evaluation between mild to moderate ECC and severe ECC, post treatment showed statistically significant decline of leptin levels in comparison to baseline values.

Conclusions: Salivary leptin has a potential to be recognized as a reliable future prognostic and diagnostic inflammatory marker in children with ECC.

Keywords: Salivary Leptin, Biomarker, Saliva, ELISA, Early Childhood Caries.

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Introduction

The American Association of Pediatric Dentistry (AAPD) has described Early Childhood Caries (ECC) as presence of one or more cavitated, non cavitated lesions, any restored tooth surface or tooth/teeth missing due to caries in children belonging to an age of or younger than seventy one months.[1] The detection of dental caries has been restricted to visual and radiographic aids.

Dental caries is an inflammatory response of dental pulp which can be put forth as the aggregation of cells which are inflammatory in nature thereby provoking the secretion of defence cells in the host such as Interleukin (IL-1, IL-6, IL-8) group of cells and the Tumour Necrosis Factor cells (TNF).[3]

Leptin is a polypeptide measuring 16 Kilodaltons (KDa) and is a non-glycosylated hormone.[4] Leptin performs a wide range of biologic functions which include homeostasis of lymphoid organs, osteogenesis, immune responses, and haematopoiesis and antilipemic effects.[5] Leptin is involved in the inflammatory responses such as induction of acute phase protein synthesis and stimulation of macrophages and /or T cells.[6] Leptin along with its receptors are structurally and functionally similar to the groups belonging to Interleukin family (IL-1, IL-6, IL-8).[7] Leptin is seen in chronic periapical lesions due to infection of dental pulp usually as a sequel to dental caries, and recently gingiva was also considered as a cellular source of leptin which is found in the gingival crevicular fluid of both, healthy and inflamed gingiva.[10-11] Recently, it has been documented that leptin has direct consequences on the pulpal stem cells, thereby being a chief regulator of the cells of mesenchymal somatic origin.[11] Pulpitis is signalized by the aggregation of cells such as dendritic cells, neutrophils, macrophages and the lymphocytes.[6] Molecules like leptin which are at an interface between metabolism and immunity reflect the abnormal immune responses. Development of latest therapeutic approaches for anti-leptin therapy are taking pace thus the blockade of leptin with antagonists could inhibit the activity of bioavailable leptin and reduce its pro-inflammatory effects.[8-9]

Early childhood caries (ECC) is a multifactorial disease and has been an enigma to researchers since past decades. There has been a correlation between ECC and dietary factors.[1-3] Thus, leptin bridges the gap between nutritional status and immune competence, serving as a primary regulator of the two.[6] However, the role of leptin in ECC remains unclear and this study could be an effort towards validating this molecule as an easily accessible and prognostic marker in clinical settings in future; hence the inevitability to perform this study. Hence the study was conducted with the objective to assess and quantify salivary leptin levels in healthy children and in children with Mild to

Moderate and Severe ECC, (S-ECC) pre and post treatment of carious lesions.

Materıals And Methods

The study was conducted in the Department of Paediatric and Preventive Dentistry in collaboration with Department of Biochemistry at the host University. The permission of the Institutional Ethical Committee was obtained prior to the study. Informed consent was taken from the parents of the children participating in the study.

Sample size distribution

60 children within the age group of 3-5 years visiting the Department of Pediatric and Preventive Dentistry at the parent institute were selected for the study. The children were divided into 3 groups of 20 each:

Group 1 – Caries free children as controls.

Group 2 – Children with mild to moderate early childhood caries (ECC) (1 or more cavitated, filled smooth surfaces in primary maxillary anterior teeth or dmfs score >4)

Group 3 – Children with severe early childhood caries (S-ECC) (1 or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces constitutes S-ECC.)[12]

The inclusion criteria comprised of: (1) Children between the age group of 3-5 years who were willing to participate in the study. (2) Children affected by mild to moderate ECC without pulpal involvement. (3) Children affected by severe ECC with not more than 3-4 pulpally involved carious lesions. (4) Healthy children with no dental caries belonging to the same age group and who are willing to participate in the study. The exclusion criteria comprised of: (1) Children who are medically compromised and with special healthcare needs. (2) Uncooperative children (3) Children who were on antibiotics, non-steroidal anti-inflammatory drugs in past 3 months which may curb the inflammatory expression cells. (4) Patients presenting with generalized gingival inflammation.

A thorough dental examination was carried out on all children using WHO Oral Health Assessment Form for children 2013 and the oral findings were recorded for each patient and a profile was maintained for ease of follow up till completion of the study. Baseline salivary samples were collected from all the three groups.

Saliva sample collection

Saliva samples were collected from the children in Group 1, Group 2 and Group 3. All samples of unstimulated saliva were collected within a 10 minutes period between 9 am and 11 am to minimize any possible effect of diurnal variation.[3] Saliva samples were collected by Draining method or the passive drooling method, in which the subjects was asked to sit up straight with the head bent down and mouth open to spit the saliva passively from the lower lip to drool approximately 1–2 millilitres (ml) of unstimulated whole resting saliva into the non- graduated sterile plastic test tube for 5 minutes.[13] Samples were then transported to Central Research Laboratory and centrifuged at 4000 revolutions per minute (rpm) for 15 minutes at 4ºC.The supernatants were stored at -70ºC. [13]

Rehabilitative intervention

The children from group 2 and group 3 were treated according to the requirement for caries control, which included advice on oral health education, diet counselling, oral prophylaxis, restorative procedures, topical fluoride application and pulp therapy. The patients in group 2 and group 3 were recalled after a period of 2 months and post caries control saliva sample was collected. The collected saliva for all the groups was analysed for salivary leptin levels by using salivary ELISA kit.

ELISA for determination of salivary levels of leptin

High-sensitivity commercially available Human Leptin Enzyme-Linked Immuno Sorbent Assay (ELISA) kit was used for determining the levels of leptin in saliva samples of the subjects.[14]

All 60 saliva samples (with the volumes 2 ml) collected by using Salivates in 10 minutes were used for this study after rinsing their mouths thoroughly with water.

Each supernatant was divided into three aliquots and stored at−70 C until analysis. All samples were stored without adding any preservatives (only overnight wait).[14] Each well was filled with a wash buffer of 400 microliters (μL) and then the solution was pulled out using a squirt bottle, manifold dispenser, or auto washer. The plate was then blocked by adding 300 μL of Block Buffer to each well. This was then incubated at room temperature for 1-2 hours. Leptin conjugate reagent was added in the standard and the testing wells and covered with a plate sealer followed by incubation at room temperature for 1 hour. Optical Density of every well was assessed promptly using a microplate reader set to 450 nanometers (nm). The salivary leptin levels were then calibrated in ng/ml and tabulated. [14]

Statistical Analysis

Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States). Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). Inter group comparison (>2 groups) was done using one way ANOVA followed by pair wise comparison using post hoc power analysis. Intra group comparison was done using paired t test (upto 2 observations) Comparison of frequencies of categories of variables with groups was done using chi square test. For all the statistical tests, $p < 0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%. * = statistically significant difference ($p < 0.05$)

Results

The children who reported to the Department of Pediatric and Preventive Dentistry, within the age group of

3 to 5 years, were screened for the presence of dental caries. 20 children with no clinical signs of dental caries and who had good oral hygiene were recruited as the controls, by convenience sampling. In the mild to moderate ECC group (Group 2) and Severe ECC (S-ECC) group (Group 3), a total of 20 children each were recruited as study group before beginning the caries management protocol. Out of the total 60 children, 42 were females and 18 males. Upon completion of the study, a total of 3 patients in Group 2 and 1 patient in Group 3 who failed to appear and follow through with the study protocol were excluded in the mild to moderate ECC and S-ECC study groups. (Table 1)

The mean value of the salivary leptin levels estimated in the control group (Group1) of children was 7.63 nanogram per milliliter (ng/ml) (±0.529 ng/ml) Similarly, mean values for the pretreatment salivary leptin levels estimated in the mild to moderate ECC (Group2) and S-ECC (Group 3) study groups were 8.89 ng/ml (±0.613n/ml) and 11.81ng/ml (±0.531ng/ml), respectively. (Table 2) The post treatment (caries control) salivary leptin levels estimated in the mild to moderate ECC and S-ECC study groups were 8.06 ng/ml (±0.55ng/ml) and 7.51 ng/ml (±0.72ng/ml), respectively after a span of 2 months. (Table 3)

In an intra group comparison of Group 2, the mean of leptin levels before intervention and after intervention was 8.80 and 8.06 respectively. Using paired t test, it was derived that there was a statically significant difference seen for the values between the time intervals (p> 0.01) with higher values at T0. (Figure 1)

In an intra group comparison for Group 3, it was noted that the mean of leptin levels before intervention and after intervention was 11.78 and 7.51 respectively. Using paired t test, it was derived that there was a statically significant difference seen for the values between the time intervals (p> 0.01) with higher values at T0. (Figure 2)

In an intergroup comparison using one way ANOVA between Group 1, Group 2 and Group 3 before treatment (T0) was found to be highly statistically significant with a p value of 0.0 (Figure 2)

In an intergroup comparison using one way ANOVA between the two groups after 2 months post caries control (T2) was found to be highly statistically significant with a p value of 0.015. (Figure 3)

Discussion

The present study is the first to demonstrate the expression of salivary leptin in children with ECC. The leptin levels are typically elevated during infection and inflammation. Moreover, after exposure to inflammatory stimuli, the levels of circulating leptin and leptin expression in the adipose tissue increases. Thus, it appears that proinflammatory mediators such as TNF- α and IL-1, which upregulate leptin expression contributing in turn to the creation of a loop of acute phase reactants that influence each other in promoting the development of chronic inflammation.

One of the most interesting aspects that medical and dental scientific community research is the possible connection between oral inflammatory processes due to infection and systemic health.[15] Saliva serves as a medium to connect these two. The use of saliva as a diagnostic tool is limited because of lack of validation arising from brief knowledge on the understanding of biomolecules and their importance to disease etiology, combined with the insufficient high-sensitivity detection systems.[14]

ECC always commences with an initial stage; starting with white or brown spots, appearing along the gingival line. In the moderate stage, cavitation starts from the enamel reaching upto dentin and in the severe stage, the caries process progresses further into the pulpal tissue.[1-3] Therefore, our study groups were categorized as mild to moderate ECC (without pulpal involvement), and severe ECC (SECC) (with minimum of 4–6 pulpally involved teeth). We hoped that this grouping could provide an insight not only the variation of expression of leptin levels in relation to severity of carious lesions, but also the prognosis following caries control protocol.

Leptin is expressed in the serum of healthy individuals in the range of 5-18 nanograms per millilitre (ng/ml) and in the saliva in the range 1-10 nanograms per millilitre (ng/ml).[16] The release of local cytokines promotes expression of molecules implicated in mineralization.[10] The pleiotropic behaviour of leptin is reinforced by the widespread distribution of leptin receptor (LEPR).[17] LEPR shows structural similarity to the class I cytokine receptor family. According to the multifunctional role of leptin, this fully-active isoform of LEPR is expressed not only in the hypothalamus, where it takes part in energy homeostasis, but also is present on peripheral tissues as well as on hematopoietic cells and on all types of immune cells involved in both innate and adaptive immunity.[18-19]

Considering the caries control protocol, the time required for stabilization of oral biofilm following restorative procedures and for setting in of new routines of oral hygiene practices, a post treatment follow-up time period of 2 months was adopted. This is in accordance with observations made by Marsh PD, Van Der Hoeven JS *et al.*, and Winnier JJ *et al.*[20]

In the present study, a dropout of 3 children from Group 2 and a dropout of 1 child from Group 3 was observed. Data was tabulated with the estimated salivary leptin levels in those patients who complied and completed the caries control protocol set out for the study. This observation of dropouts from our study is in line with previous literature where it has been documented that parental avoidance behavior and lack of understanding of the seriousness of the implications of dental caries on the general health and wellbeing of the child. Thus, it becomes important to establish caries preventive and control protocols to engage parents in community and self-directed awareness programs. These play an important role during the early years of a child's life, even before eruption of the first primary tooth. [21-22]

In healthy children, the range of salivary leptin levels was 7.11 to 8.32 ng/ml with a mean of 7.63 ng/ml. The normal salivary leptin levels ranges between 1–10 ng/mL, directly reflecting the amount of energy stored in the

adipose tissue, thus, obese individuals typically produce higher leptin than leaner individuals. Leptin levels can also be influenced by the taste perception of individuals. This wide range of salivary leptin can be due to its instability as a result of increased LEPRs in the oral cavity.[23]

Whereas, in children with mild to moderate ECC, the range of baseline mean salivary leptin levels was 7.8 to 9.76 ng/ml with a mean of 8.9 ng/ml. This could be because, in mild-moderate ECC there is a developing nidus of bacteria and this increasing bacterial load may contribute to the rise of the pro inflammatory cytokines. Hence, an increase from the normal range can be witnessed.[3,16]

In children with severe ECC, the range of baseline mean leptin levels at T0 was 11.08 to 12.57 nanograms (ng/ml) with a mean of 11.81ng/ml. This rise in levels is in accordance with various studies conducted by Menon MM *et al.*[4] 2020 and Vrinda Sharma *et al.*[3], 2012 on the role of inflammatory cytokines in S-ECC. S-ECC is associated with an increase in pulpal involvement thereby leading to a rise in the circulating inflammatory markers.[14] An in vitro study conducted by Martin Gonzalez et al *in* 2013 to investigate the presence of leptin in healthy and inflamed human dental pulp tissue also concluded the elevated expression of leptin in the inflamed pulp tissue samples than in healthy tissues. This can be due to the role of leptin as an autocrine as well as paracrine pathway and therefore it may play a role in pulpal/periapical inflammatory and immune responses similar to that of the white adipose tissues.[16]

Thus, post caries control in mild to moderate ECC group, after a span of 2 months, the range of salivary leptin levels was observed to be 7.24 to 8.53 ng/ml with a mean of 8.069 ng/ml. This is in accordance with a study conducted by Menon et al in 2020 where they evaluated salivary IL-6 levels pre and post intervention and found a reduction in the IL-6 levels post intervention after 2 months.[4] This was as a result of reduction of bacterial colonies and inflammatory load post intervention of pulpitis.

In severe ECC group, the range of salivary leptin levels was observed to be 6.35 to 8.33 ng/ml with a mean of 7.51 ng/ml. As this group was associated with an increased pulpal inflammation, this decline in the levels of salivary leptin post caries control can be supported by the fact that leptin has direct consequences on dental pulp stem cells, and it has been reported that.[24] Leptin is associated with an increased expression of CC-chemokine ligand 20 (CCL20).[25] CCL20 expression is persuaded by stimulus with bacteria related to caries progression, invading deeply into the dentinal tubules, as well as by proinflammatory cytokines in the inflamed pulpal lesions.[26]

In a comparative evaluation between the baseline levels of healthy children and children with mild to moderate ECC, a statistically significant increase of levels was seen. While the baseline values also showed a statistically significant upsurge between mild to moderate ECC and children with severe ECC. Leptin regulates the immune response, both innate and adaptive responses, not only in normal but also in pathological conditions.[17] Consistent with this, the leptin levels are increased upon infectious and inflammatory stimuli such as lipopolysaccharides, turpentine, and cytokines.[16,27]

Similarly in a comparative assessment between children with mild to moderate ECC and children with severe ECC, a fall in the salivary leptin levels was seen post treatment as compared to the healthy children. A statistically significant fall in the levels was also witnessed between the mild to moderate ECC and severe ECC group, post caries control, thus stating that as the pulpal inflammation is controlled, there is a fall in the salivary leptin levels.

In comparison before and after intervention in children with mild to moderate ECC, the mean difference of salivary leptin levels was 0.73 ng/ml whereas in the children with severe ECC the mean difference was 4.26 ng/ml. Thus, a statistically significant difference in the levels of leptin was appreciated at the different time intervals with higher value at the baseline. As the amount of pulpal inflammation was higher and chronic in the children with severe ECC, a drastic rise and fall of the normal values were in accordance with the support of literature.[16,24]

The post treatment rehabilitative procedures helped in reducing the inflammatory and the bacterial load in the oral cavity. Cytokines are produced by the activation of monocytic macrophagic cells and act as mediators of infection, inflammation and immunological processes in defence to the bacterial irritation.[25] Pulpal symptoms are generally explained by increase in intra-pulpal pressure due to edema. The levels of salivary leptin could be correlated to the extent of inflammation and edema in the pulp, in addition to its role as a mediator of host response following tissue injury and infection. As salivary leptin is correlated to the immune defence mechanisms a period of 2 months was chosen for the inflammatory reactions to subside.

The leptin levels have decreased post caries control protocols in both the groups. Thus, indicating that undergoing caries control treatment helps to reduce their inflammatory status. An increased nidus of inflammation in the oral cavity may lead to hindrance in the immunologic, metabolic, physical and emotional development of the child.[2-3] Thus, it is important to diagnose and treat the pulpal inflammation but also is important to counsel the parents. ECC is a pathologic condition of multifactorial nature. Hence, along with the bacterial load, various dietary and oral hygiene maintenance practices also need to be incorporated to get a desirable reduction of the inflammation of the oral cavity.[3,13] Moreover, the diagnostic and prognostic significance for ECC has not been explored till date. Thus, from the present study we can conclude that salivary leptin holds a true potential to be a future prognostic and diagnostic inflammatory marker. Also, a drastic fall in the levels of leptin can be appreciated post rehabilitative intervention of ECC, stating its role in the immune and inflammatory reactions.

Conclusions

Leptin bridges the gap between nutritional status and immune competence, serving as a primary regulator of

the two. Salivary leptin holds a true potential to be a future prognostic and diagnostic inflammatory marker. Also, a drastic fall in the levels of leptin can be appreciated post rehabilitative intervention of ECC, stating its role in the immune and inflammatory reactions and in determining the status of pulp in such patients.

This study could be an effort towards validating it as a non-invasive accessible and prognostic biomarker in clinical settings in future.

Limitations and Recommendations

On the basis of the observations in our study, we would like to propose the following recommendations for future research:

1. Small sample size and shorter duration of follow up, hence requires a more exhaustive study with a larger sample size and longer follow ups in establishing the role of leptin in assessing the pulpal status of patients with ECC.

2. Further studies may be recommended for estimation of salivary leptin to establish a reference range in children. This will validate the results of our pioneer study.

3. The role and mechanism of salivary adiponectin hormone family in oral defence processes needs to be further understood in healthy and pathological conditions.

4. More longitudinal studies can be done with salivary leptin levels as a good biomarker for comparing the efficacy of various caries control treatment protocols.

Conflict of Interest

No Conflicts of Interest

Source Of Funding

Nil

Table 1: Summary of Sample recruitment and dropouts in the study.

Group	Sample size estimated (participants required)	Participants recruited (pre-treatment saliva collected) ml	Participants completed (post- treatment saliva collected) ml
Control (No ECC)	20	20	-
Mild to Moderate ECC (ECC)	20	20	17 (-3)
Severe ECC (S-ECC)	20	20	19 (-1)

Table 2: Numerical Summary for Pre-treatment salivary leptin levels in children from Group 1, Group 2 and Group 3 (ng/ml)

	Group	N	Mean (ng/ml)	Standard deviation
1.	Control (No ECC)	20	7.63	± .529
2.	Mild to Moderate ECC	20	8.89	± .613
3.	Severe ECC (S-ECC)	20	11.81	± .531

Table 3: Numerical Summary for Post-treatment salivary leptin levels (ng/ml):

	Group	N	Mean (ng/ml)	Standard Deviation
1.	Control (No ECC)	-	-	-
2.	Mild to Moderate ECC	17	8.06	± 0.55
3.	Severe ECC (S-ECC)	19	7.51	± 0.72
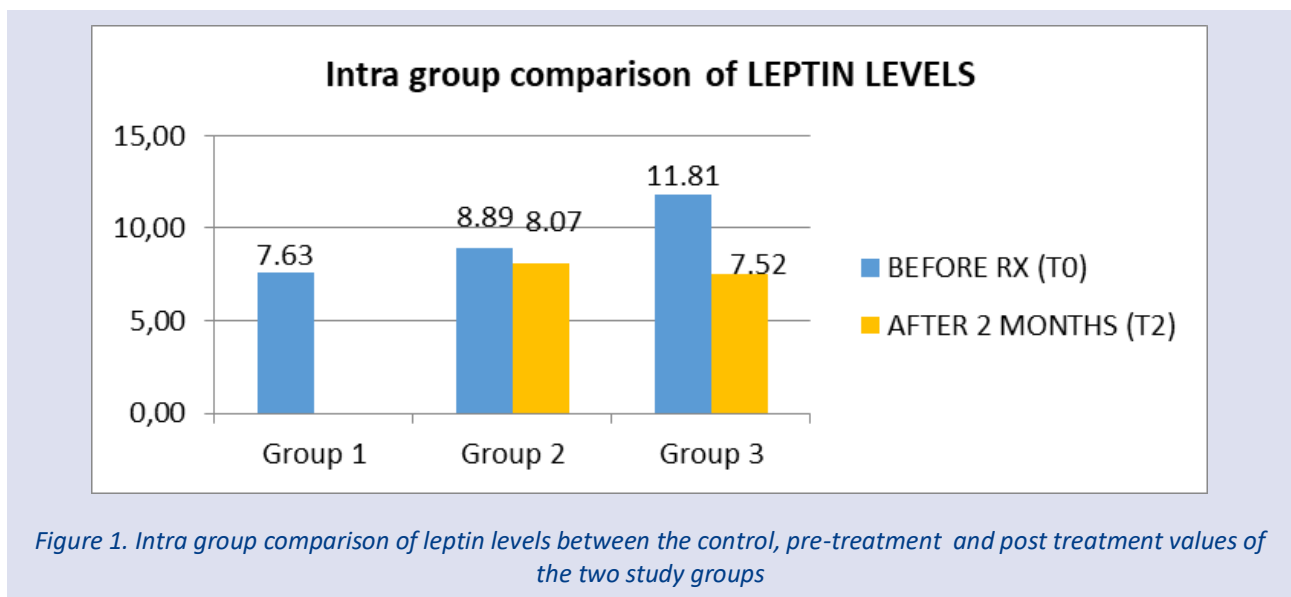
Figure 1. Intra group comparison of leptin levels between the control, pre-treatment and post treatment values of the two study groups
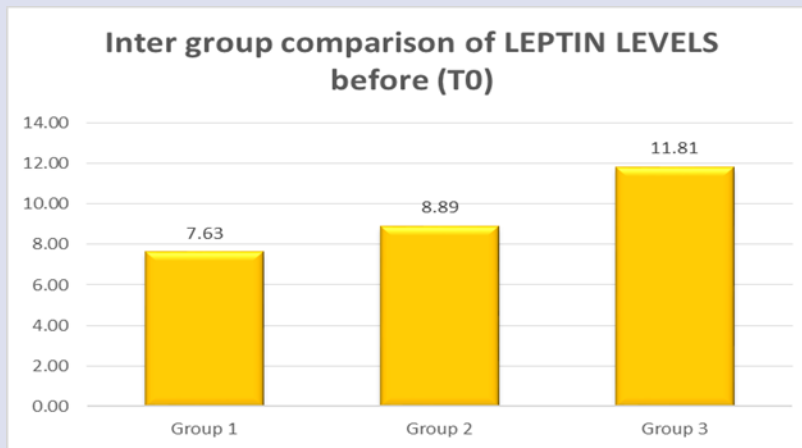
Figure 2. Intergroup comparison of leptin levels before treatment (T0)
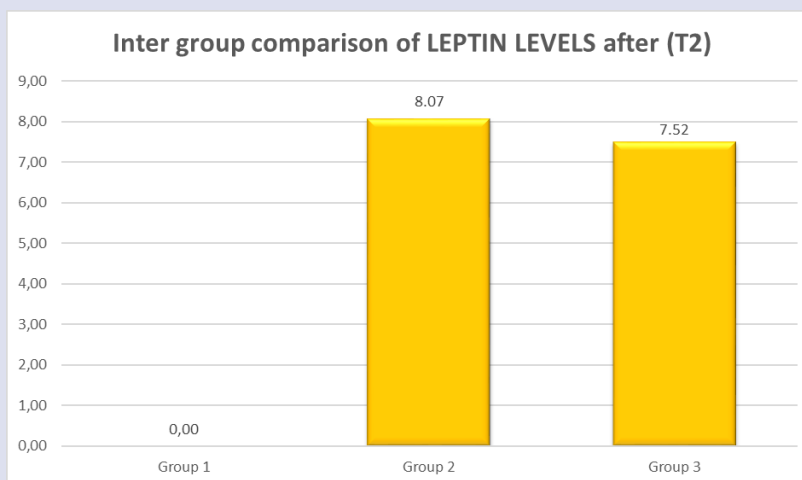
Figure 3. Inter group comparison of leptin levels after treatment (T2)   Intergroup comparison between the 3 groups before treatment (T0) was found to be highly statistically significant with a p value of

0.0 Inter group comparison between the 3 groups after 2 months post caries control (T2) was found to be highly statistically significant with a p value of 0.015.

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Cumhuriyet Dental Journal

Perspectives of Students and Educators on the Effects of the COVID-19 Pandemic on Undergraduate Dental Education

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ABSTRACT

Objectives: The novel coronavirus pandemic has become a real challenge for provision of dental education. The aim of this survey study was to evaluate the opinions of dental faculty educators and students about online education after e-learning experience.

Materials and Methods: The research was conducted on educators and 4th and 5th grade students at Ankara University, Faculty of Dentistry. Questionnaire items for educators and students were categorized into the following factors: educators'/students' personal and professional skills and attitudes towards online education, online theoretical education, online practical training, online exams, handling and quality of education during pandemic. The data obtained was recorded as percentage (%) and interpreted.

Results: 281 undergraduate students and 52 educators participated in this survey study. 36.7% of the students and 24.5% of the educators stated that online courses were as successful as face-to-face education. In addition, 84.2% of the students stated that online learning was not sufficient to acquire clinical skills. 55.9% of the students reported that the educators' effective use of communication methods (videos, surveys, etc.) increased their motivation. 89% of students and 69.8% of educators thought that students should attend in a postgraduate program to acquire clinical skills that they have not acquired due to the pandemic.

Conclusions: The results of this study showed that academicians hold a negative view of distance learning as they think online theoretical courses, practical training and exams impaired the quality of learning. On the other hand, dentistry students have a positive attitude towards online theoretical education but are concerned about clinical skill acquisition.

Keywords: Covid-19, Dental Education, Distance Learning.

Öğrenci ve Eğitmenlerin Covid-19 Pandemisinin Diş Hekimliği Lisans Eğitimine olan Etkilerine Bakış Açıları

ÖZ

Amaç: Yeni koronavirüs pandemisi, diş hekimliği eğitiminin sürdürülmesi açısından zorlayıcı bir durum haline gelmiştir. Bu anket çalışmasının amacı, diş hekimliği fakültesi eğitmen ve öğrencilerinin e-öğrenme deneyimi sonrası online eğitim hakkındaki görüşlerini değerlendirmektir.

Gereç ve Yöntemler: Araştırma, Ankara Üniversitesi Diş Hekimliği Fakültesi'ndeki eğitmenler ile 4. ve 5. sınıf öğrencileri üzerinde gerçekleştirilmiştir. Eğitmenler ve öğrenciler için anket maddeleri şu kriterlere göre kategorize edilmiştir: eğitmenlerin/öğrencilerin çevrimiçi eğitime yönelik kişisel ve mesleki beceri ve tutumları, çevrimiçi teorik eğitim, çevrimiçi pratik eğitim, çevrimiçi sınavlar, pandemi sırasında eğitimin ele alınması ve kalitesi. Elde edilen bulgular % olarak kaydedilmiş ve yorumlanmıştır.

Bulgular: Bu anket çalışmasına 281 lisans öğrencisi ve 52 eğitmen katılmıştır. Öğrencilerin %36,7'si eğitmenlerin ise %24,5'i çevrimiçi derslerin yüz yüze eğitim kadar başarılı olduğunu belirtmiştir. Ayrıca, öğrencilerin %84,2'si çevrimiçi öğrenmenin klinik beceri kazanmak için yeterli olmadığını belirtmiştir. Öğrencilerin %55,9'u eğitmenlerin iletişim yöntemlerini (videolar, anketler vb.) etkin kullanmasını onların motivasyonunu artırdığını bildirmiştir. Öğrencilerin %89'u ve eğitmenlerin %69,8'i öğrencilerin pandemi nedeniyle edinemediği klinik becerileri elde etmek için bir lisansüstü programa kaydolması gerektiğini düşünmektedir.

Sonuçlar: Bu çalışmanın sonuçları, akademisyenlerin çevrimiçi teorik derslerin, uygulamalı eğitimlerin ve sınavların öğrenmenin kalitesini bozduğunu düşündükleri için uzaktan eğitime olumsuz baktıklarını göstermiştir. Öte yandan, diş hekimliği öğrencileri çevrimiçi teorik eğitime karşı olumlu bir tutuma sahip olmakla birlikte, klinik beceri kazanımı konusunda endişelidirler.

Anahtar Kelimeler: Covid-19, Diş Hekimliği Eğitimi, Uzaktan Öğrenme.

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Introduction

The novel coronavirus (COVID-19) pandemic has become a real challenge for healthcare providers around the world and has significantly affected the dental professionals in practices, universities and research institutions.[1-3] Dental procedures by their nature have a high risk of COVID-19 infection due to face-to-face communication with patients and the dental team. Clinical studies indicate that most of the dental procedures involving use of rotary handpieces generate considerable amount of contaminated and potentially infectious aerosol and droplets.[4-7] Due to high risk of COVID-19 transmission, in-person procedures in both undergraduate and graduate dental education came to a halt globally, and theoretical and clinical learning processes shifted to being conducted online.[1,8,9]

Academic dental institutions have, since March 2020, closed teaching clinics and sent students home to continue didactic learning in accordance with governmental stay-at-home measures for months. It was challenging for faculty members to adapt the entire curriculum and evaluation methods in a short period of time. Dental faculties managed to produce an online curriculum by utilizing teleconference and file-sharing platforms to deliver virtual lectures. In addition, preclinical simulation activities have stopped at many institutions.[1,2,8,9]

On the other hand, although the coronavirus pandemic has caused many difficulties for provision of dental education, it was also an opportunity for the dental educators to modernize their teaching approaches using novel digital concepts.[4]

In light of the foregoing facts, the aim of this study was to evaluate the student and educator experience of distance learning/teaching, their level of motivation and attitude toward distance education, and the barriers encountered when learning and teaching online after one year of online education.

Materials and Methods

Ethical Approval and Inclusion Criteria

The ethical board approval required for the study was obtained from Ankara University, Faculty of Dentistry Clinical Research Ethics Board (No:14/4, 09.12.2020). Participation in the study was voluntary for both students and educators. The study included 52 academicians and 281 undergraduate students (4th and 5th grade) who have been involved in online theoretical and practical education in e-learning period.

During this time, the theoretical courses were given on a dedicated internet platform with 20-minute virtual sessions. To avoid disruptions in clinical training, online videos were uploaded to the system.

Conducting the Survey and Contents of Survey Sub-headings

The survey was delivered to the participants by e-mail or through social media platforms by using the "Google Forms" application. Variables of the survey research were classified into 9 sub-headings for both students and educators.

Questionnaire items for students were divided into the following categories: (1) students' personal and professional skills and attitudes toward online education, (2) students' own conditions, (3) online theoretical education, (4) online practical training, (5) online exams, (6) educator's attitudes toward online education, (7) handling and quality of education during the pandemic, (8) school administration attitudes, and (9) recommendations for better online learning experience.

Questionnaire items for educators were categorized into the following factors: (1) educators' personal and professional skills and attitudes towards online education, (2) lecturers' own conditions, (3) online theoretical education, (4) online practical training, (5) online exams, (6) students' interest and participation, (7) handling and quality of education during pandemic, (8) the school administration attitudes, and (9) recommendations for better online learning experience.

The survey was rated on a 5-point Likert scale with the responses of 1) Agree, 2) Strongly Agree, 3) Undecided, 4) Disagree, 5) Strongly Disagree.

The findings were obtained and evaluated by additing the percentages of agree and strongly agree for the positive response of the participants to a question. Similarly, the findings were obtained and evaluated by adding the percentages of disagree and strongly disagree for the negative response of the participants to a question.

Results

52 educators and 281 undergraduate students participated in this survey study. The main answers to the survey questions were as follows.

During the online education process, 82.7% of the students stated that they had enough technological knowledge to follow the online courses. On the other hand, 32% of the students reported that they easily adapted to online education conditions. 41.2% of the students reported that they had regularly attend online classes. However, 28% of the students stated that they had not have technological resources (laptop, webcam, microphone) for online education (Table 1). While 69.8% of the educators reported that they easily adapted to the online education conditions, 35.9% said that the courses they gave had no educational value (Table 1).

36.7% of the students stated that online theoretical courses were as successful as face-to-face courses. 72% of the students said that their theoretical education skills could only be increased in a face-to-face courses. The majority of the students (84.2%) stated that online learning was not sufficient to acquire clinical skills (Table 2). 24.5% of the educators stated that online theoretical courses were as successful as face-to-face courses. Only 9.4% of the educators stated that they were more comfortable in online theoretical lessons than in face-to-

face lessons. For online practical training, 81.2% of the trainers reported that training videos could be useful for improving practical skills (Table 2). 56.4% of the students reported that the online course materials were sufficient for them to be successful in the exams. 60.5% of the students reported that they were more successful in online exams than face-to-face exams (Table 3). 74% of the educators stated that online exams could not accurately determine the knowledge level of the student (Table 3).

55.9% of the students reported that the educators' effective use of communication methods (videos, surveys, etc.) increased their motivation. 61.6% of the students reported that the educators were not as competent in online education as in face-to-face education (Table 4). 77.4% of the educators stated that the interaction between the student and the educator was better in face-to-face education (Table 4).

21.4% of the students stated that they preferred the suspension of education completely during the Covid-19 pandemic. 89% of students thought that they should attend in a postgraduate program (PhD or Dental Speciality Exam - DSE) in order to acquire clinical skills that they could not acquire due to the pandemic (Table 5). 69.8% of the educators agreed with this opinion (Table 5).

68% of the students stated that the quality of education could be improved by increasing the online equipment. The vast majority of students (85.1%) thought that increasing visual materials in online education could reduce the deficiencies in education (Table 6). 90.4% of the educators thought that improving the online education facilities at the university would increase the quality of distance learning (Table 6).

Discussion

Educational processes in the field of dentistry include an education model in which theoretical knowledge is supported by clinical practice and applications. Also, face-to-face, mutual and open communication in dental education increases trust and cooperation.[9,10] However, the COVID-19 pandemic caused disruption in education, as in other interactive processes. Moreover, dental educators and clinical students are at high risk of contamination in potentially infected environments where aerosol-generating procedures are performed intensively.[7,11,12]

In this context, the immediate effects of COVID-19 on dental education were noticed very soon after the announcement of the need for "social distancing" and minimizing all face-to-face communication including teaching and educational activities. Because dental schools are different than other dental practices and institutions, as they have large open clinics and a need for supervising dentists to move between patients, all dental schools and post-graduate teaching providers stopped their routine face-to-face educational sessions and hands-on pre-clinical teaching as well as the supervised clinical training and shifted to alternative methods of teaching delivery and assessment such as online lectures, webinars,

problem-solving sessions, written reports and computer-based exams.[1-4]

Much of the dental literature on e-learning has concentrated on students' experience to such innovations.[13-17] There is also little information available about how dental teachers perceive e-learning approaches. Very little is also known about how dental teachers experience e-learning approaches. In this regard, the purpose of this study is to assess students' and educators' perspectives on the effects of the COVID-19 pandemic on undergratuate dental education and the educational model, which has taken on a new dimension as a result of the pandemic. Our study included educators who have been teaching online classes and students who have been attending distance learning for the past academic year. The student survey group was drawn from 4th and 5th grade students who had received both face-to-face and online education. In recent years, online survey applications have become a frequently used method in survey studies in the literature with the help of rapid developments in technology. This method offers fast and low-cost access to a large number of participants.[18] For this reason and considering the social distancing measures brought by the COVID-19 pandemic, the online survey forms in our study were delivered to the participants via social media or e-mail in order to reach a higher sample size.

This survey was rated on a 5-point Likert scale with the responses of 1) Agree, 2) Strongly Agree, 3) Undecided, 4) Disagree, 5) Strongly Disagree. In this type of scale, there is an expression that states an attitude or opinion about the subject/opinions being researched, and options that indicate different rates of endorsement of this statement. Participants are offered multiple options on a scale in order to determine the level of endorsement of the opinion/topic being researched.[19]

In a recent survey focusing on the European management of the COVID-19 crisis, 90% of dental schools reported using online pedagogical software tools, 72% used live or streamed videos, 48% provided links to further online materials, 65% participated in organizing virtual meetings and, less frequently, small-scale working groups, social media groups or journal clubs.[20] New forms of classroom conference and lectures, using video conference systems were implemented at many institutions.[21-24] Generally, students were found to have positive impressions despite technical problems and related stresses. While these platforms are advantageous because they are already structured and universal, they require improvements, constant maintenance, and a high-quality internet connection. Although face-to-face education is a more effective communication modality for clinical case-based discussions; remote, internet-based discussions on virtual platforms enable a more relaxed discussion ambience.[8] Easy adaptation of current graduate students to online education may also be explained by their belonging to Generation Z. Generation Z takes advantage of all the opportunities of the information age and closely follows technological

developments, internet environment, social media and different communication channels. These individuals are highly capable of multi-tasking with equally high levels of oral, cognitive and learning skills.[25] However, according to the results of this study, only 32% of the students said that they easily adapted to e-learning conditions. However, this may be attributed to the increased level of anxiety that might have been caused by the abrupt introduction of an extraordinary education setting without preliminary preparation, together with the sudden pandemic process, rather than the students' inability of adapting to distance learning.[26] The study's authors believe that students' ability to adapt to distance education will improve over time. On the other hand, 28% of students stated that they lacked proper technical means such as computers and web cameras for online learning, and 32% of them expressed concern on how to tackle problems they may encounter while using the online education platform. In this respect, the technological shortcomings can be explained by the low-income levels of some students' families, and the difficulty of overcoming the problems encountered can be due to the fact that distance education was not widely used in dentistry before the COVID-19 pandemic.[27] In this context, providing students with necessary technological equipment, the preparation of manuals that will help them troubleshoot most basic problems they may encounter in online education processes, and establishing dedicated helpdesks will be beneficial. Whereas, a sizeable majority of the educators have stated that they could easily adapt to online education conditions (69.8%), their personal technological equipment was adequate for online education (81.1%), and their universities' infrastructure for distance learning were sufficient (66%). Considering that the educatiors are generally belonging to Generation X, these results are promising and show good adaptation levels. However, in case of technological expertise, only a small proportion of the educators stated using effective communication methods (h5p, forums, surveys, etc.) which is in line with the properties of Generation X.[28] University administrations should provide practical training opportunities to educators related to these issues.

Although online education is indispensable during the pandemic period, the advantages or disadvantages of online education over face-to-face education have become a matter of debate. Indeed, in a study conducted by Abbasi *et al.*[29] on medical and dental school students, 85% of the students reported that online learning was less effective than face-to-face learning. Hattar et al.[26], reported that 5th grade students sensed that they actually missed important learning experiences especially the clinical sessions. Similarly, in this study, 72% of the students for theoretical lessons and 89.1% of the students for clinical education expressed belief that skills can only be improved via face-to-face teaching. Only a small portion of the educators (24.5%) stated that online theoretical classes are as successful as face-to-face classes. Moreover, 40% of the educators said that the duration of the classes was not long enough to teach the

contents of the class. On clinical practical training, a big majority of the educators (81.2%) said practical videos will be beneficial whereas only 49% of the educators stated that clinical conditions could be simulated for online practical training. Drawing from these findings, we may say that improvements should be made to the adaption of educators to online education conditions. Indeed, we believe that students' opinions and suggestions about the educators will be enlightening. In this respect, 55.9% of the students said their motivation for participation in the classes are increased when educators used effective communication methods such as videos, surveys, etc. In addition to this, only 45.4% of the students said that their educators continuously looked for ways to self-improve themselves for better online teaching. Given that dental training mainly hinges on the mutual communication of the student and the educator,[10,30] we believe that the educators should be willing to act in line with the students' feedback and suggestion and self-correct and strive for a better-quality training.

Another potential setback the educators are concerned about in distance learning is the efficacy of online exams.[31] A vast majority of the educators (74%) expressed belief that online exams could not fully assess the level of knowledge. In this respect, 79.3% of the educators said that they searched for ways to improve question formulation techniques for online exams while a sizeable part of the educators (81.2%) said online exams encouraged cheating. In this context, modern information technology (IT) can be used for high quality exams. By means of exam precautions like the lockdown browser, students cannot search on the internet (like Google and so on) to find an answer during the internet examination. However, it is obvious that an examination platform for dental education will also need to be developed.[32] 71.1% of the students stated that the exams should not be interactive or video-based. For online education, open book exams may be preferred to traditional or closed book exams. The main premise for open book exams is that teachers can devise questions that require students to answer in more critical and analytical ways thus encouraging high-order thinking skills in their students; as compared to closed book or traditional exams that tend to encourage rote learning and more superficial application of knowledge. The COVID-19 national lockdown resulted in re-structuring of assessments to open-book formats so learners could complete their examinations at home and has provided a novel opportunity to reflect on examination practices.[33]

Case-based discussions/exams are important learning strategy, applied in several dental specialties and courses. Virtual patient (VP)-based learning consists of simulating clinical cases to improve students' skills in decision making and diagnosis.[3,8] The findings of the present survey also showed that nearly half of the students suggested that the inclusion of more case planning that feature clinical visual materials in online education can reduce the shortcomings in practical training.

On the other hand, regarding the problems encountered during the pandemic, a small portion of the students stated that they could convey their problems to the administrators (29.9%) and that they could find solutions to these problems through administrators (23.2%). 41.9% of the students said that infrastructure problems at their universities substantially reduced the quality of online education. In order to resolve the pandemic-related problems or setbacks, which have been confirmed by the responses of students and educators who participated in our study, and to make improvements, the suggestions presented here and their implementation in an appropriate framework are of utmost importance. In the last section of our study, we examined feedback from students and educators to potential suggestions. In this respect, a substantial portion of the students (85.1%) have suggested that online education should include more case planning that feature clinical visual materials. Moreover, students suggested that even during the pandemic, partial face-to-face teaching should be offered side by side distance learning (51.1%), website recommendations by educators for access to reliable learning resources (83.9%) and education management and planning should involve more feedback from students (89.4%).

As for suggestion by educators, a great majority of the educators (90.4%) said that improvements to university's online infrastructure and diversification of ways for access to distance education will increase the quality of education, and 94.3% of the educators called for improved access to online learning tools such as computers, microphones, web cameras, etc. On a similar rate with the students, 90.6% of the educators emphasized that feedback should be given more place within education plans.

Considering that the education model in dentistry has changed and differentiated with the COVID-19 pandemic, Deery[1] evaluated the impacts of COVID-19 on the provision of dental education in the 67 dental schools in the United States and stated that schools should re-evaluate their policies and curricula and incorporate appropriate methods of distance learning permanently into their teaching.

Considering our new reality, it is possible to continue teaching theoretical content, keeping students' unequal access to quality internet in mind, especially those with low income. However, few effective alternatives are available to dental students today, considering pre-clinical and clinical scenarios. The challenges will be greater in developing countries. Unequal student access to quality internet can compromise achievement in remote activities. To return to clinical activities, major investments must be made in dental school clinics, to adapt to the new biosafety reality of the post-pandemic period.[1,8] Moreover, in an educational setting such as dental training whereby interaction between students and educators play a crucial role, their feedback should be subject of regular scrutiny and necessary revision and adjustments must be made in line with their suggestions. Besides, it is acceptable to suggest that infrastructural services should be improved considering that distance learning will a permanent part of dental education even though dental training is a process that is mostly based on clinical practice.

Conclusions

The results of this survey study showed that academicians hold a negative view of distance learning as they think remote theoretical courses and practical training, and online exams impaired the quality of learning. Results of this survey study demonstrated that dental students have a positive attitude towards online theoretical education but are concerned about clinical skill acquisition.

Online education, which has forced its way to become a part dental education due to the COVID-19 pandemic, may remain as a supporting feature in the future for several advantages it presents such as repeatable learning tools, effective communication skills, instant evaluation, and visual education materials.

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Conflict of interest

The authors declare no conflict of interest.

Table 1: Factors Related to Students' and Educators' Own Attributes and Conditions

	Agree (%)	Strongly Agree (%)	Undecided (%)	Disagree (%)	Strongly disagree (%)
Factors Related to Students' Own Attributes					
I have enough computer technology skills to follow online classes.	36.6	46.1	8.5	5.3	3.5
I can easily adapt to online education conditions.	18.7	13.3	23.7	31.3	13
I know how to solve problems in the online education system when I face one.	24.6	8.7	34.7	21.5	10.5
I regularly attend online classes.	31.3	9.9	30.6	21.5	6.7
I abide by the rules of online education.	51.8	34.9	8.8	2.8	1.7
Factors Related to Students' Own Conditions					

I do not have adequate resources (laptop, webcam, microphone etc.) for online education.	17.3	10.7	11.5	38	22.5
I am having problems connecting to internet.	23.9	13.7	27.1	25.7	9.6
Sharing my computer with family members is causing issues attending online classes.	18	12.3	7.4	32	30.3
I am forced to attend online classes elsewhere other than my house (another house or institution, at neighbors' or cafe, etc.) due to internet connection issues.	3.5	5.3	7.7	35.6	47.9
Factors Related to Educators' Own Attributes					
I can easily adapt to online education conditions.	37.7	32.1	18.9	5.7	5.6
Online classes I teach have no educative value.	18.9	17	22.6	32.1	9.4
My computer technology skills are not enough to online classes.	62.3	15.1	13.2	5.7	3.7
I am not informed enough to troubleshoot problems encountered during online classes.	47.2	9.4	15.1	18.9	9.4
I abide by the rules of distance learning.	50.9	34	9.4	1.9	3.8
I know how to handle problems I face in online education environment.	28.3	9.4	37.7	20.8	3.8
Factors Related to Educators' Own Conditions					
I have adequate resources (laptop, webcam, microphone etc.) for online education.	41.5	39.6	5.7	9.4	3.8
The equipment I own conforms to the distance learning infrastructure of our university.	41.5	24.5	13.2	17	3.8
I am having internet issues during online classes.	30.2	17	11.3	32.1	9.4

Table 2: Student and Educator Opinion on Online Theoretical and Clinical Training

	Agree (%)	Strongly Agree (%)	Undecided (%)	Disagree (%)	Strongly Disagree
Student Opinion on Factors Related to Online Theoretical Training					
Online theoretical classes are as successful as face-to-face classes.	18	18.7	14.7	20.1	28.5
"Learning management system" guides are sufficient enough for me to get acquainted with online education environment.	28.2	14.1	31.6	16.2	9.9
Duration of online classes is sufficient.	29.6	12	17.3	24.6	16.5
I can follow online classes more easily than I can do with face-to-face classes.	25	28.9	12.3	10.6	23.2
Online classes are held regularly and on time according to schedule.	48.6	15.5	22.9	8.1	4.9
I feel I am taught efficiently after online courses.	19.7	8.1	28.9	24.3	19
Repeatable online sessions boost my learning capacity.	35.6	46.5	11.6	4.2	2.1
I believe theoretical training skills can only be improved in a face-to-face setting.	45	27	15.1	6.1	6.8
Student Opinion on Factors Related to Online Practical Training					
Practical training videos are good for me to improve my skills.	23.6	8.5	33.4	14.8	19.7
Watching practical training videos hones my clinical skills.	24.3	6.7	26.8	20.8	21.4
Online learning is not sufficient enough for me to gain clinical skills.	24.3	59.9	11.6	0.7	3.5
I believe clinical training skills can only be improved in a face-to-face or a clinical setting.	29.2	59.9	6.7	2.1	2.1
Educator Opinion on Factors Related to Online Theoretical Training					
Duration of online classes is long enough to teach the content.	43.4	5.7	10.9	17	23
I always offer my students additional accessible resources during my classes.	47.2	26.4	15.1	11.3	0
Online theoretical classes are as successful as face-to-face classes.	15.1	9.4	28.3	26.4	20.8
I provide students feedback about their mistakes in their assignments.	32.0	5.7	15.1	30.2	17
I am more comfortable with online theoretical classes than I am with face-to-face classes.	1.9	7.5	17	43.4	30.2
I regularly upload my teaching material to the learning management system.	45.3	43.4	9.4	0	1.9
Online classes are not as effective as in-person classes.	41.5	22.6	26.4	3.8	5.7
My motivation is negatively affected if number of students attending my classes is low.	39.6	34	7.5	17	1.9
I do use effective communication methods (h5p, forums, surveys, etc.) in my classes.	18.9	1.9	28.3	35.8	15.1
Educator Opinion on Factors Related to Online Practical Training					
Practical training videos are good for improving skills.	47.2	34	15.0	0	3.8
I regularly update my documents to provide students resources regarding practical training.	45.3	24.5	24.5	3.8	1.9
Online practical education does not help gain critical thinking skills.	15.1	30.2	28.3	17	9.4
I try to improve my students' skills of choosing the right method for diagnosis and treatment by planning cases in online practical training.	32.1	30.1	20.8	11.3	5.7
I strive to simulate clinical conditions during online practical training sessions.	22.6	26.4	30.2	15.1	5.7

Table 3: Students' and Educators' Opinion on Online Exams

	Agree (%)	Strongly Agree (%)	Undecided (%)	Disagree (%)	Strongly Disagree (%)
Students' Opinion on Online Exams					
Online course material is sufficient enough for me to achieve success in exams.	38.4	18	24.6	11.3	7.7
I believe my level of knowledge is fully measured by online exams.	30.6	13.4	24.3	13.7	18
I get more anxious during online exams than I get in in-person education.	14.1	12	17.3	28.9	27.7
I am more successful in online exams than I am in face-to-face learning.	41.5	19	31.3	4.9	3.3
I believe that exams should be interactive or held via video-conferencing rather than being multiple-choice.	6.7	1.4	20.8	19.7	51.4
Online exams should be composed of open-ended (classical) questions.	4.9	1.1	25.7	22.9	45.4
I have connection issues during online exams.	31	9.9	33.8	18.3	7
Educators' Opinion on Online Exams					
Online exams can not assess knowledge level correctly.	39.6	34.4	11.3	5.3	9.4
I strive to improve my methods of formulating questions for online exams.	32.1	47.2	9.4	7.5	3.8
Online exams encourage cheating.	34	47.2	17	1.8	0

Table 4: Students' and Educators' Opinion on Interrelated Situations

	Agree (%)	Strongly Agree (%)	Undecided (%)	Disagree (%)	Strongly Disagree (%)
Student Opinion about Educator					
Effective use of communication methods (videos, surveys, etc.) by educators boosts my motivation to attend classes.	40.1	15.8	30.6	8.6	4.9
Getting feedback from educators helps me learn more easily.	54.6	21.5	16.9	3.5	3.5
Educators in online education are not as competent as they are in face-to-face education.	37.7	23.9	18.3	13.7	6.4
Educators offer learning resources for each class.	30.3	6.3	29.9	24.3	9.2
Slides shown by educator and content of the course affect my participation in classes.	45.4	37.3	9.5	4.6	3.2
I can easily reach educators and convey my problems to them outside online classes.	31.7	9.9	30.6	20.1	7.7
Educators are constantly self-improving for better quality online teaching.	38.4	7	29.2	15.5	9.9
Educator Opinion on Factors Related to Student Attention and Class Participation					
I believe that students have a low level of motivation to participate in online classes.	49.1	30.2	9.4	1.9	9.4
My students feel more comfortable asking questions during online sessions than in face-to-face classes.	30.2	9.4	18.9	24.5	17
I believe that interaction between the student and the educator is better in face-to-face education.	28.3	49.1	13.2	7.5	1.9
My students are pleased to receive feedback about their mistakes during online education.	32.1	15.1	49.1	0	3.7

Table 5: Students' and Educators' Opinion on the Pandemic Period and Administrative Attitude of the Institution

	Agree (%)	Strongly Agree (%)	Undecided (%)	Disagree (%)	Strongly Disagree (%)
Student Opinion on Factors Related to the Pandemic					
I'd rather education was suspended altogether during the pandemic.	6.3	15.1	9.9	32.7	36
I'd rather receive face-to-face education during the pandemic.	11.6	21.5	21.1	20.4	25.4
Classes during the pandemic are strengthening my communication with my friends and instructors.	15.8	19	20.4	27.8	17
When the pandemic is over and clinical practices resume, clinical training should be planned on a priority basis.	25.7	60.2	10.6	1.1	2.4
I believe I should be enrolled in a postgraduate program (PhD or Dental Specialist Exam – DSE) to obtain clinical skills I failed to gain due to the pandemic.	23.2	65.8	7.4	2.8	0.8
Online education in post-pandemic era should be replaced by face-to-face education entirely.	21.8	25	23.2	16.9	13.1

Student Opinion on Factors Related to Administrative Attitude of Institution					
I can communicate my problems to managing authorities.	24.6	5.3	34.9	24.3	10.9
I can solve my problems through managing authorities.	19.7	3.5	39.4	23.9	13.5
Shortcomings in internet connection or infrastructure on the part of my university substantially takes away from the quality of online education.	26.1	15.8	37	16.9	4.2
Faculty management asks about our thoughts on drawbacks of online education and heeds our solution offers.	28.9	7.7	36.6	14.8	12
Faculty's student affairs office finds solutions to my problems.	15.8	3.2	40.5	21.1	19.4
Educator Opinion on Factors Related to the Pandemic					
I'd rather education was suspended altogether during the pandemic.	3.8	22.6	24.5	35.8	13.3
Face-to-face clinical practice should be conducted during the pandemic.	15.1	15.1	17	30.2	22.6
Students who graduate during the pandemic will lack clinical skills.	26.4	66	7.6	0	0
Students who graduate during the pandemic should improve practical skills through tutorials and workshops before they attend to independent clinical practice.	26.4	66	3.8	3.8	0
Students who graduate during the pandemic should receive postgraduate training (PhD or Dental Specialist Exam – DUS) before they attend to independent clinical practice.	32.1	37.7	22.6	7.6	0
Educator Opinion on Factors Related to Administrative Attitude of Institution					
I can communicate my problems to managing authorities.	41.5	39.6	11.3	1.9	5.7
I can solve my problems through managing authorities.	15.1	15.1	22.6	34	13.2
Shortcomings in internet connection or infrastructure on the part of my university substantially takes away from the quality of online education.	32.1	18.9	3.8	28.3	16.9

Table 6: Solution Offers by Students and Educators

	Agree (%)	Strongly Agree (%)	Undecided (%)	Disagree (%)	Strongly Disagree (%)
Solution Offers by Students					
Quality of education can be improved through live streaming from physical classroom and clinics by increasing online infrastructure.	36.3	31.7	21.1	5.6	5.3
Inclusion of more case planning that feature clinical visual materials in online education can reduce our shortcomings in practical training.	44.7	40.4	10.9	2.5	1.5
Learning process can be improved by incorporating collective discussion sessions into online education.	35.6	18.7	26.1	16.2	3.4
Instead of full-time online education, partial face-to-face learning will be beneficial.	20.8	30.3	23.9	8.5	16.5
Educators' sharing of safe internet sites as resources will help boost my clinical skills.	45.3	38.6	10.9	2.8	2.4
Student feedbacks should occupy more space in educational planning.	43.7	45.7	9.1	1.4	0.1
Solution Offers by Educators					
The improvement of my university's online education infrastructure and diversification of options to access to education will increase the quality of distance learning.	41.4	49	3.8	0.1	5.7
My university should provide educators the necessary equipment (PC, microphone, webcam, etc.) for distance education.	30.2	64.1	0.1	0	5.6
Post pandemic education planning should include programs to compensate for losses especially in clinical practice.	32.1	64.2	3.7	0	0
Faculty managements should provide professional assistance to produce of practical videos.	18.9	71.7	3.8	0	5.6
Solutions should be offered for all stakeholders to increase the quality of education.	24.5	66	3.8	0	5.7
Student and educator feedback should be included in the educational planning to a greater extent.	34	56.6	7.4	0	2

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Comparative Evaluation of the Shaping Ability of Five Different Nickel-Titanium Rotary File Systems in Simulated Canals

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Research Article

ABSTRACT

Objectives: The aim of this study was to compare the simulated canal shaping efficiencies of five different NiTi rotary file systems.

Materials and Methods: In the study, 100 transparent resin blocks with J-shaped canals were randomly divided into five groups (n = 20). Simulated canals were shaped with VDW.Rotate (VR), TruNatomy (TRN), HyFlex CM (HF), EdgeFile X7 (EF), or ProTaper Next (PTN) files. Ten measuring points were detected on the pre- and post-preparation images taken from the blocks and superimposed. After preparation, the total canal width and the amount of transportation were calculated for the determined measuring levels. Zipping and ledge formation, instrument fracture and deformation, and change in working length were evaluated. The data were statistically analyzed with the Kolmogorov-Smirnov test, one-way ANOVA, Tukey test, Chi-Square test, and a Monte Carlo version of the Fisher Exact tests. The error level was taken as 0.05.

Results: There were significant differences between the groups at all measuring levels in terms of total canal width after instrumentation (p = 0.001). Significant differences in the amount of transportation were found between the groups (p = 0.001) except at levels 4 (p = 0.169) and 10 (p = 0.054). Zip and instrument fractures did not occur in any group. 3 EF size 25/.04 files were deformed (p = 0.021). There was no significant difference between the groups in terms of ledge formation and working length change (p > 0.05).

Conclusions: According to findings obtained in the study, transportation occurred at all 10 measuring levels with all file systems used. HF and EF systems were found to be more reliable in terms of transportation in the middle and coronal regions. Wider canal preparation was obtained with the PTN system in the middle and coronal regions.

Keywords: Root canal transportation, NiTi, shaping efficiency, simulated canal.

Beş Farklı Nikel-Titanyum Döner Eğe Sisteminin Yapay Kanallarda Şekillendirme Etkinliklerinin Karşılaştırmalı Olarak Değerlendirilmesi

ÖZ

Amaç: Bu çalışmanın amacı, beş farklı NiTi döner eğe sisteminin yapay kanalları şekillendirme etkinliklerinin karşılaştırılmasıdır.

Gereç ve Yöntemler: Çalışmada 100 adet J şekilli kanala sahip şeffaf rezin blok, rastgele beş gruba ayrıldı (n = 20). Yapay kanallar VDW.Rotate (VR), TruNatomy (TRN), HyFlex CM (HF), EdgeFile X7 (EF) veya ProTaper Next (PTN) eğeleriyle şekillendirildi. Bloklardan alınan ve çakıştırılan preparasyon öncesi ve sonrası görüntülerinin üzerinde 10 ölçüm seviyesi tespit edildi. Preparasyon sonrası toplam kanal genişliği ve transportasyon miktarı belirlenen ölçüm seviyeleri için hesaplandı. Zip, basamak oluşumu, alet kırığı, deformasyon ve çalışma boyunda meydana gelen değişim değerlendirildi. Veriler Kolmogorov-Smirnov testi, tek yönlü varyans analizi, Tukey testi, Khi-Kare testi ve Fisher Exact testlerden Monte Carlo modeliyle istatistiksel olarak analiz edilerek yanılma düzeyi 0,05 olarak alındı.

Bulgular: Tüm ölçüm seviyelerinde preparasyon sonrası toplam kanal genişliği yönünden gruplar arasında anlamlı farklar bulundu (p = 0,001). Seviye 4 (p = 0,169) ve 10 (p = 0,054) dışındaki ölçüm seviyelerinde gruplar arasında transportasyon miktarı açısından önemli farklılıklar bulundu (p = 0,001). Hiçbir grupta zip ve alet kırığı oluşmadı. 3 tane EF 25/.04 boyutlu eğe deforme oldu (p = 0,021). Basamak oluşumu ve çalışma boyu değişimi yönünden ise gruplar arasında anlamlı fark gözlenmedi (p > 0,05).

Sonuçlar: Çalışmada elde edilen bulgulara göre kullanılan tüm eğe sistemleriyle 10 ölçüm seviyesinde de transportasyon meydana geldi. HF ve EF sistem orta ve koronal bölgede transportasyon yönünden daha güvenilir bulundu. PTN sistemiyle orta ve koronal bölgede daha geniş kanal preparasyonu elde edildi.

Anahtar Kelimeler: Kök kanal transportasyonu, NiTi, şekillendirme etkinliği, yapay kanal.

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Introduction

Adequate cleaning, shaping, and a three-dimensional sealing of the root canal system are required for successful root canal treatment.[1,2] Many endodontic file systems and shaping methods have been developed to maintain the original shape of the canal and to avoid complications during root canal preparation. Design features and manufacturing methods can significantly affect the clinical performance of NiTi files.

Newly designed VDW.Rotate system (VR; VDW, Munich, Germany) that preserves pericervical dentin has an off-centered design and a constant taper. Its double-bladed adapted S-shaped cross-section increases cutting efficiency and reduces the screw-in effect. The heat treatment applied to the files increases flexibility and fatigue resistance.[3,4] TruNatomy (TRN; Dentsply Sirona, Maillefer, Ballaigues, Switzerland) is also a newly introduced heat-treated file system that allows it to be pre-bent with high flexibility, manufactured from thin NiTi wire with a maximum 0.8 mm flute diameter instead of 1.2 mm. TRN instruments; preserve structural dentin and tooth integrity due to instrument geometry, regressive taper, and slim NiTi wire design.[3,5] HyFlex CM (HF; Coltene Whaledent, Cuyahoga Falls, OH) is a more flexible rotary file system with controlled memory. Hyflex CM files are made of NiTi alloy, which contains a lower percentage of nickel weight (52.1%) by weight than conventional NiTi alloys.[6] EdgeFile X7 system (EF; EdgeEndo; Albuquerque, NM) has a constant taper, triangular cross-section, and variable helix angle. The files are made of an annealed heat-treated NiTi alloy called Fire-Wire, which is claimed to increase the cyclic fatigue resistance and flexibility but reduce the shape memory effect inherent of NiTi files.[7,8] ProTaper Next (PTN; Dentsply Maillefer, Ballaigues, Switzerland) is a variable taper file system with an off-centered rectangular cross-section manufactured with M-Wire technology, which is reported to increase cyclic fatigue resistance and flexibility.[7,9]

The present in vitro study aimed to examine the shaping efficiency of the above-mentioned NiTi rotary file systems whose mechanical properties and flexibility were improved by applying heat treatment.

Materials and Methods

The present study design was approved by the Non-Interventional Clinical Research Ethics Committee of Sivas Cumhuriyet University with the decision number 2020-08/14. One hundred transparent resin blocks (FlexMaster Übungsblocks, Ref. V040245, Germany) with approximately 18.5 mm long canals having curvatures ranging from 30 to 38 degrees were used for this study. Resin blocks were randomly divided (https://www.randomizer.org/) into five groups (n = 20):

Group 1 – VR NiTi rotary file system: The simulated canals were shaped using VR files (size 15/.04 file with 1.3 Ncm torque, size 20/.05 file with 2.1 Ncm torque, and size 25/.04 file with 2.3 Ncm torque, respectively) with an up-and-down movement at 350 rpm until each file reached the working length.

Group 2 – TRN NiTi rotary file system: The simulated canals were shaped using TRN size 17/.02 and 26/.04 files with an up-and-down movement at 500 rpm and 1.5 Ncm torque until each file reached the working length.

Group 3 – HF NiTi rotary file system: The simulated canals were shaped using HF size 20/.04 and 25/.04 files with an up-and-down movement at 500 rpm and 2.5 Ncm torque until each file reached the working length.

Group 4 – EF NiTi rotary file system: Coronal parts of the simulated canals were shaped using EF size 25/.04 files with an up-and-down movement at 300 rpm and 3 Ncm torque until resistance was felt in the canal. Afterward, the preparation of the canals was completed to the working length using EF size 20/.04 and 25/.04 files at the same torque and speed settings.

Group 5 – PTN NiTi rotary file system: The simulated canals were shaped using PTN size 17/.04 and 25/.06 files with an up-and-down movement at 300 rpm and 3 Ncm torque until each file reached the working length.

The canal patency of each block was checked with a size 10 K-file (Shenzhen Denco Medical Co., Ltd., China) before shaping. The canal length was measured. The working length was established for each canal by subtracting 0.5 mm from the canal length. The blocks were covered with aluminum foil to prevent the canal from being seen. A glide path was created with a size 15 K-file for each canal. Each canal was shaped using the group's rotary file system and EndoMatic Endo Motor (Guilin Woodpecker Medical Instrument Co. Ltd., China). Copious irrigation with distilled water was performed after each instrument change and during the shaping processes. Each file was used to prepare only four canals.

Standardized pre- and post-instrumentation images obtained with a digital camera (Canon Digital IXUS 80 IS) were superimposed using Adobe Photoshop CS3 (Adobe System, San Jose, CA, USA) program. A total of 10 different points were marked from apical to coronal with a distance of 1 mm with the image analysis program (ImageJ 1.42q, National Institutes of Health, USA) on the superimposed images (Figure 1). The initial measuring level was determined to be 1.5 mm away from the apical end of the artificial root canal. The total canal width of the instrumented canal was measured for each level and recorded. In addition, a total of 20 distances were measured for each canal to determine the amount of resin removed from the inner and outer sides of the curve. The amount of transportation was recorded for each measuring level as the value of the difference between the amount of resin removed from the inner and outer sides of the canal. Images were evaluated in terms of zipping and ledge formation. Instrument deformation and separation were examined. The difference between the working length before preparation and the working distance measured by placing the master apical file in the canal after preparation was recorded as the change in working length.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics version 22.0 (SPSS, Inc., Chicago, IL, USA). Since the parametric test assumptions were fulfilled in the evaluation of the data (Kolmogorov-Smirnov), the one-way analysis of variance was used when comparing more than two independent groups, the Tukey test was used to find the group or groups that make a difference, and Chi-Square test was used to evaluate the data obtained by counting. When the assumptions were not met, a Monte Carlo version of the Fisher Exact tests was used and the error level was taken as 0.05.

Results

Table 1 shows the mean total canal widths at each measuring level for all groups. There were significant differences between the groups at all levels in terms of the total mean instrumented canal widths (p = 0.001). TRN caused more canal widening than the PTN, VR, and HF groups at level 1 (p < 0.05). The mean canal width value of the EF group was higher than the VR, HF, and PTN groups at levels 1 and 2 (p < 0.05). EF removed more resin than the VR, TRN, and HF at levels 3 and 4 (p < 0.05). The PTN group showed a greater amount of resin removal than the VR, TRN, and HF groups at levels 4 and 5 (p < 0.05). The mean canal widths of the PTN group were higher than all groups at levels 6-10 (p < 0.05). The total resin removal with TRN was greater than VR, HF, and EF at levels 7 and 8 (p < 0.05). For levels 9 and 10, while there were no differences in canal widths between VR and EF groups, there were differences between all other groups (p < 0.05).

The amount of absolute canal transportation for the measuring levels is detailed in Table 2. Significant differences in the amount of transportation were found between the groups (p = 0.001) except at levels 4 (p = 0.169) and 10 (p = 0.054). For level 1, TRN produced less transportation than VR, HF, and PTN; EF produced less transportation than PTN (p < 0.05). Less canal transportation was created by VR, TRN, and EF than PTN at level 2. The amount of canal transportation related to TRN was also less than HF at this level (p < 0.05). At level 3, PTN resulted in more canal transportation than VR and EF; HF produced more canal transportation than VR, TRN, and EF (p < 0.05). The mean absolute transportation at level 5 was statistically least with HF, while more canal transportation occurred with PTN compared to TRN, HF, and EF (p < 0.05). At level 6, TRN induced less canal transportation than PTN; HF and EF created less canal transportation than VR, TRN, and PTN (p < 0.05). The absolute transportation with PTN, VR, and TRN was greater than HF and EF at level 7 (p < 0.05). At level 8, PTN and TRN produced more transportation than HF and EF; VR produced more transportation than HF (p < 0.05). Transportation was significantly less following the use of TRN rather than VR, HF, and PTN at level 9 (p < 0.05).

Instrument fractures did not occur in any group; however, 3 EF size 25/.04 files were deformed (p = 0.021). No zips were observed. Ledge formation occurred in canals instrumented using PTN (1 ledge), VR (2 ledges), and EF (2 ledges) systems (p = 0.554). None of the canals became blocked with resin debris for all groups. There was no significant difference between the groups in terms of change in working length ranging from 0.115 to 0.237 (p = 0.122).

Discussion

In this study, simulated canals in transparent resin blocks with a severe curve according to Schneider's classification[10] were used to evaluate the shaping efficiency of five different NiTi rotary file systems. The use of simulated resin canals has limitations because their surface texture, hardness, and cross-section differ from those of real teeth.[11] However, resin blocks allow standardization of canal length, diameter, curvature angle, and radius of curvature.[12]

In the present study, TRN showed greater total canal width than VR, HF, and PTN at level 1 and this was attributed to the larger tip diameter of the TRN master apical file. However, similar canal widths were observed in the TRN group with the VR and HF groups at levels 2-5. This finding might be related to the regressive taper and slim design of TRN files. Thus, it can be said that the structural dentin and tooth integrity were better preserved in the curvature region. The PTN group had the highest total widths at levels 4-10. It may be explained by the larger taper of the PTN files. A previous study[13] comparing ProTaper Gold, TRN, VR, and Reciproc Blue files reported that the removed tooth structure was minimal in the TRN and VR groups. They stated that this result is related to the smaller taper sizes of the files and their slim design features. In this study, larger canal width was also obtained with PTN at levels 4-10 compared to TRN and VR systems.

Differences in the cross-sections, tip designs, taper angles, and metallurgical properties of the files and instrumentation techniques are effective in root canal transportation.[14-17] Saber et al.[18] found no significant difference in canal transportation between PTN, iRaCe, and HF files in the apical region. However, they reported significantly greater canal straightening with PTN files. Huang et al.[19] showed that HyFlex EDM caused greater volume increases than HFCM and PTN at all levels of the canal while HF removed the least resin in the coronal region compared to other files. They reported that this result could be related to the taper of the instruments. The use of the HF system also resulted in less canal transportation in the apical 2 mm. They attributed this finding to the flexibility of CM alloy. Likewise, in the present study, HF removed significantly less resin than the PTN system at all measuring levels 4-10. However, HF and PTN created a similar amount of transportation at levels 1-4, while PTN produced more canal transportation than HF at levels 5-8.

Kim et al.[20] reported that TRN caused less canal transportation than ProTaper Gold and WaveOne Gold at the 3 and 5 mm levels in simulated S-shaped resin canals because it is made of thin NiTi wire and has a small regressive taper. In the present study, TRN showed significantly less canal transportation than PTN at levels 1, 2, 5, and 6, while no

difference was observed between the two groups at levels 3 and 4. This might be related to the larger taper of the PTN system and a decrease in flexibility. These findings show that TRN files provide safe preparation, especially in the apical and middle regions of the canal.

Wu *et al.*[21] showed that after the preparation of single-rooted teeth with a curvature of 21°–39°, the apical sealability of root canal obturation was adversely affected by apical transportation greater than 0.3 mm. In this study, when all measuring levels were assessed maximum amount of canal transportation was 0.128 mm. It was 0.077 mm at levels 1-3.

Large sizes files might cause zipping and perforation due to their tendency to straighten inside the canal. The decrease in flexibility of the larger size files was associated with the formation of zip.[22] No zip formation was observed in any group in this study due to the metallurgical properties of the files, their flexibility, and the use of the files by the manufacturer's instructions.

The working length loss may occur due to canal straightening and the canals becoming blocked with resin debris during canal enlargement.[6,23] It has been reported that heat-treated NiTi files preserve the original shape of the canal better and cause less loss in working length.[24] In this study, none of the canals became blocked with resin residues. The change in working length in the range of 0.11-0.24 could be occurred due to the straightening of the canals.

Conclusions

All file systems created canal transportation at each measurement point. However, they exhibited minimal transportation values within the range of 0.001-0.128 mm. These mean values show that all file systems used are reliable in terms of canal transportation. The EF system provided a wider preparation in the apical region compared to other files, while the system was found to be reliable in terms of transportation. Wider canal preparation was obtained with the PTN system in the middle and coronal regions. Since the EF size 25/.04 file showed more deformation, controlled clinical use of this file is recommended, especially in teeth with narrow and curved canals.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

Table 1. The mean total canal widths at each measuring level for different file systems.

	VDW. Rotate	TruNatomy	HyFlex CM	EdgeFile X7	ProTaper Next	p values
Level 1	0.343 ± 0.166[a]	0.377 ± 0.013[b]	0.342 ± 0.015[a]	0.376 ± 0.021[b]	0.350 ± 0.019[a]	**p = 0.001***
Level 2	0.393 ± 0.021[a]	0.412 ± 0.023[ab]	0.397 ± 0.021[a]	0.431 ± 0.021[b]	0.410 ± 0.024[a]	**p = 0.001***
Level 3	0.436 ± 0.020[a]	0.433 ± 0.014[a]	0.439 ± 0.020[a]	0.458 ± 0.018[b]	0.448 ± 0.022[ab]	**p = 0.001***
Level 4	0.469 ± 0.017[a]	0.460 ± 0.024[a]	0.467 ± 0.016[a]	0.489 ± 0.022[b]	0.493 ± 0.018[b]	**p = 0.001***
Level 5	0.499 ± 0.021[a]	0.478 ± 0.102[a]	0.498 ± 0.009[a]	0.520 ± 0.023[ab]	0.560 ± 0.021[b]	**p = 0.001***
Level 6	0.535 ± 0.018[a]	0.548 ± 0.016[a]	0.539 ± 0.015[a]	0.544 ± 0.020[a]	0.635 ± 0.019[b]	**p = 0.001***
Level 7	0.575 ± 0.016[a]	0.620 ± 0.026[b]	0.581 ± 0.011[a]	0.587 ± 0.016[a]	0.697 ± 0.023[c]	**p = 0.001***
Level 8	0.611 ± 0.017[a]	0.684 ± 0.023[b]	0.605 ± 0.014[a]	0.621 ± 0.016[a]	0.756 ± 0.033[c]	**p = 0.001***
Level 9	0.655 ± 0.018[a]	0.725 ± 0.016[b]	0.630 ± 0.014[c]	0.663 ± 0.016[a]	0.831 ± 0.024[d]	**p = 0.001***
Level 10	0.702 ± 0.014[a]	0.768 ± 0.015[b]	0.660 ± 0.014[c]	0.697 ± 0.015[a]	0.894 ± 0.020[d]	**p = 0.001***

Superscripts with different letters indicate the significance of differences between groups. (*p < 0.05)

Table 2. Mean values of absolute canal transportation (mm) at each measuring level for different file systems.

	VDW. Rotate	TruNatomy	HyFlex CM	EdgeFile X7	ProTaper Next	p values
Level 1	0.042± 0.025[ac]	0.012 ± 0.032[b]	0.041 ± 0.027[ac]	0.015 ± 0.033[ab]	0.048 ± 0.032[c]	**p = 0.001***
Level 2	0.035 ± 0.024[ab]	0.029 ± 0.040[a]	0.059 ± 0.033[b]	0.040 ± 0.035[ab]	0.077 ± 0.031[cb]	**p = 0.001***
Level 3	0.011 ± 0.024[a]	0.018 ± 0.039[ac]	0.050 ± 0.037[b]	0.003 ± 0.030[a]	0.042 ± 0.026[cb]	**p = 0.001***
Level 4	0.031 ± 0.024[a]	0.007 ± 0.039[a]	0.026 ± 0.027[a]	0.001 ± 0.148[a]	0.007 ± 0.030[a]	**p = 0.169**
Level 5	0.058 ± 0.024[ac]	0.034 ± 0.035[a]	0.005 ± 0.017[b]	0.033 ± 0.031[a]	0.067 ± 0.039[c]	**p = 0.001***
Level 6	0.114 ± 0.023[ac]	0.086 ± 0.034[a]	0.032 ± 0.023[b]	0.042 ± 0.042[b]	0.128 ± 0.052[c]	**p = 0.001***
Level 7	0.101 ± 0.032[a]	0.115 ± 0.041[a]	0.029 ± 0.022[b]	0.042 ± 0.041[b]	0.114 ± 0.069[a]	**p = 0.001***
Level 8	0.049 ± 0.035[ac]	0.067 ± 0.039[a]	0.001 ± 0.022[b]	0.012 ± 0.038[cb]	0.067 ± 0.063[a]	**p = 0.001***
Level 9	0.009 ± 0.033[a]	0.042 ± 0.037[b]	0.005 ± 0.027[a]	0.011 ± 0.041[ab]	0.007 ± 0.042[a]	**p = 0.001***
Level 10	0.009 ± 0.039[a]	0.019 ± 0.041[a]	0.009 ± 0.031[a]	0.008 ± 0.044[a]	0.013 ± 0.040[a]	**p = 0.054**

Superscripts with different letters indicate the significance of differences between groups. (*p < 0.05)
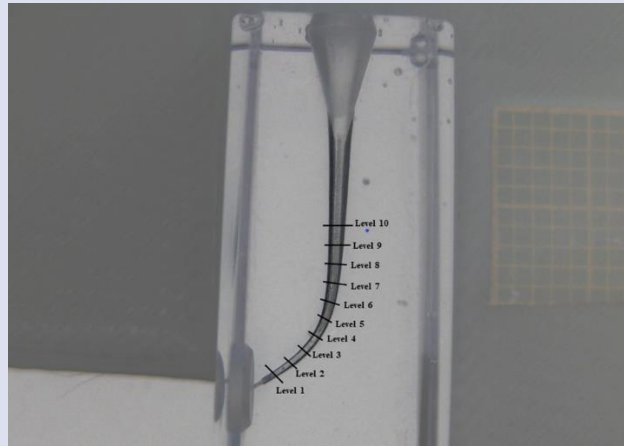
Figure 1. Superimposed pre- and post-instrumentation images with 10 measuring levels.

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Cumhuriyet Dental Journal

Micro-Computed Tomography Assesment of Structural Microporosity and Marginal Gaps in Different Flowable Composites Placed with Different Instruments

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Research Article

ABSTRACT

Objectives: *The aim of this study was to evaluate the structural microporosity (MP) and marginal gap (MG) of different flowable composite resins placed with different instruments using micro-computed tomography (μCT).* ***Materials and Methods:*** *Standard Class II MOD cavities were prepared on 108 lower third molar teeth. Three different flowable composite resins; Filtek Bulk-fill, SDR Bulk-fill and I-Flow conventional flowable composite resins were applied to the cavities using a sharp explorer, a microbrush or an injector. After they were covered with a paste-like nanohybrid composite resin, μCT images were examined in terms of MP and MG. Statistical analysis of the data was performed using two-way ANOVA and Tukey's post hoc tests.* ***Results:*** *MP was observed less in explorer group than microbrush and injector groups (p<0.05). There was no significant difference between microbrush and injector groups (p>0.05). i-FLOW flowable composite resin showed the highest MP rate compared to the other two groups (p<0.05). There was no significant difference between SDR and Filtek Bulk-fill in MP (p > 0.05). In terms of MG, i-FLOW presented higher MG values than other two materials (p<0.05), while the instruments showed similar results (p>0.05).* ***Conclusions:*** *Additional occluso-gingivally vibration of flowable materials with an explorer may be useful for placement. Bulk-fill flowable composite resins exhibited better MP and MG than conventional flowable composite resin used in this study.*

Keywords: *Flowable composite resins, Bulk-fill, μCT, Microporosity, Marginal gap.*

Farklı Aletlerle Yerleştirilmiş Farklı Akışkan Kompozitlerde Yapısal Mikropörözite ve Marjinal Gaplerin Mikro Bilgisayarlı Tomografi Değerlendirmesi

ÖZ

Amaç: *Bu çalışmanın amacı, mikro bilgisayarlı tomografi (μCT) kullanarak farklı aletlerle yerleştirilen farklı akışkan kompozit rezinlerin yapısal mikro pörözite (MP) ve marjinal gap oluşumunu (MG) değerlendirmektir.* ***Gereç ve Yöntemler:*** *Bu çalışmada 108 adet alt üçüncü molar dişe standart Sınıf II MOD kaviteler hazırlandı. Üç farklı akışkan kompozit rezin (Filtek Bulk-fill, SDR Bulk-fill ve I-Flow konvansiyonel akışkan kompozit), kavitelere keskin bir sond, bir mikrofırça veya bir enjektör kullanılarak uygulandı. Akışkan kompozit yüzeyleri pasta tipi bir nanohibrit kompozit ile kapatıldıktan sonra μCT görüntüleri MP ve MG açısından incelendi. Verilerin istatistiksel analizi, iki yönlü ANOVA ve Tukey's post hoc testleri kullanılarak yapıldı.* ***Bulgular:*** *MP, sond grubunda mikrofırça ve enjektör gruplarına göre daha az gözlendi (p<0,05). Mikrofırça ve enjektör grupları arasında anlamlı fark yoktu (p>0,05). i-FLOW akışkan kompozit, diğer iki gruba kıyasla en yüksek MP oranını gösterdi (p<0,05). MP'de SDR ve Filtek Bulk-fill arasında anlamlı bir fark yoktu (p>0,05). MG açısından, i-FLOW diğer iki materyalden daha yüksek MG değerleri sunarken (p<0,05), uygulama aletleri açısından anlamlı fark gözlenmedi (p>0,05).* ***Sonuçlar:*** *Akışkan materyallerin bir sond ile ilave oklüzo-gingival vibrasyonla uygulanması faydalı olabilir. Bulk-fill kompozit rezinler, MP ve MG açısından geleneksel akıcı kompozit rezinden daha iyidir.*

Anahtar Kelimeler: *Akışkan kompozit rezinler, Bulk-fill, μCT, Mikropörözite, Marjinal gap.*

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Introduction

Flowable composite resins are often preferred as a base material under paste-like resin composite restorations. These materials have advantages such as low thermal permeability, compatible elastic modulus with dental tissues and easy penetration to anatomical details.[1]

Flowable composites are presented in an injector on the market. Clinicians apply directly to the cavity with the help of this injector or they apply additional fitting with the instruments such as explorer or microbrush. Flowable composites are known to be less viscous due to their low filler content, and microporosities (MP) or microgaps (MG) may remain in body structure and marginal areas during placement.[2] MP and MG occured in the composite resin structure lead to weakening of the physical and mechanical properties of the restoration.[1]

MP and MG formation may mostly be occurred by increased thickness and amount of applied material.[3] In addition, flowable composite resins may present higher amount of MP and MG in larger cavities such as Class II.[4] The formation of MG disrupts the compatibility of the material with the cavity walls that may cause microleakage and secondary caries, especially in areas difficult to reach such as gingival wall.[4]

Bulk-fill composite resins, which have been introduced to the market in recent years, can be applied in 4-6 mm thickness.[5] Some modifications in translucency, photoinitiator, the filler content and/or the organic matrix have been made to increase the curing depth in bulk-fill composites. Bulk-fill composite resins are more rigid with higher elastic modulus and more plastic (show higher plastic deformation and creep values) when compared to regular flowable composite resins. They also generally show lower mechanical properties than conventional composite resins.[6] Bulk-fill composite resins can be classified as high-viscosity and low-viscosity bulk-fill composites.[7] Low-viscosity bulk-fill composites may more likely expected that they show relatively higher rate of MP and MG due to higher thickness of material applied.

Defining MP and MG properties of materials can provide the dentist to make objective selection and can thus help in the difficult task of choosing between numerous materials and material brands. In the literature there is not sufficient study on MG and MP properties of flowable composite resins especially on bulk-fill composite resins. The aim of this study is to investigate the MP and MG formation of two different bulk-fill flowable composite resins and a conventional flowable composite resin placed with three different instruments using μCT. In the present study, the null hypothesis were as follows:

1) μCT would be an effective tool for the evaluation of MG and MP formation.
2) The use of a sharp explorer would reduce MP and MG formation.
3) Different flowable materials would present different level of MP and MG.

Materials and Methods

This study protocol was approved by Erciyes University Clinical Research Ethics Committee with the protocol number 96681246-2017/115.

Cavity preparations and adhesiveprocedures

In this study, 108 lower third molar teeth were used which were stored in 0.1% thymol solution until use. Standard MOD cavities (4 mm bucco-lingual width and 4 mm depth for the occlusal cavity, 4 mm bucco-lingual width and 6 mm depth for the proximal cavity) were prepared using a cylindrical diamond bur (959 KR 018; Komet Dental, Lemgo, Germany).

After cavity preparation, the teeth were washed with air/water spray and dried. After applying the Supermat Matrix System (Hawe Neos Dental, Gentilino, Switzerland) to the teeth, Clearfil Tri-S Bond (Kuraray Medical Inc, Kurashiki, Japan) which is a one-step self-etch adhesive was applied by rubbing with a disposable microbrush to the entire cavity walls for 10 sec. It was dried by blowing mild air for 5 sec and light cured by a LED light device (Valo, 1000 mW/ cm^2, Ultradent Products Inc, South Jordan, USA) for 10 sec. All restorative procedures were performed by an experienced operator.

Creating of the groups

Materials, manufacturers and compositions used in the study were showed in Table 1.

Table 1. Materials, manufacturers and compositions used in the study

Material	Manufacturer	Composition
Clearfil Tri-S Bond Plus Lot#000004	Kuraray Medical Inc., Okayama, Japan	MDP, Bis-GMA, HEMA, Colloidal silica, Ethanol, Water, Qamforokinon, Initiators, accelerators
Filtek Z550 Lot#N617603	3 M ESPE, St. Paul, MN, USA	Silanized ceramic, Bisphenol A polyethylene glycol, Dietherdimetacrylate, Bis-GMA, Silanized silica, UDMA
Filtek Bulk Fill Flowable Lot#N666493	3 M ESPE, St. Paul, MN, USA	Bis-GMA, UDMA, Bis-EMA, Procrylate resins, zirconia / silica filler, ytterbium trifluoride filler
Surefil SDR Flow Lot#09301	Dentsply Caulk, Milford, USA	Polymerization regulators, Dimethacrylate resins, Modified UDMA, TEGDMA, Ba-B-F-Al silicate glass, SiO$_2$, Amorphous Sr-Al silicate glass, TiO$_2$
i-Flow Lot#151208	i-Dental, Medicinos Linja, UAB, Lithuania	Bis-GMA, UDMA, TEGDMA, Bis-EMA and barium borosilicate glass fillers

Abbreviations: Bis-GMA, Bis-phenol A diglycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloxydexyl dihydrogen phosphate; UDMA, Urethane Dimethacrylate; Bis-EMA, Ethoxylated Bisphenol A dimethacrylate; TEGDMA, Polyethylene glycodimetacrylate.

The samples were divided into three main groups according to the instrument used (explorer, microbrush, and injector) and then subdivided into three subgroups according to the materials used i-FLOW (i-dental, Medicinos Linja UAB, Lithuania), Filtek Bulk-Fill Flowable (3M ESPE, St. Paul, MN, USA), and Surefil SDR Bulk-fill (Dentsply, Caulk, Milford, USA).

Restorative procedures

Injector group: The tip of the injector was contacted with the cavity floor and injected with a slight movement horizontally and occluso-gingivally for 3-4 sec.

Explorer group: Following injector application, additional occluso-gingivally movement was applied for 3-4 sec with a sharp explorer (Hu-Friedy, Chicago, IL, USA).

Microbrush group: The same motion as in the explorer method was repeated with a microbrush.

The total thickness of each layer did not exceed 2 mm in i-FLOW group. In the SDR and Filtek Bulk-fill groups, the cavities were filled up with bulk-fill composite to 4 mm and the top layer (2mm) of the cavities were left for the conventional paste-like resin composite. Flowable composite resins were polymerized with the same LED light device for 20 sec. Then it was covered with a nanohybrid resin composite (Filtek Z550, 3M ESPE, St Paul, MN, USA) and polymerized with LED light device for 20 sec.

Finishing and polishing procedures were completed under water cooling using yellow belt diamond burs and polishing discs (SofLex PopOn, 3M ESPE, St. Paul, USA).

µCT evaluation

A high-resolution micro-CT (Skyscan 1272, Bruker CCT, Kontich, Belgium) was used to evaluate the MP and MG of the samples. A 4 mm part of each restorations were scanned to evaluate MP and MG. A total of 900 micro-CT images were taken from each restoration. Display settings were set to acceleration voltage: 80kV, beam flow: 125 µA, Al filter: 1mm, resolution: 4 µm, rotation: 360°, and step: 0.400°. Images were analyzed for 3D reconstruction using an image analysis software (Mimics software, version 18, Materialize, Leuven, Belgium). The region of interest for each image was manually selected for 3D space calculation.

Statistical Analysis

The normality of data was tested using Kolmogorov-Smirnov test and it was decided to use parametric tests. The data were analyzed using a two-way ANOVA and Tukey's post-hoc tests using a statistical program (SPSS 20.0, SPSS Inc., Chicago, IL, USA) ($\alpha = 0.05$).

Results

The mean MP and MG percentage (%), standard deviations and statistical differences of the groups are given in Table 2. Also, some µCT images of microporosity and microgaps from the groups are represented in Figure 1-3.

Table 2. Mean, standard deviation, statistical differences of microporosity and microgap values.

|	Groups and Subgroups	Statistical differences	P values	Mean	Standard Deviation	Statistical differences	P values	Mean	Standard Deviation
			Microporosity (%)				**Microgap (%)**		
Instruments	Explorer	a		0.16	0.09	a		0.35	0.19
	Microbrush	b	0.034	0.26	0.13	a	0.68	0.35	0.22
	Injector	b		0.28	0.17	a		0.27	0.24
Materials	İ-Flow Flowable	X		0.38	0.14	X		0.42	0.19
	SDR Bulkfill	Y	0.029	0.12	0.11	Y	0.041	0.27	0.14
	Filtek Bulkfill	Y		0.18	0.08	Y		0.29	0.11
Subgroups	İ-flow + Injector	D		0.53	0.13	C,D		0.46	0.22
	İ-flow + microbrush	B,C		0.37	0.13	C,D		0.41	0.19
	İ-flow + explorer	A,B		0.24	0.07	C,D		0.39	0.25
	SDR + Injector	A		0.05	0.02	A		0.15	0.11
	SDR + microbrush	A,B	0.00	0.26	0.09	C,D	0.001	0.36	0.15
	SDR + explorer	A		0.06	0.03	B,C		0.29	0.13
	Filtek + Injector	A,B		0.25	0.09	A,B		0.21	0.14
	Filtek + microbrush	A		0.15	0.06	B,C		0.29	0.19
	Filtek + explorer	A		0.15	0.07	C,D		0.38	0.29
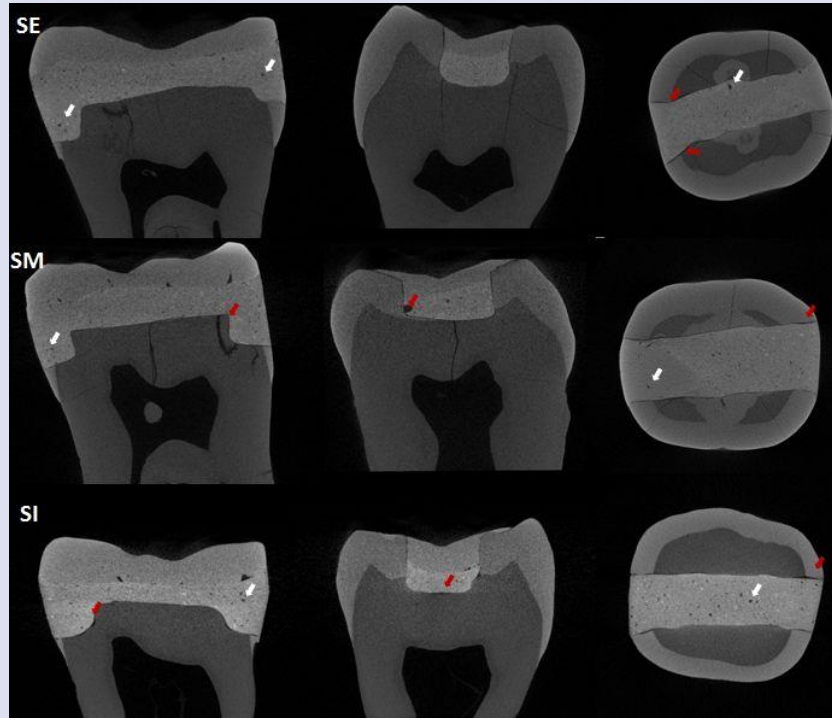
Figure 1. µCT images of microporosity and microgaps from SDR Bulk-fill group. SE, SDR + Explorer; SM, SDR + Microbrush; SI, SDR + Injector.
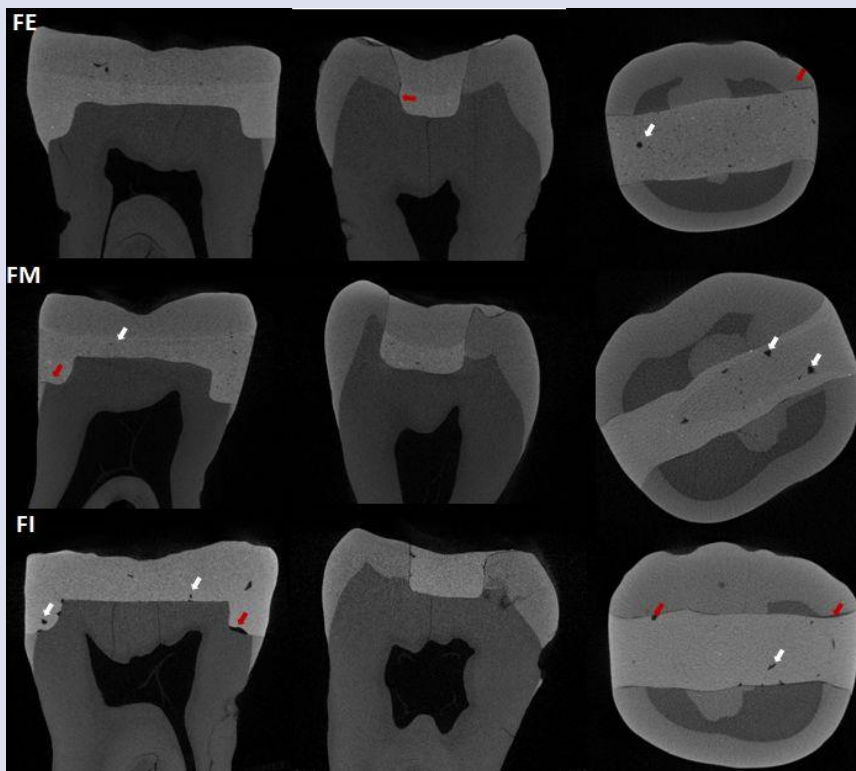

Figure 2. µCT images of microporosity and microgaps from Filtek Bulk-fill group. FE, Filtek Bulk-fill + Explorer; FM, Filtek Bulk-fill + Microbrush; FI, Filtek Bulk-fill + Injector.
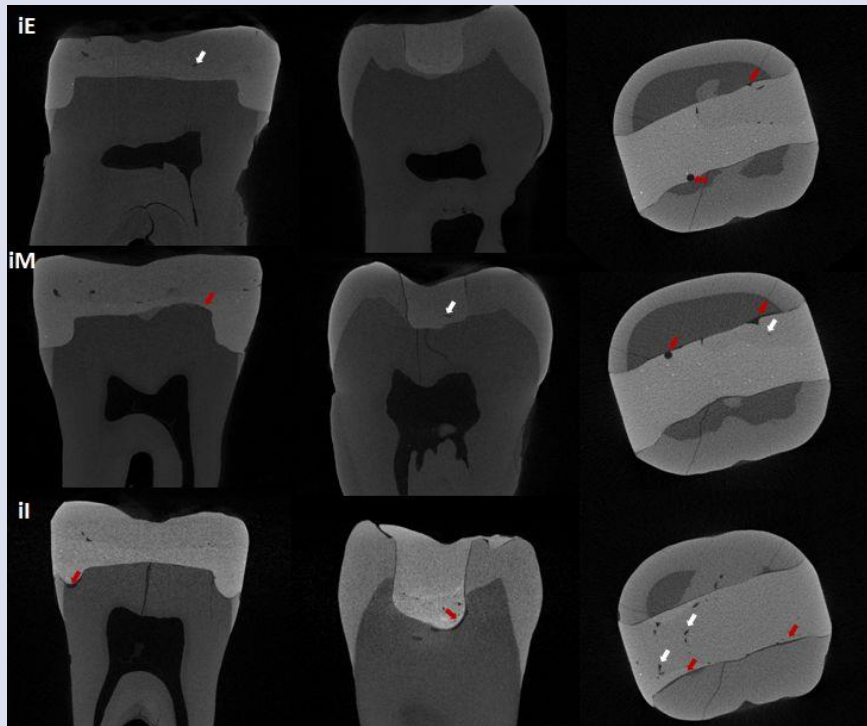
Figure 3. µCT images of microporosity and microgaps from i-FLOW group. iE, i-FLOW + Explorer; iM, i-FLOW + Microbrush; iI, i-FLOW + Injector.

Microporosity assesment

The mean MP formation in flowable composite resins placed with the help of an explorer (0.16±0.09%) was significantly less than the injector (0.28±0.17%) and microbrush (0.26±0.13%) ($p<0.05$). However, no significant difference was found between injector and microbrush group ($p>0.05$).

When compared to the used materials in terms of MP formation, SDR Bulk-fill (0.12±0.11%) and Filtek Bulk-fill (0.18±0.08%) were found to be similar (p>0.05) while i-FLOW (0.38±0.14%) showed significantly higher MP ($p<0.05$).

In SDR group, the lowest MP values were obtained regardless of the method applied and there was no statistical difference between the methods ($p>0.05$). Filtek Bulk-fill composite resin was found to be more successful in terms of MP with the application of explorer and microbrush than the injector method, but there was no statistical difference between these three groups ($p>0.05$).

i-FLOW flowable composite resin presented significantly higher MP values in injector applications than the explorer and microbrush ($p<0.05$). The explorer method was found to be the best, followed by the microbrush method, and there was no statistical significant difference between the explorer and microbrush groups ($p>0.05$).

Microgap assesment

All instruments showed similar results in terms of MG ($p>0.05$).

When compared the used materials, i-FLOW presented the highest MG values (0.42±0.19%) ($p<0.05$). SDR (0.29±0.14%) and Filtek Bulk-fill composite resins (0.27±0.11%) presented similar MG values ($p>0.05$).

When all composite resins were separately evaluated in terms of instrument used, different instruments did not change the MG values ($p>0.05$).

Discussion

Tooth-colored composite resin materials are frequently preferred by both patients and clinicians in the restoration of posterior teeth as in anterior teeth. Packable composite resins are used in the posterior region due to their good physical and mechanical strength against chewing forces.[8,9] On the other hand, flowable composite resins are recommended as liners under posterior composite resins due to their low viscosity, increased elasticity and wettability.[10] It is thought that the use of low-viscosity flowable composite resins together with the posterior composites will allow restorations with better marginal adaptation and less microleakage, since flowable composite resins better fill the irregular inner surfaces of the cavity.[11,12] Flowable composite resins are also used as liners since they can act as a flexible intermediate layer that helps relieve stresses during polymerization shrinkage of the restorative material.[13] For this reason, this study focused on the use of flowable composites as a liner under conventional composite resin.

During placement of the composite resins, MP and MG may be caused by the iatrogenic factors, as well as microstructure and chemical structure of the materials.

MP and MG can affect the durability of the material, discoloration, microleakage, wear, and polishing properties[14], and consequently can negatively affectthe longevity of the restoration.[15] The chemical structure and geometric shape of the instruments used during the placement of the material may also affect the formation of MP and MG.[16] Since the probability of MP and MG is higher in the flowable materials[2], the MP and MG of three different flowable composites placed using three different instruments were examined in this study.

This study was performed using a micro-CT which is an important device in the analysis of polymerization shrinkage, gap formation, marginalintegrity.[17,18] Micro-CT is also a non-invasive method because it provides highly sensitive and quantitative results and allows the analysis of the samples without causing any stress, distortion, crack or any damage.[19] In this study, the µCT method clearly revealed the difference between the measured materials and provided measurable quantitative values. Therefore, the first hypothesis of the study was accepted.

In the present study, all materials presented MP and MG formation. However, according to the results of the study the use of an explorer reduced the formation of MP, but did not change MG. Therefore, the second hypothesis of the study was partially accepted. This effect of explorer on MP may be caused by sharp geometry of the tip and rigid structure of instrument. The vibrations that occur during back and forth movements of the explorer may activate the molecules to move that consequently eliminating the voids.

The injector and microbrush did not show the same effect as the explorer. The geometry of the tip of the injector may not be thin sufficiently and has a lumen which may cause air bubbles during movement. And microbrush has a non-metallic soft bristle structure trapping material or air into it consequently creating of new bubbles during movement.

In the study, SDR and Filtek Bulk-fill composite resins were presented less MP compared to i-FLOW. Therefore the third hypotheses of the study was also accepted. Undoubtedly, the chemical structure of the three flowable resin materials used in study is not the same totally that may affect to formation of MP and MG. The thickness of the resin placed to cavity may also affect the formation of MP and MG. However, i-FLOW was placed up to 2 mm of layers because it is a conventional flowable composite resin while the other two bulk-fill composite resins were applied as 4 mm monolayer. i-FLOW offered higher MP and MG, while it was expected that it would offer less MP and MG because of its lower thickness of each layer placed than bulk-fill resins. This may be related to the fact that bulk-fill composite resins have different chemical structure (fillers, organic monomer matrix, etc.) compared to conventional composite resins. In addition, the polymerization shrinkage may affect the MP and MG formation. The fact that bulk-fill composites showed less MP and MG in our study may be a result of the bulk-fill composite resins showing less polymerization shrinkage, as stated in a meta-analysis by Cidreira Boaro et al.[20]

The MG values of the different instruments used in this study was similar. This may be caused by surface tension of the adhesive agent applied to the cavity before the composite resin placement as well as intermolecular attraction force between the adhesive and composite resins that may compensate the difference between the instruments. In present study, it was found that both bulk-fill resins offered less MG than conventional flowable resin. Similarly, Kapoor et al.[21] found better adaptation and less cavity formation in the pulpal wall when bulk-fill resins were used compared to conventional composite resins.

In a study by Hirata et al. [3], when sonic application was performed, they found that bulk-fill composite resins showed more void formation compared to conventional composite resin and they reported that SDR flowable resin should not be used with sonic application. They attributed this to the changes in the rheological structure of the material caused by sonic application that increases the flow of material offered by the manufacturer. They also argued that the small voids already present in the material structure become larger voids with the sonic effect. However, in the present study, sonic application was not applied. This difference between the results of this study and our study may be caused by the differences in the method.

In this study, MOD cavities were prepared in molar teeth. It should not be underestimated that different materials and different cavities with smaller c-factor may present different MP and MG values. In addition, in the present study, a bonding agent was applied to the cavity before the restorative procedures in order to mimic the clinical conditions. It is not clear whether this bonding application changes the MP and MG values. Further studies involving different size of cavities, methods or materials are needed.

Conclusions

Within the limitation of this study, it can be concluded;
1. µCT is an effective tool for the evaluation of MG and MP formation.
2. Additional occluso-gingivally vibration of flowable materials with an explorer may be useful in the placement.

Bulk-fill flowable composite resins exhibited better MP and MG than conventional flowable composite resin used in this study.

Acknowledgement

Conflicts of Interest

The authors of the present study declare no conflict of interest.

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Cumhuriyet Dental Journal

The 100 Most Cited Articles on Dental Anomalies: A Bibliometric Analysis

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Review	ABSTRACT
	Objectives: *To identify and analyze the 100 most-cited articles in the field of dental anomalies.* ***Materials and Methods*** *A search was performed in the Thompson Reuters Web of Science (WOS) database on May 31, 2021. The search strategy was performed using keywords obtained from the Medical Subject Headings (MeSH) index. The following data were extracted and analyzed: number of citations, title, authors, year of publication, title of scientific journals, type of study and subject area.* ***Results:*** *The search strategy resulted in 69,014 articles and the most cited studies were concentrated between 1945-2012. Twenty-four percent of all articles were published in orthodontic journals. The number of citations ranged from 98 to 482 and The United States was the most prolific country. Numerical anomalies and structural changes were the most studied thematic fields (79 articles) and laboratory studies were the most cited type of study.* ***Conclusions:*** *Numerical anomalies and structural changes were the most studied thematic fields. There was a predominance of laboratory studies and The United States was responsible for most of the articles included.*
	Keywords: *Bibliometrics; Citation analysis; Dental anomalies.*

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Introduction

Dental anomalies (DA) are changes in the number, shape, structure, size and pattern of tooth eruption resulting from disturbances during the morphodifferentiation stages of tooth germ development.[1]

Different prevalence rates have been reported, however this wide range of values reflects the differences in race and age of the samples, as well as the diagnostic criteria used in research.[2,3] The etiology of DA is multifactorial, multilevel and multidimensional.[4] Complex interactions between genetic, epigenetic and environmental factors seem to explain the emergence of these abnormalities.[5]

From a clinical point of view, changes in the size, number, and pattern of tooth eruption can lead to the development of malocclusions.[6] Moreover, changes in morphology and color can compromise patients' esthetics.[7,8] Early diagnosis, combined with proper treatment are essential to achieve occlusal, functional and esthetic harmony.[9]

Several studies have been published evaluating the prevalence, etiology and treatment of the most varied types of dental anomalies. This high scientific production has generated new knowledge, improved technologies

and encouraged the critical and reflective spirit of professionals.[10] In this scenario, bibliometric studies emerge as a macroscopic and voluminous view of scientific literature, and help to identify authors, journals, countries, institutions and the most influential research themes in the areas studied.[11]

Bibliometrics is a scientific mapping method for applying quantitative and statistical analysis to monitor and analyze the structure and growth of science.[12,13] The number of citations of an article is a bibliometric tool that has been used to assess and describe the impact of a research publication over time.[14,15] Furthermore, this number may express the capacity of this article to generate changes in clinical practice and also to guide future researches.[16]

At present, there are no studies evaluating research related to DA, so the aim of this study was to use these bibliometric resources to identify the 100 most cited articles on this topic.

A bibliometric study was conducted to identify and analyze the 100 most cited articles on dental anomalies. The studies were selected by means of a search performed in the Thompson Reuters Web of Science (WOS) database, owned by Clarivariate Analytics on May 31, 2021. An additional search was performed in the

Scopus database, for use in making comparisons at a later stage with regard to the total number of citations.

The search strategy was performed using the keywords obtained from the Medical Subject Headings (MeSH) index and combined with the TS field tag (Topic) and the Boolean OR operator, using the advanced search tool in the section "All databases" (Table 1).

Materials and Methods

A universal micro-hybrid resin composite (Z250; Filtek Z250, 3M ESPE, St Paul, MN, USA), two resin-based bulk-fill restoratives (FOB; Filtek One Bulk Fill, 3M ESPE, St Paul, MN, USA and EBF; Estelite Bulk-Fill Flow, Tokuyama Dental Corporation, Tokyo, Japan), a glass ionomer-based bulk-fill restorative (Equia FF; Equia Forte Fil, GC Dental Products Corp, Tokyo, Japan), a bulk-fill alkasite restorative (CN; Cention N) and an indirect composite resin (Gradia P; Gradia Plus, GC Dental Products Corp, Tokyo, Japan) were tested in the present study. The shades of the materials were A2 except for HB-DA2 was used in Gradia Plus group. The composition and polymerization or setting procedures of the materials are described in Table 1.

The filter "Dentistry, Oral Surgery & Medicine" was applied to direct the search, however no restrictions were placed on the year of publication or the language. Inclusion criteria were full articles focused on dental anomalies, so editorials and conference articles were excluded. The articles were selected independently by two researchers (MEFS and GVS) and differences of opinion were resolved by consensus.

The search retrieved a total of 69,014 items that were ranked in descending order according to the total number of citations. Articles with the same number of citations were ranked taking into account the year of publication, so that more recent studies received higher ranking. The evaluation stopped at the 100th most cited article.

For each item selected, the following data were extracted and stored in an Excel spreadsheet (Microsoft Office for Mac 2011 package): (a) number of citations; (b) title; (c) authors (name, number, order of authorship, country); (d) year of publication; (e) title of scientific journals; (f) type of study; (g) subject field and (h) keywords. Study designs were classified this way: literature reviews, laboratory studies (in vitro, in vivo, in situ, ex vivo), epidemiological study, controlled clinical trial, prevalence and incidence study, randomized clinical trial, clinical practice guide, control case, diagnostic study, tracking study, experimental research and observational study.

Thematic fields included the following themes: (a) structural alteration (amelogenesis imperfecta, osteogenesis imperfecta, invaginated tooth, dentinogenesis imperfecta, MIH and enamel hypoplasia); (b) eruption trajectory (palatally displaced canine, transposition, ectopic eruption of permanent first molars, ankylosis); (c) change in number (supernumerary and tooth agenesis); (d) changes in the number of roots; (e)

odontogenic tumors (odontoma); (f) changes in shape (fusion and twinning) and (g) changes in size (microdontia and macrodontia).

Statistical analysis was performed using IBM® SPSS® (Statistical Package for the Social Sciences) version 20.0; Statsoft, Tulsa, Okla for frequency of descriptive measures. VOSviewer software was used to generate keyword co-occurrence networks.

Results

The search strategy resulted in 69,014 articles in Web of Science and 62,542 articles in Scopus. Articles found in Web of Science were ranked. Table 2 shows the ranking of the 100 most cited articles, in descending order of number of citations, ranging from 98 to 482 and totaling 17,230 citations, with an annual average of 226.71. The most cited article was by Vastardis, Heleni et al., with 482 citations, published in the United States in 1986, which addresses the genetic etiology of tooth agenesis. Relative to study designs, 23% were laboratory studies, 16% literature reviews and 11% epidemiological studies which, when added together, represented half of all articles included in the list (Table 3).

In total, 324 authors were involved in the articles retrieved, with each publication having an average of 4.05 authors per article. The contribution of 17 authors with more than three studies on the list amounted to 39 articles, and 7711 citations. Simmer, JP was the author with the largest number of articles included, totaling seven studies and 879 citations (Table 4).

The most cited studies were concentrated between 1945-2012. Figure 1 shows the years in which there were more than three publications with a high citation rate, showing a low production between 1940 and 1980. In addition, the highest productivity per year was observed in 2001 (nine studies).

The articles selected were published in 42 different journals. Figure 2 shows the list of 13 journals with the most articles included in the ranking, and which accounted for 66% of all citations. American Journal of Orthodontics and Dentofacial Orthopedics (AJO-DO) (13), The Angle Orthodontist (6), Journal of Dental Research (6) and Nature Genetics (6) were the journals with the greatest contribution to the list, totaling 5,740 citations.

Number anomalies and structural changes were the most studied thematic fields, totaling 79 articles (Figure 3). Of all the articles on number of anomalies, 90.63% assessed tooth agenesis and 9.37% supernumerary teeth. While, of the total of 47 studies on structural alteration, more than 50% were on Amelogenesis imperfecta (15), Enamel hypoplasia (11) and Molar-Incisor Hypomineralization (6).

A total of 389 keywords were identified, with Hypodontia, Oligodontia, Expression and Dental anomalies being the most expressive ones (Figure 4). Only five countries accounted for almost 80% of the articles with the highest number of citations. The United States contributed 40% of all publications listed and 7,108

citations. Subsequently, Sweden, Finland, England and Australia collaborated with a total of 38 publications and 6,399 citations (Figure 5).

Discussion

To the best of the authors' knowledge, this is a pioneer and unique bibliometric research in this area. The sample size was defined in 100 studies to provide a manageable and significant number of articles to be analyzed, according to several previously published works.[17-21]

Generally, a highly cited article is seen as a milestone and can positively influence research and clinical practice.[22] Our search resulted in 69,014 articles in the Web of Science and 62,542 articles in Scopus. Articles that exceed the limit of 100 citations are arbitrarily considered as "classics".[23] Of all the articles included, 98% had more than 100 citations, and 27% were cited more than 200 times, which showed a high scientific impact. The two studies that led the ranking had 482 and 473 citations, and both addressed tooth agenesis as the main theme.

The articles were published between 1945 and 2012, however a higher concentration of studies (53%) was published after the 2000s, which may represent a late consolidation of research on the subject. The largest number of articles per year was found in 2001 (nine), with 66.66% on structural anomalies. This increase observed on this date was due to the fact that MIH was first described in this year, which prompted a higher quantity of researches on the subject.

Laboratory studies (23%) and literature reviews (16%) were the most cited types of studies. Reviews play an important role in gathering information on a specific topic, and laboratory studies are the basis for research in any biomedical area.[24] In the analyzed articles, the laboratory studies included in the list focused on the investigation of the etiology of tooth agenesis (34%), in several studies gene investigations and in vitro analyzes were carried out. These studies add to the evidence that agenesis and its associated abnormalities are under genetic control.[25]

Furthermore, literature reviews especially addressed structural anomalies (50%), such as enamel hypoplasia and amelogenesis imperfecta. No systematic review of dental anomalies was highly cited. Considering that these types of studies are the basis for research and clinical practice in evidence-based dentistry, the lack of research with a higher level of scientific rigor is clear.

According to the results, the 100 most cited articles were published in a total of 42 journals, 60% were published in dental journals, 26% in journals in the field of Oral Biology, 9% in journals of Medicine, and 5% in Anthropological area. Of the 60 articles published in dental journals, 40% were published in orthodontic journals (American Journal of Orthodontics and Dentofacial Orthopedics; The Angle Orthodontist; European Journal of Orthodontics). This was expected since dental anomalies are frequently observed in orthodontic patients and must be considered because they could complicate the dental and orthodontic treatments. Moreover, the early detection of a single dental anomaly may allow timely orthodontic intervention.[25,26] In addition, of the 26 articles published in journals specialized in oral biology, 77% addressed the genetic aspects of dental anomalies, especially tooth agenesis (35%).

The keywords provided in the articles included were also evaluated using a co-occurrence network mapping. This type of map is a tool that allows the research domain in a certain field to be summarized, visualized and examined. In the present analysis, a total of 389 keywords were identified. "Hypodontics" was the most prevalent word (14), followed by "dental anomalies" (10), "expression" and "oligodontics", both appeared 9 times. This result was expected since tooth agenesis was the most studied dental anomaly and corresponded to 27% of all articles in the ranking.

The 100 most cited articles were produced predominantly by the United States (40%), in agreement with several bibliometric studies in the area of health.[27-29] This result probably reflected the large number of researchers who belong to this country, which was also the leading country in the number of medical research publications.[18] Furthermore, the United States has a large scientific community and generous science funding policies.[30]

In total, 324 authors were involved in the articles that were ranked, with each publication having an average of 4.05 authors. The top 17 most prolific authors contributed 39 articles and accounted for almost half of the total number of citations (45%). These authors worked mainly on three themes: anomalies of eruption trajectory, structure, and number, demonstrating their outstanding contributions in the area. The 3 authors with the highest number of citations were Ericson, S (1086), Kurol, J (1086) and Wright, JT (1024). It is interesting to emphasize that Ericson, S and Kurol, J contributed five articles to the ranking, in which they were the only authors of the articles, with Ericson, S always being the first author of the articles. This partnership focused exclusively on studies on the trajectory of permanent canine eruption. Vastardis, H was the author responsible for the publication with the highest number of citations and addressed the etiology of tooth agenesis. Still on the same theme, the author produced another study (20th) with a high number of citations (237). It is noteworthy that studies involving the etiological factors of dental anomalies (46%) and, in particular, the genetic aspects involved in tooth agenesis (37%) aroused great interest among the academic community (3,497 citations).

One of the limitations of the present study was the lack of time restrictions, favoring older publications. Naturally, studies published in previous years had more time to be cited, irrespective of their scientific value. In addition, two databases were consulted to identify the articles, but only the Web of Science was used to rank the studies. However, WOS is the oldest, most widely used, and trusted research database, publications, and citations that

covers nearly 34,000 journals today.[31] More research is needed to address and overcome present limitations.

Conclusions

The majority of the articles were laboratory studies, and it was possible to observe a lack of studies of the systematic review type.

Number anomalies and structural changes were the most studied thematic fields. Orthodontics journals accounted for 24% of all surveys and United States was the most prolific country.

This study provides a source of quality information for researchers, academics and students and allows for analysis and prediction of the trend of future research.

Table 1: Search strategy

TS =	(Tooth Abnormalit* OR Anodontia OR Hypodontia OR Oligodontia OR Hyperdontia OR Shape anomal* OR Tooth Ankylosis OR Dens evaginatus OR Dens in Dente OR Enamel hypoplasia OR Amelogenesis Imperfecta OR Dentin Dysplasia OR Dentinogenesis Imperfecta OR Fused Teeth OR Gemination OR Dilaceration OR Concrescence OR Odontodysplasia OR Tooth, Supernumerary OR Tooth Eruption, Ectopic OR Taurodontism OR Distoangulation OR Hypercementosis OR Macrodontia OR Microdontia OR Impaction OR Ectopic tooth eruption OR Ectopic eruption OR Tooth Transposition OR Talon cusp OR Enamel pearls OR Giroversion OR Dental anomal* OR Dental agenesis OR Tooth agenesis OR Palatally displaced canine OR Dental Fusion OR Supranumerary OR Odontoma OR Congenitally missing tooth OR Congenitally missing teeth OR Peg-shaped)

Table 2: The top-100 most-cited papers in dental anomalies

	Article	Number of citations
1	VASTARDIS, Heleni et al. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. **Nature genetics**, v. 13, n. 4, p. 417-421, 1996.	482
2	POLDER, Bart J. et al. A meta-analysis of the prevalence of dental agenesis of permanent teeth. **Community dentistry and oral epidemiology**, v. 32, n. 3, p. 217-226, 2004.	473
3	GOODMAN, Alan H.; ROSE, Jerome C. Assessment of systemic physiological perturbations from dental enamel hypoplasias and associated histological structures. **American Journal of Physical Anthropology**, v. 33, n. S11, p. 59-110, 1990.	427
4	LAMMI, Laura et al. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. **The American Journal of Human Genetics**, v. 74, n. 5, p. 1043-1050, 2004.	403
5	VAN DEN BOOGAARD, Marie-José H. et al. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. **Nature genetics**, v. 24, n. 4, p. 342-343, 2000.	403
6	STOCKTON, David W. et al. Mutation of PAX9 is associated with oligodontia. **Nature genetics**, v. 24, n. 1, p. 18-19, 2000.	355
7	GIBSON, Carolyn W. et al. Amelogenin-deficient mice display an amelogenesis imperfecta phenotype. **Journal of Biological Chemistry**, v. 276, n. 34, p. 31871-31875, 2001.	337
8	WEERHEIJM, K. L.; JALEVIK, B.; ALALUUSUA, Satu. Molar-incisor hypomineralisation. **Caries research**, v. 35, n. 5, p. 390, 2001.	289
9	ERICSON, Sune; KUROL, Jüri. Early treatment of palatally erupting maxillary canines by extraction of the primary canines. **European Journal of Orthodontics**, v. 10, n. 4, p. 283-295, 1988.	288
10	SREENATH, Taduru et al. Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. **Journal of Biological Chemistry**, v. 278, n. 27, p. 24874-24880, 2003.	286
11	PECK, Sheldon; PECK, Leena; KATAJA, Matti. The palatally displaced canine as a dental anomaly of genetic origin. **The Angle Orthodontist**, v. 64, n. 4, p. 250-256, 1994.	286
12	WITKOP JR, C. J. Amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia revisited: problems in classification. **Journal of Oral Pathology & Medicine**, v. 17, n. 9-10, p. 547-553, 1988.	286
13	BROOK, A. H. A unifying aetiological explanation for anomalies of human tooth number and size. **Archives of oral biology,** v. 29, n. 5, p. 373-378, 1984.	265
14	GROVER, Pushpinder S.; LORTON, Lewis. The incidence of unerupted permanent teeth and related clinical cases. **Oral Surgery, Oral Medicine, Oral Pathology**, v. 59, n. 4, p. 420-425, 1985.	259
15	SHIELDS, E. D.; BIXLER, D.; EL-KAFRAWY, A. M. A proposed classification for heritable human dentine defects with a description of a new entity. **Archives of oral biology**, v. 18, n. 4, p. 543-IN7, 1973.	253
16	ERICSON, Sune; KUROL, Jüri. Radiographic examination of ectopically erupting maxillary canines. **American Journal of orthodontics and Dentofacial orthopedics**, v. 91, n. 6, p. 483-492, 1987.	250
17	XIAO, Shangxi et al. Dentinogenesis imperfecta 1 with or without progressive hearing loss is associated with distinct mutations in DSPP. **Nature genetics**, v. 27, n. 2, p. 201-204, 2001.	240
18	BACCETTI, T. A controlled study of associated dental anomalies. **The Angle Orthodontist**, v. 68, n. 3, p. 267-274, 1998.	239
19	RANTA, Reijo. A review of tooth formation in children with cleft lip/palate. **American Journal of Orthodontics and Dentofacial Orthopedics**, v. 90, n. 1, p. 11-18, 1986.	239
20	VASTARDIS, Heleni. The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. **American Journal of Orthodontics and Dentofacial Orthopedics**, v. 117, n. 6, p. 650-656, 2000.	237
21	ERICSON, Sune; KUROL, Jüri. Resorption of incisors after ectopic eruption of maxillary canines: a CT study. **The Angle Orthodontist**, v. 70, n. 6, p. 415-423, 2000.	218
22	HILLSON, S.; BOND, S. Relationship of enamel hypoplasia to the pattern of tooth crown growth: A discussion. **American Journal of Physical Anthropology**, v. 104, n. 1, p. 89–103, set. 1997.	217
23	GOODMAN, Alan H.; ARMELAGOS, George J.; ROSE, Jerome C. Enamel hypoplasias as indicators of stress in three prehistoric populations from Illinois. **Human biology**, p. 515-528, 1980.	212
24	THESLEFF, Irma. The genetic basis of tooth development and dental defects. **American Journal of Medical Genetics Part A**, v. 140, n. 23, p. 2530-2535, 2006.	211

25	LAGERSTRÖM, Maria et al. A deletion in the amelogenin gene (AMG) causes X-linked amelogenesis imperfecta (AIH1). **Genomics**, v. 10, n. 4, p. 971-975, 1991.	210
26	JACOBY, Harry. The etiology of maxillary canine impactions. **American journal of orthodontics**, v. 84, n. 2, p. 125-132, 1983.	201
27	ZHANG, Xiaohai et al. DSPP mutation in dentinogenesis imperfecta Shields type II. **Nature genetics**, v. 27, n. 2, p. 151-152, 2001.	200
28	HART, P. S. et al. Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. **Journal of medical genetics**, v. 41, n. 7, p. 545-549, 2004.	187
29	CATERINA, John J. et al. Enamelysin (matrix metalloproteinase 20)-deficient mice display an amelogenesis imperfecta phenotype. **Journal of Biological Chemistry**, v. 277, n. 51, p. 49598-49604, 2002.	186
30	ERICSON, Sune; KUROL, Jüri. Radiographlc assessment of maxillary canine eruption in children with clinical signs of eruption disturbance. **The European Journal of Orthodontics**, v. 8, n. 3, p. 133-140, 1986.	179
31	LEPPANIEMI, A.; LUKINMAA, Pirjo-Liisa; ALALUUSUA, Satu. Nonfluoride hypomineralizations in the permanent first molars and their impact on the treatment need. **Caries research**, v. 35, n. 1, p. 36-40, 2001.	173
32	PHILIPSEN, H. P. et al. Mixed odontogenic tumours and odontomas. Considerations on interrelationship. Review of the literature and presentation of 134 new cases of odontomas. **Oral oncology**, v. 33, n. 2, p. 86-99, 1997.	168
33	PECK, Leena; PECK, Sheldon; ATTIA, Yves. Maxillary canine-first premolar transposition, associated dental anomalies and genetic basis. **The Angle Orthodontist**, v. 63, n. 2, p. 99-109, 1993.	168
34	CRAWFORD, Peter JM; ALDRED, Michael; BLOCH-ZUPAN, Agnes. Amelogenesis imperfecta. **Orphanet journal of rare diseases**, v. 2, n. 1, p. 1-11, 2007.	166
35	KIM, J. W. et al. MMP-20 mutation in autosomal recessive pigmented hypomaturation amelogenesis imperfecta. **Journal of medical genetics**, v. 42, n. 3, p. 271-275, 2005.	164
36	DAVIS, P. Jane. Hypodontia and hyperdontia of permanent teeth in Hong Kong schoolchildren. **Community Dentistry and Oral Epidemiology**, v. 15, n. 4, p. 218-220, 1987.	164
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43	DE COSTER, P. J. et al. Dental agenesis: genetic and clinical perspectives. **Journal of Oral Pathology & Medicine,** v. 38, n. 1, p. 1-17, 2009.	147
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89	RØLLING, S. Hypodontia of permanent teeth in Danish schoolchildren. **European Journal of Oral Sciences**, v. 88, n. 5, p. 365–369, out. 1980.	107
90	KIM, Y.; LEE, S.-J.; WOO, J. Morphology of Maxillary First and Second Molars Analyzed by Cone-Beam Computed Tomography in a Korean Population: Variations in the Number of Roots and Canals and the Incidence of Fusion. **Journal of Endodontics**, v. 38, n. 8, p. 1063–1068, ago. 2012.	105
91	SIMMER, J. P. et al. Hypomaturation Enamel Defects in Klk4 Knockout/LacZ Knockin Mice. **Journal of Biological Chemistry**, v. 284, n. 28, p. 19110–19121, jul. 2009.	105
92	PECK, S.; PECK, L.; KATAJA, M. Concomitant occurrence of canine malposition and tooth agenesis: Evidence of orofacial genetic fields. **American Journal of Orthodontics and Dentofacial Orthopedics**, v. 122, n. 6, p. 657–660, dez. 2002.	105
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98	SEOW, W. Kim. Enamel hypoplasia in the primary dentition: a review. **ASDC journal of dentistry for children**, v. 58, n. 6, p. 441-452, 1991.	102
99	BAILLEUL-FORESTIER, I. et al. The genetic basis of inherited anomalies of the teeth. **European Journal of Medical Genetics**, v. 51, n. 4, p. 273–291, jul. 2008.	99
100	DAS, P. et al. Haploinsufficiency of PAX9 is associated with autosomal dominant hypodontia. **Human Genetics**, v. 110, n. 4, p. 371–376, 14 mar. 2002	98

Table 3: Categorization of articles in terms of study design

Study design	N° of articles
Laboratory studies	23
Literature reviews	16
Epidemiological studies	11
Controlled clinical trial	7
Prevalence and Incidence Study	5
Randomized clinical trial	5
Clinical Practice Guide	5
Control case	5
Diagnostic study	3
Tracking Study	3
Experimental research	3
Observational study	3

Table 4: Authors with three or more papers in the 100 most-cited papers in dental anomalies

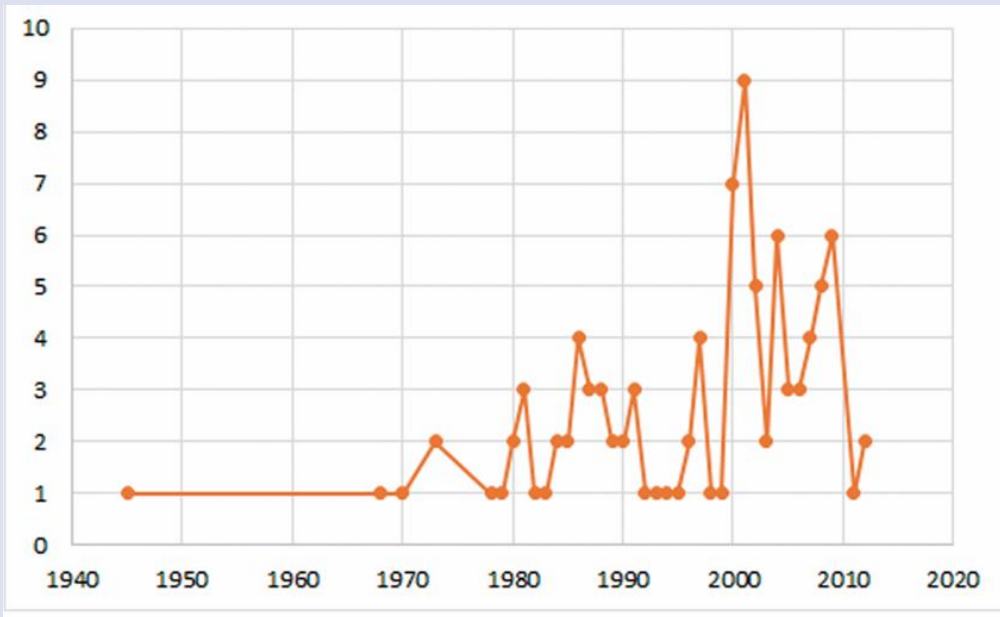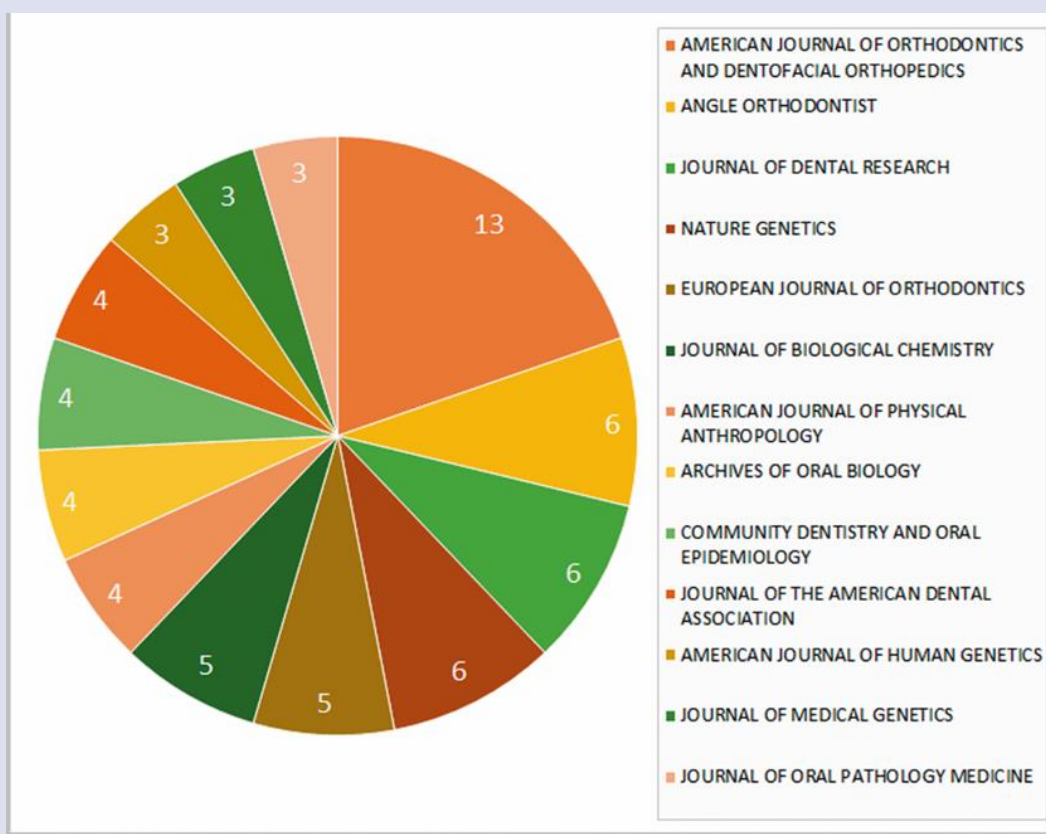| Name	First author	Co-author	N° of citations	Total
ERICSON S	5	0	1086	5
KUROL J	0	5	1086	5
WRIGHT JT	0	5	1024	5
GOODMAN AH	4	0	929	4
SIMMER JP	2	5	879	7
VASTARDIS H	3	0	850	3
PECK L	1	4	782	5
PECK S	4	1	782	5
ROSE JC	0	3	779	3
THESLEFF I	1	2	734	3
NIEMINEN P	2	1	666	3
HU JCC	2	3	650	5
ALALUUSUA S	1	2	585	3
D'SOUZA RN	0	3	569	3
KATAJA M	0	3	500	3
KIM JW	3	0	435	3
DIXON MJ	1	2	365	3

Figure 1. Number of articles per year

Figure 2. The 13 journals in which the top-100 cited articles were published
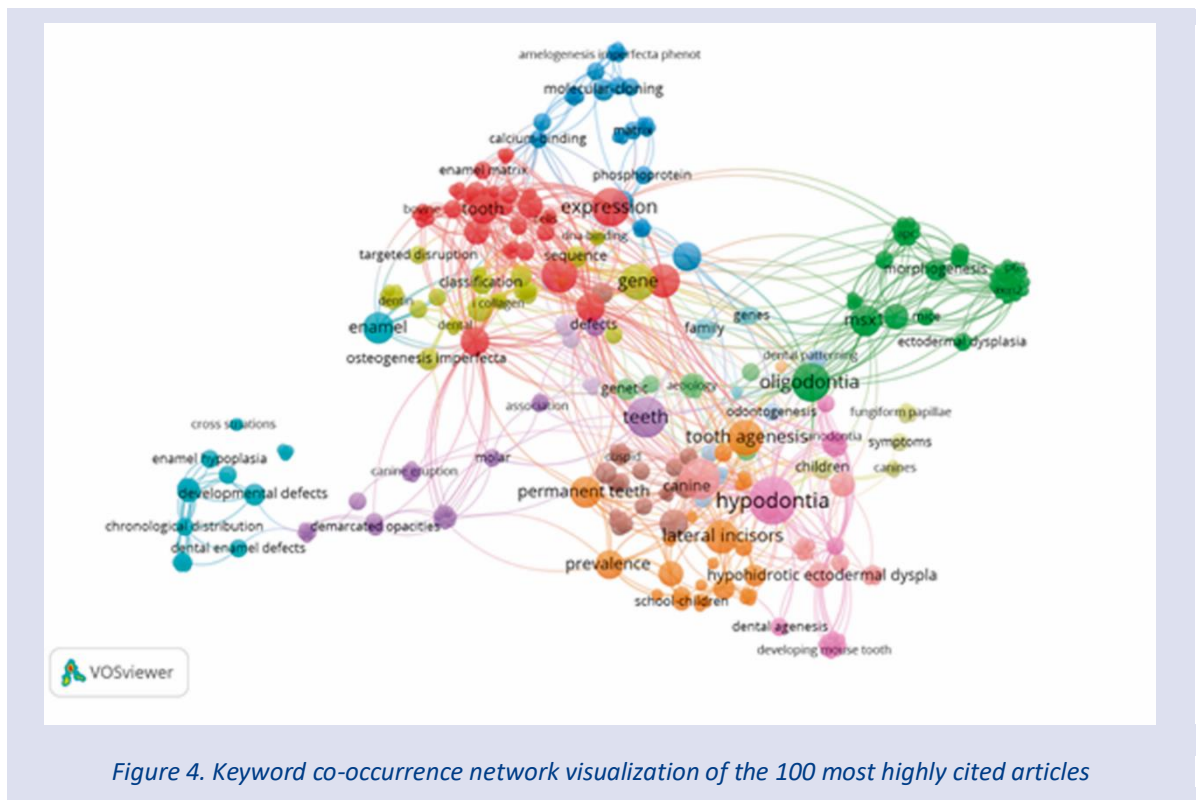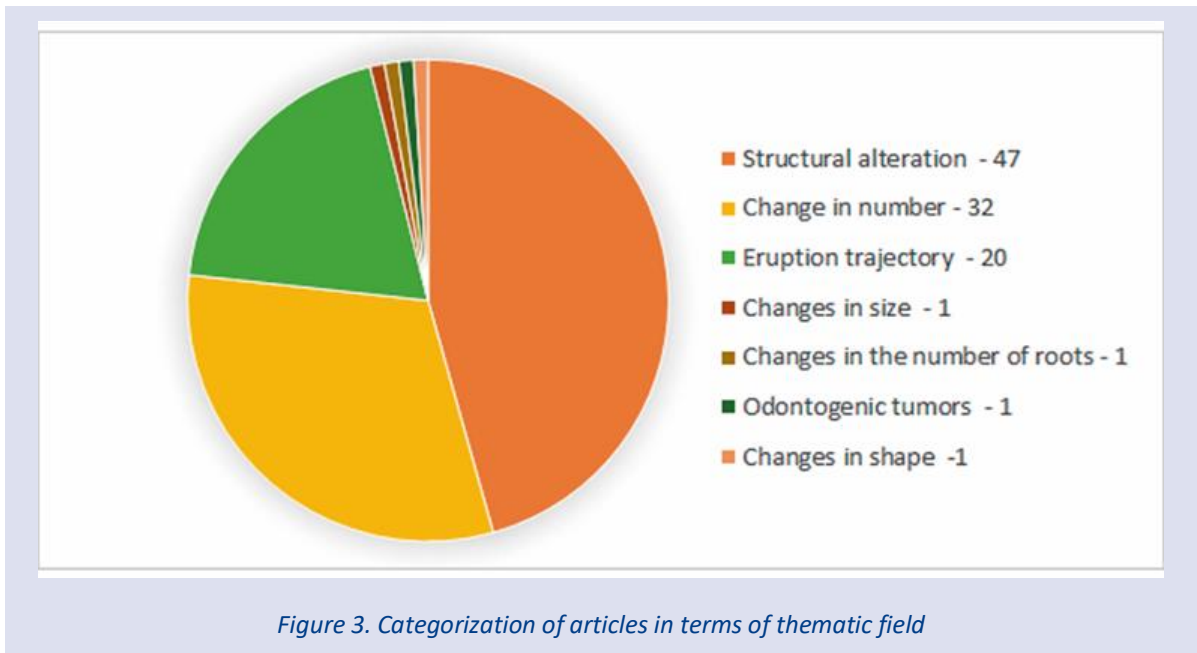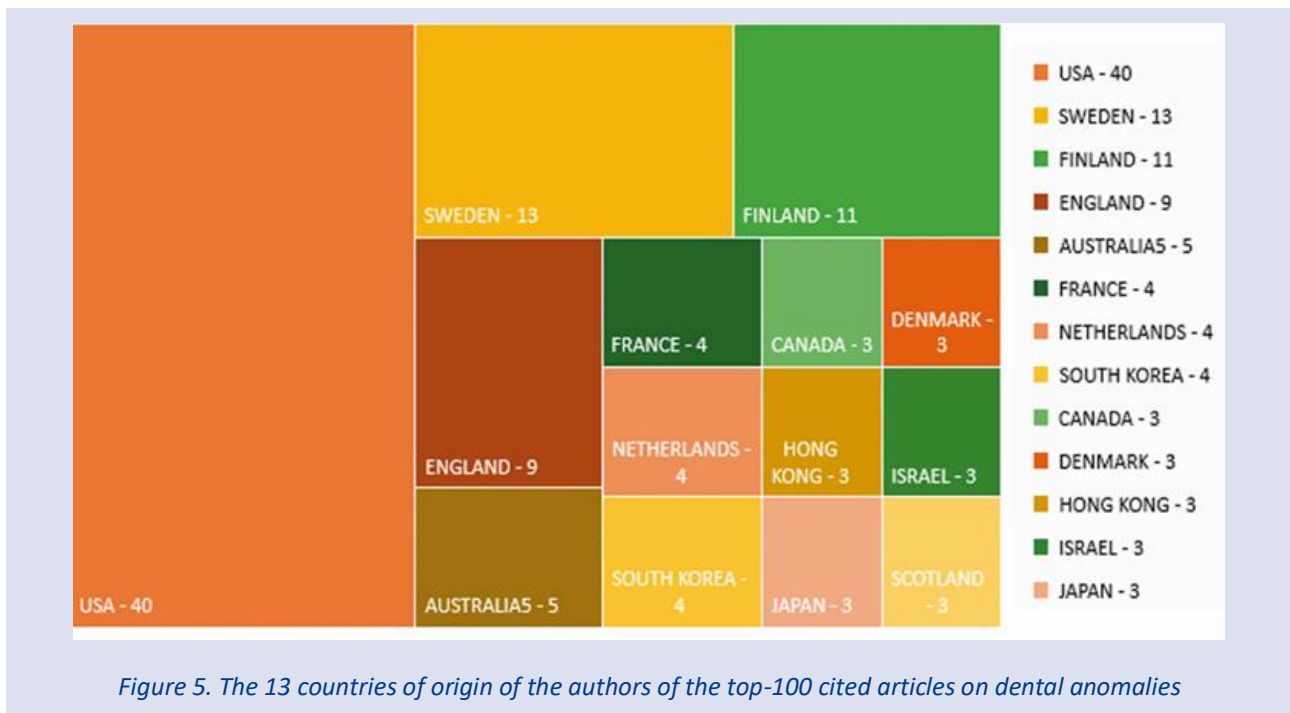
Figure 3. Categorization of articles in terms of thematic field

Figure 4. Keyword co-occurrence network visualization of the 100 most highly cited articles

Figure 5. The 13 countries of origin of the authors of the top-100 cited articles on dental anomalies

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Is Sclerostin Antibody an Effective Agent for Alveolar Bone Regeneration in Animal Models? A Scoping Review

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Review

ABSTRACT

Objectives: The use of Sclerostin Antibody(Scl-Ab) as a bone anabolic agent has shown significant benefit in bone disorders in preclinical animal models and human clinical trials. The objectives of this scoping review is to determine whether sclerostin antibody is an effective agent for alveolar bone regeneration in animal models and if sclerostin antibody is effective in syndrome/endocrine related diseases which may result in the reduction of alveolar bone quality.

Materials and Methods: An online search was conducted to locate published animal studies in the databases such as Medline/PubMed, Scopus, Web of Science, Google scholar. The articles published in the international peer-reviewed literature in the English language, from January 2010 up to and including February 2021 are included in this review. The initial search from the mentioned database resulted in 555 articles for review. Further, a search in the references led to additional 4 articles. After title and abstract screening and removing the duplicates, 9 articles were subjected to full text screening to determine their eligibility. Three articles were excluded and the remaining 6 articles were included in the review. The parameters describing bone quality and quantitiy such as, Bone Mineral Density (g/cm2), bone volume fraction (BVF), trabecular thickness in alveolar bone, Percentage of bone volume/tissue volume (BV/TV), were determined to ascertain the effects of Scl-Ab on alveolar bone regeneration.

Results: Scl-Ab was found to be effective in improving the bone quality and quantity. Scl-Ab has the potential to improve Bone Mineral Density (g/cm2), bone volume fraction (BVF), trabecular thickness in alveolar bone, Percentage of bone volume/tissue volume (BV/TV) and other parameters. Scl-Ab can improve the quality of bone in conditions that impairs the quality and density of bone such as osteoporosis, Down syndrome.

Conclusions: It was observed that Scl-Ab was useful in improving the quality and quantitiy of bone lost due to local infections such as periodontal diseases as well as reduced bone density associated with diseases and conditions affecting osteoblast activity. The review concluded that Scl-Ab promotes alveolar bone augmentation and improves bone quality without surgical interventions.

Keywords: Alveolar bone, Animal models, Preclinical models, Regeneration, Sclerostin, Sclerostin antibody.

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Introduction

Periodontitis is a chronic inflammatory disease that affects the supporting structures of the tooth, which often presents with bleeding on gentle probing, loss of attachment and increased gingival crevicular fluid. This non-communicable disease is triggered by bacteria and their endotoxins, resulting in immunological and humoral response[1,2] with the production of proinflammatory cytokines and other biomolecules.[2] These signaling markers participate in upregulating the inflammatory cascade causing further destruction of the periodontal structures including the alveolar bone defects in the advanced stages of periodontal disease. Hence, various therapeutic modalities are being attempted to regenerate/ restore the lost periodontal structures. To achieve regeneration of alveolar bone, various anabolic agents or bone antiresorptive agents have been experimented with. Due to the various biological, biomechanical factors, achieving periodontal tissue regeneration remains a major clinical challenge.

In the quest to understand the human body, researchers have come across the interesting working of the beta-catenin pathway and sclerostin molecule in the functioning of osteocytes which have been shown to accomplish several functions that are crucial for bone formation and turnover.[3] Sclerostin, a 213-amino-acid glycoprotein expressed mainly by osteocytes, is a vital messenger in the communications between osteocytes and osteoblasts that impedes osteoblast differentiation and regulates bone resorption [Figure 1].[4,5] According to the genetic studies, mutations in the SOST(Sclerostin) gene encoding sclerostin, are responsible for higher bone mass

and density in the skull, mandible, ribs, clavicles and all the long bones[6-8] and similar results have been observed in animal studies. However, overexpression of the SOST gene leads to the development of osteopenia in mice.[9] Thus, recognizing the inhibitory effect of sclerostin on bone formation, research has led to the development of sclerostin-neutralizing antibody (Scl-Ab). Sclerostin and its inhibitors exhibit a potential role in prosthetic, regenerative and preventive therapy in dentistry. The sclerostin neutralizing antibody has been investigated for bone disorders in preclinical animal models and human clinical trials. Systemic administration of Scl-Ab to female rats with osteopenia due to ovariectomy-induced estrogen deficiency, or to aged (sixteen-month-old) male rats, was shown to increase the bone formation at various bone sites and improve bone mass, mineral apposition rate, and bone strength.[10-12] The systemic delivery of Scl-Ab has demonstrated its efficacy in treating generalized bone loss across various studies.[13,14]

Figure 1. Schematic representation of the effect of Sclerostin and Sclerostin-Antibody on Bone
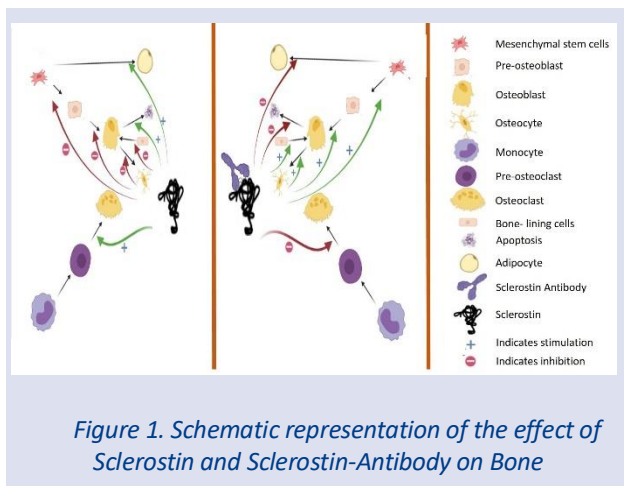
Based on the literature above, the use of Scl-Ab as a bone anabolic agent may have potential benefits in the treatment of alveolar bony defects. However, no human trials have been conducted in which Scl-Ab has been used for alveolar bone regeneration and hence treatment of alveolar bony defects in animal models using Scl-Ab has drawn considerable attention. The study of regeneration of bones in animal models using Scl-Ab has analysed the effect over a varied range of experimental conditions including the effectiveness of the agent in experimental periodontitis, surgical bone defects and around implants. The dosage of drug administered and the route of administration that has been used is also different in the studies that have been done. An understanding of the best mode of drug administration, the effectiveness of each dosage, the complications associated with the use of the drug is essential for the translation of animal research into human clinical trials.

A Scoping review will provide an overview of the available research evidence of the effectiveness of Scl-Ab on alveolar bone regeneration. It is used to identify the number, nature, and characteristics of the primary research in terms of effectiveness of Scl-Ab on alveolar bone regeneration as the topic has not yet been extensively reviewed and the existing research is heterogeneous in nature. It also helps to summarize and disseminate research findings as well as determine the research gaps in the literature on the topic. Studies on pre-clinical animal models constitute the currently available evidence on the topic and hence this scoping review encompasses the animal studies conducted to ascertain the effectiveness of Scl-Ab on alveolar bone regeneration. Literature search and protocol databases did not identify any systematic or scoping review on the topic. The aim of the present scoping review of animal studies is to determine the effectiveness of Scl-Ab in alveolar bone regeneration. The articles published in the international peer-reviewed literature in the English language, from January 2010 up to and including February 2021 have been included in this review. This would help identify areas for future research, and also help develop strategies for research implementation.

Review question

The review has been registered in the Open Science frame registries (Registration DOI:10.17605/OSF.IO/DQY5F). In preparing the scoping review with the objective to determine the effectiveness of Scl-Ab in alveolar bone regeneration, we examined the following review questions: Is sclerostin antibody an effective agent for alveolar bone regeneration in animal models? Is sclerostin antibody effective in syndrome/endocrine related diseases which may result in the reduction of alveolar bone quality?

Inclusion and exclusion criteria
Studies Included

Animal studies in which the treatment arm includes Scl-Ab administration for alveolar bone preservation or regeneration were included. Rodents models and canine models which are most commonly used for periodontal research were the animal models included in the study. There were no restrictions regarding the route and the dosage of application. Clinical trials and ex-vivo studies were excluded.

Concept

The use of Scl-Ab for bone disorders has been studied in preclinical animal models and human clinical trials. However, its use in alveolar bone regeneration has seen limited research exposure with studies confined to animal research. This review studied the concept of the role of Scl-Ab on alveolar bone preservation and regeneration in animal models. Studies in which there is the use of Scl-Ab for purposes other than healing and regeneration of alveolar bone defects were excluded.

Context

Alveolar bone regeneration in patients with periodontal disease and bone defects has been an avenue that has been studied extensively. Studies have shown that osteocytes have a mechanosensory function via sclerostin which helps in bone preservation. Alveolar bone regeneration using Scl-Ab is a less explored territory with research on pre-clinical

models being the currently available evidence. This review included animal studies on the effectiveness of Scl-Ab on alveolar bone regeneration. There were no restrictions regarding the gender or age of the animal model being used. However, Studies utilizing bisphosphonates and other agents that facilitate bone formation were excluded.

Types of Sources

This scoping review considered experimental animal studies for inclusion. Animal studies exploring qualitative and quantitative data pertaining to the topic were included. The databases, Medline/PubMed, SCOPUS and Web of Science were used as sources of evidence.

Methods

Information sources and search strategy

Articles on the topic were identified through an initial limited search of Medline/PubMed. A full search strategy for SCOPUS and Web of Science was developed using the text words in the titles and abstracts of relevant articles as well as the index terms used in the description of the article. The reference list of all included sources of evidence was screened for additional studies. All articles from January 2010 to February 2021 about animal studies regarding the efficacy of Scl-Ab in the treatment of alveolar defects were included in the review. The articles published in the international peer-reviewed literature in the English language were included.

The search terms used were (((Animal model) OR Rodent) AND Alveolar bone defect) AND Anti sclerostin antibody) OR Sclerostin antibody) AND Alveolar bone regeneration) OR Bone regeneration) AND Bone fill. The search details in PubMed was ((("models, animal"[MeSH Terms] OR ("models"[All Fields] AND "animal"[All Fields]) OR "animal models"[All Fields] OR ("animal"[All Fields] AND "model"[All Fields]) OR "animal model"[All Fields]) OR ("rodentia"[MeSH Terms] OR "rodentia"[All Fields] OR "rodent"[All Fields])) AND (Alveolar[All Fields] AND ("bone and bones"[MeSH Terms] OR ("bone"[All Fields] AND "bones"[All Fields]) OR "bone and bones"[All Fields] OR "bone"[All Fields]) AND defect [All Fields])) AND (Anti[All Fields] AND sclerostin[All Fields] AND ("immunoglobulins"[MeSH Terms] OR "immunoglobulins"[All Fields] OR "antibody"[All Fields] OR "antibodies"[MeSH Terms] OR "antibodies"[All Fields])) OR (Sclerostin[All Fields] AND ("immunoglobulins"[MeSH Terms] OR "immunoglobulins"[All Fields] OR "antibody"[All Fields] OR "antibodies"[MeSH Terms] OR "antibodies"[All Fields])) AND (Alveolar[All Fields] AND ("bone regeneration"[MeSH Terms] OR ("bone"[All Fields] AND "regeneration"[All Fields]) OR "bone regeneration"[All Fields])) OR ("bone regeneration"[MeSH Terms] OR ("bone"[All Fields] AND "regeneration"[All Fields]) OR "bone regeneration"[All Fields]) AND (("bone and bones"[MeSH Terms] OR ("bone"[All Fields] AND "bones"[All Fields]) OR "bone and bones"[All Fields] OR "bone"[All Fields]) AND fill[All Fields]) AND ("2011/02/05"[PDat] : "2021/02/01"[PDat]).

Following the search, all relevant citations were collected and uploaded into Mendeley reference manager (version 1.19.4) and duplicates were removed. Titles and abstracts were screened by two independent reviewers (LP, SB) for assessment against the inclusion criteria for the review. Potentially relevant articles were identified and retrieved in full and were assessed in detail by the reviewers (LP, SB) and the reasons for the exclusion of articles were recorded. Any disagreements between the reviewers regarding the inclusion of the articles during the selection process were resolved through discussion or consultation with additional reviewers. The results of the search and the study inclusion process have been presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for scoping review (PRISMA-ScR)[15] flow diagram [Figure 2].
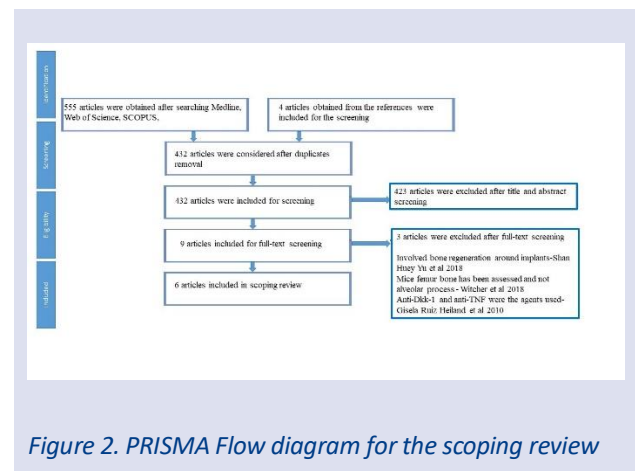

Figure 2. PRISMA Flow diagram for the scoping review

Data Extraction

Data were extracted from papers included in the scoping review by two independent reviewers (LP, SB). Disagreements, if any, that arose between the reviewers were resolved through discussion or consultation with additional reviewers (JV, MK). The data extracted included details like the author, year, country, Animal used, genetic modifications/mutations of the animal used, ethical clearance, type of disease model, the grouping of the animal models based on drug used, the dosage of the drug used, route of administration, type of disease model (Supplementary file 1), parameters assessed (Table 1) such as Bone Mineral Density (BMD) (g/cm2), bone volume fraction (BVF), Serum Osteocalcin (ng/ml), N-terminal propeptide of procollagen type I, tartrate-resistant acid phosphatase 5b, Trabecular thickness (Tb.Th) in alveolar bone, Percentage of bone volume/tissue volume (BV/TV), Mean tooth to alveolar crest length, mm (SD), Trabecular separation(mm), Trabecular Number (Tb.N), BFR/BS (bone formation rate/bone surface) for alveolar and basal bone,Osteocyte (Ocy) surface area(μm^2), Ocy dendrite length(μm), Ocy total cell Volume (μm^3) presented either in qualitative or quantitative form and other parameters assessing changes in bone structure and regeneration of periodontal supporting tissues. The six articles included in this review were analysed and the data obtained have been described in a narrative summary.

Results

The initial search from the mentioned database resulted in 555 articles for review. Further, a search in the references led to additional 4 articles. After removing the duplicates, 9 articles were subjected to full text screening to determine their eligibility. Three articles [Shan Huey Yu *et al.* 2018, Witcher *et al.* 2018, Ruiz Heiland *et al.* 2010][16-18] were excluded and the remaining 6 articles [Yao Yao etal2020, M Liu *et al.* 2018, Chen *et al.* 2015, Taut *et al.* 2013, Ren *et al.* 2015, Tamplen *et al.* 2018] [19-24] were included in the review.

Characteristics of sources of evidence

The animal models used in the study ranged from Sprague Dawley rats[19-22], Periostin Knock-out mice(PKO), Double Knockout mice(DKO),mice with the C57BL/6 background[23], Ts65 male mice and wild type euploid littermate.[24] The characteristics of the articles included are given in Supplementary file 1 and the parameters assessed in the included studies are given in Table 1.

Table 1: Parameters assessed

S No	Parameter Assessed	Yao Yao et al 2020	Taut AD et al. 2013	Liu et al 2018	Tamplen M et al 2018	Ren Y et al 2015	Chen et al 2015
1	Length of new bone (mm)	✓					
2	Linear bridging bone (%)	✓					
3	Area of new bone (mm2)	✓					
4	Bone fill %	✓					
5	New cementum	✓					
6	New cementum length (mm)	✓					
7	New cementum length (%)	✓					
8	Root resorption	✓					
9	Bone volume	✓					
10	Bone fill	✓					
11	Bone mineral density/bone mineral content	✓	✓	✓			✓
12	Bone volume fraction (BVF)		✓	✓			✓
13	Linear alveolar bone loss (ABL) in millimeters		✓				
14	Osteoprotegerin(pg/ml)						✓
15	Serum P1NP(ng/ml)		✓	✓			
16	Serum Osteocalcin (ng/ml)		✓	✓			✓
17	Trap5b		✓	✓			✓
18	Trabecular thickness (Tb.Th) in alveolar bone		✓		✓		✓
19	BFR/BS (bone formation rate/bone surface) for alveolar and basal bone/ Mineral Apposition Rate(MAR)		✓				✓
20	ES/BS (eroded surface/bone surface) for alveolar and basal bone		✓				
21	mRNA expression for the genes encoding sclerostin (Sost)		✓				
22	mRNA expression for the genes encoding Dkk1		✓				
23	Maxillary alveolar ridge volume		✓				
24	Maxillary height loss		✓				
25	Percentage of bone volume/tissue volume (BV/TV)				✓	✓	
26	Mean tooth to alveolar crest length, mm (SD)/ CEJ-Alveolar bone crest(mm)				✓		✓
27	Trabecular spacing, mm (SD)				✓		✓
28	Ratio of SOST positive cells/total cells					✓	
29	Ocy surface area(μm2)					✓	
30	Ocy dendrite length(μm)					✓	
31	Ocy total cell Volume (μm3)					✓	
32	Dendrite numbers					✓	
33	Cementum-enamel junction (cm2)					✓	
34	Qualitative assessment: TRAP, DMP1, OSX, Biglycan, Decorin, Collagen					✓	
35	Trabecular Number (Tb. N) (I/mm)						✓
36	CTx-1(ng/mL)						✓
37	Mineral Apposition Rate(MARμm/d)						✓

Critical appraisal of an individual source of evidence

Length of new bone (mm), Linear bridging bone (%), Area of new bone (mm2), Bone fill %, New cementum, New cementum length (mm), New cementum length (%), Root resorption, Bone volume (BV), Bone fill (BF) have been assessed in the study by Yao Yao *et al.* 2020.[19] In this study it was shown that local application of Scl-Ab microspheres (MS) did not have an improved effect on length of new bone formation (2.43±0.14mm), Linear bridging bone (%) (89.9±4.8) , Area of new bone (mm^2) (0.83±0.09), Bone fill % (45.3±4.1), New cementum length (mm) (0.38±0.10), New cementum length (%) (32.4±8.5) ,Root resorption (6/12) as compared to systemic administration of Sclerostin antibody (Sys Scl-*Ab*), Empty microspheres(Emp), Controls (c) [length of new bone formation (Sys Scl-Ab-2.69±0.09, Emp- 2.60±0.12, C- 2.52±0.07), Linear bridging bone (%) (Sys Scl-Ab-97.3±1.8, Emp- 93.8±3.4, C- 96.4±2.0) , Area of new bone (mm2) (Sys Scl-Ab-1.03±0.05, Emp- 0.89±0.06, C- 0.92±0.07), Bone fill % (Sys Scl-Ab-61.2±3.0, Emp- 46.0±2.8, C- 53.9±3.5), New cementum length (mm) (Sys Scl-Ab-0.58±0.12, Emp- 0.40±0.09, C- 0.39±0.08), New cementum length (%) (Sys Scl-Ab-52.5±9.8, C- 32.8±7.1) and Root resorption (Sys Scl-Ab-1/9, C-3/12)]. In terms of new cementum length percentage (%) and the number of roots having root resorption, the locally administered Scl-Ab microspheres gave better results as compared to empty microspheres (new cementum length %-31.9±7.5, root resorption-7/12). The number of teeth with new cementum formation was similar for local and systemic administration of Scl-Ab but reduced for Empty microspheres and control group [New cementum for local Scl-Ab- (7/12), New cementum (Sys Scl-Ab-4/9, Emp- 6/12, C- 6/12)]. BV, BF and BMD showed a significant difference in the systemic Scl-Ab group as compared to the microsphere groups.

Bone mineral density/bone mineral content has been assessed in four of the included studies (Yao Yao etal2020, M Liu *et al.* 2018, Chen *et al.* 2015, Taut *et al.* 2013).[19-22] The group which received systemic Scl-Ab showed significantly higher BMD compared to locally delivered Scl-Ab MS (Yao Yao *et al.* 2020).[19] In the study by Taut *et al.* 2013[22], it was shown that twice-weekly subcutaneous administration of 25 mg/kg Scl-Ab for 3 weeks, increased the serum concentration of osteocalcin and P1NP which are bone formation markers and increased the mineral apposition rate(MAR) in the alveolar bone. Twice weekly subcutaneous administration of 25 mg/kg Scl-Ab for 6 weeks showed significantly greater BMD and BVF in ovariectomized(OVX) rats with ligature induced periodontitis as compared with vehicle treated controls[21]. Assessment of percentage change in bone mineral content (BMC) by Liu *et al.* 2018[20] showed that DKK1 antibody (DAB) in combination with Scl-Ab had a significantly more increase in BMC as compared to Scl-Ab alone.

M Liu *et al.* 2018[20], Chen *et al.* 2015[21], Taut *et al.* 2013[22] have assessed the bone volume fraction(BVF) post Scl-Ab administration. Scl-Ab group had higher BVF as compared to the control group in the studies by Chen *et al.* 2015[21] (P< 0.001) and Taut *et al.*[22] (P<0.05). The study by Taut *et al.*[22] also showed that a 6 week treatment with systemic

Scl-Ab resulted in the reversal of ligature-induced bone loss. M Liu *et al.* 2018[20] have shown that five weeks of Scl-Ab or Scl-Ab+DAB therapy initiated 9 weeks post-surgery, restores BVF to levels greater than that with vehicles and controls. Assessment of linear Alveolar Bone Loss (ABL) in the study by Taut *et al.*[22] showed that 6 weeks of systemic Scl-Ab administration resulted in statistically significant improvement in linear ABL (p<0.05) as compared to vehicle treatment.

Serum osteocalcin levels and serum Tartrate-resistant acid phosphatase (TRAP) 5b are markers of bone metabolism and their levels have been assessed in studies by M Liu *et al.* 2018[20], Chen *et al.* 2015[21], Taut *et al.* 2013[22], while Serum Procollagen 1 Intact N-Terminal Propeptide (P1NP) levels, another marker of bone metabolism has been assessed in studies by M Liu *et al.* 2018[20] and Taut *et al.* 2013.[22] In the study by Chen *et al.*[21], a significant elevation in the serum osteocalcin and osteoprotegerin and a decrease in serum TRAP5b were noted after 6 weeks of treatment with Scl-Ab. Type I Collagen Cross-Linked C-Telopeptide (CTX-1) which is a bone resorption marker, was found to be increased in ovariectomized (OVX) rats when compared to sham and control groups even if Scl-Ab was administered, however, the levels were significantly lower than that in the ovariectomized (OVX) rats receiving vehicle instead of Scl-Ab (P=0.001). Liu *et al.*[20] have found a significant increase in serum P1NP in the SAB group (P<0.05 versus Veh), and a significant reduction in serum TRACP-5b in the Scl-Ab +DAB group (P<0.05). There was no mention regarding the serum osteocalcin levels in the result section of the study. Taut *et al.*[22] have shown an increase with respect to serum osteocalcin levels in comparison to intact (p=0.0019) and vehicle (p=0.0001) groups after 3 weeks of treatment and at 6 weeks after the treatment commenced (p=0.034). However, Scl-Ab did not demonstrate any statistical difference between the intact (p=0.10) and vehicle-treated Experimental Periodontitis (EP) (p = 0.058) groups at 6 weeks in serum P1NP levels although the difference was significant at 3 weeks. Scl-Ab treatment after ligature-induced EP did not produce any change in serum TRAP 5b levels during the therapeutic phase.

M Liu *et al.* 2018, Chen *et al.* 2015, Tamplen *et al.* 2018[20,21,24] have assessed the effect of Scl-Ab on trabecular thickness (Tb.Th) in the alveolar bone. Tamplen *et al.* have found that the trabecular thickness in alveolar bone was higher for Scl-Ab [0.177 (0.01) in wild type mice and 0.167 (0.02) in Ts65 mice] as compared to the vehicle [0.154 (0.01)]. A similar result was seen in the study by M Liu *et al.*[20], where an increased Tb.Th was found in Scl-Ab and Scl-Ab+DAB groups as compared to controls. The microarchitecture parameters, Tb.Th and Tb.N was higher in the OVX + Ligature + Scl-Ab group as compared with the OVX + Ligature + Vehicle group (P = 0.001) in the study by Chen *et al.*[21]

Bone formation rate/bone surface (BFR/BS) and Mineral apposition rate (MAR) for alveolar and basal bone assessed in studies by Liu *et al.*[20] and Chen *et al.*[21] shows that a significantly greater BFR/BS (bone formation rate/bone surface) in alveolar and basal bone was noted in

the Scl-Ab and Scl-Ab+DAB groups versus Veh, with Scl-Ab+DAB demonstrating a higher BFR/BS in basal bone compared to Scl-Ab[20]. In the study by Chen *et al.*[21] Scl-Ab and Scl-Ab+DAB groups had significantly lower eroded surface/bone surface (ES/BS) values in comparison with OVX-Veh rats. MAR was significantly increased in the OVX + Ligature + Scl-Ab group compared with the Sham +Ligature + Vehicle (P = 0.003) and OVX + Ligature + Vehicle groups (P = 0.001).

mRNA expression for the genes encoding sclerostin (Sost), mRNA expression for the genes encoding Dkk1, Maxillary alveolar ridge volume, Maxillary height loss has been assessed in a study by Liu *et al.*[20] The alveolar osteocytes in the non-extracted and extracted maxilla expressed high levels of Sost mRNA while Dkk1 was moderately expressed by osteocytes in the non-extracted maxillae. Molar extraction resulted in an increase in Dkk1 levels within 1 week and the Dkk1 mRNA expression remained high even after 2 weeks of extraction. The Dkk1 levels returned to non-extracted levels 3 to 5 weeks post-extraction.

The percentage of bone volume/tissue volume (BV/TV) has been assessed by Tamplen *et al.*[24] and Ren *et al.*[23] Volumetric analysis in the study by Tamplen *et al.*[24] has shown that the average mandibular bone volume (bone volume per total volume) was higher in wild-type mice treated with Scl-Ab as compared with vehicle-treated mice. Ren *et al.*[23], have shown through microCT data, the restoration of Bone Volume (BV) in the PKO mice qualitatively and quantitatively as a result of Scl-Ab treatment.

Mean tooth to alveolar crest length, (mm)/ CEJ-Alveolar bone crest(mm), Trabecular spacing, mm (SD) were assessed in studies by Tamplen *et al.*[24] and Chen *et al.*[21] A significant decrease in the average CEJ-ABC distance was noted in both the studies as a result of Scl-Ab treatment. Tamplen *et al.*[24] have found that trabecular spacing was least for Scl-Ab treated wild type mice [0.073, (0.005)] as compared to vehicle treated and Scl-Ab treated Ts65 mice [0.079, (0.004)] while it was highest for Ts65 mice [0.085, (0.007)] and a similar result has been suggested in the study by Chen *et al.*[21]

Quantitative assessment of Ocy surface area(μm^2), Ocy dendrite length(μm), Ocy total cell Volume (μm^3), Dendrite numbers and qualitative assessment of TRAP, DMP1, OSX, Biglycan, Decorin, Collagen was done by Ren Y *et al.*[23] The statistical analysis revealed that there were significant differences in the quantitative parameters assessed among WT, PKO mice treated with Scl-Ab for 8 weeks (P< 0.01) with a higher value evident in specimens treated with Scl-Ab. The molecular markers and collagen were shown to be restored in the DKO mice and the PKO mice treated with Scl-Ab for 8 weeks.

The effect of Scl-Ab on surgical defect has been evaluated in the study by Yao Yao *et al.*[19] Quantitative μCT measurements showed a 40% greater bone mineral density, bone volume, bone fill in the systemic Scl-Ab group than the control, empty MS, Scl-Ab MS groups. It was observed that there was increased bone bridging and osteogenesis in the systemic Scl-Ab group.

The effect of Scl-Ab on experimental periodontitis has been studied by Taut *et al.*[22] and Chen *et al.*[21] Six weeks of Scl-Ab restored the bone quality and quantity to levels comparable to intact control and brought about an increased bone apposition rate.[22] Similar results have been observed in the study by Chen *et al.*[21] Taut *et al.*[22] have also shown that systemic Scl-Ab can be effective in the prevention of the progression of periodontal disease

Tamplen *et al.*[24] and Chen *et al.*[21] have assessed the effect of Scl-Ab on alveolar bone quality in models with metabolic diseases/disorders that affect the bone quantity and quantity. Tamplen *et al.*[24] have found that the low bone mass phenotype of Down syndrome mandibular bone in the Ts65 mice can be completely normalized using Scl-Ab treatment. An increase in serum osteocalcin and osteoprotegerin has been shown in the study by Chen *et al.*[21] while levels of serum tartrate-resistant acid phosphatase and CTx-1 decreased with the administration of Scl-Ab resulting in increased alveolar bone mass in OVX rats with estrogen deficiency osteopenia plus periodontitis.

Effect on underloaded alveolar bone following extraction of teeth was studied by Liu *et al.*[20] The study demonstrated that systemic Scl-Ab administration improved the volume and height of atrophic alveolar ridges and DAB had a synergistic effect when used in combination with Scl-Ab. Complete reversal of bone loss in the opposing mandible as a result of hypo-occlusion was achieved with the use of Scl-Ab and Scl-Ab +DAB.

It has been shown in the study by Taut *et al.*[22] that after 6 weeks of treatment, BVF and TMD values in the experimental periodontitis group with Scl-Ab administration were similar to those of healthy controls. In the study by Liu *et al.*, it was observed that by treatment week 15, the Scl-Ab group had 42% and the Scl-Ab+DAB group had 81% greater alveolar ridge volume when compared to extracted Veh controls (both P<0.05) and a significant gain in ridge height was also observed within 2 and 4 weeks of treatment initiation. The Scl-Ab+DAB group achieved complete height recovery by week 9 while the Scl-Ab group had only about two-thirds of alveolar bone height recovery in 15 weeks. Summarization of the findings is given in Supplementary file 2.

Summarization of response to the review questions

Scl-Ab has the potential to improve the quantity and quality of alveolar bone by improving Bone Mineral Density (g/cm2), bone volume fraction (BVF), trabecular thickness in alveolar bone, Percentage of bone volume/tissue volume (BV/TV) and other parameters.

Scl-Ab can improve the quality of bone in conditions that impairs the quality and density of bone such as osteoporosis, Down syndrome.

Risk of bias

SYRCLE's (SYstematic Review Center for Laboratory animal Experimentation) tool was used for assessing the risk of bias.[25] Low risk of bias was recorded in terms of

attrition bias and other sources of bias for all included studies.[19,20,21,22,23,24] Reporting bias was unclear for the study by Liu *et al.* 2018[20] as there was no mention regarding the serum osteocalcin levels in the result section of the study. All other studies had a low reporting bias. Unclear risk of bias was noted for sequence generation and baseline characteristics in studies by Yao Yao *et al.*2020, Chen *et al.* 2015, Taut *et al.* 2013, Ren *et al.* 2015, Tamplen *et al.* 2018 [19,21,22,23,24] as well as in allocation concealment, random outcome assessment in studies by Yao Yao etal2020, M Liu *et al.* 2018, Chen *et al.* 2015, Taut *et al.* 2013, Ren *et al.* 2015, Tamplen *et al.* 2018[19,20,21,22,23,24], and in random housing of animals [M Liu *et al.* 2018, Taut *et al.* 2013, Ren *et al.* 2015, Tamplen *et al.* 2018].[20,22,23,24] Random housing of animals had a low risk of bias in studies by Yao Yao *et al.* 2020 [19] and Chen *et al.* 2015.[21] The study by Yao Yao *et al.*[19] had a low risk of bias in terms of blinding of caregivers/investigators as well as outcome assessors while the other included studies had a high risk of bias in terms of these two criteria. The risk of bias assessment across studies and within studies is given in Figure 3 and Figure 4.
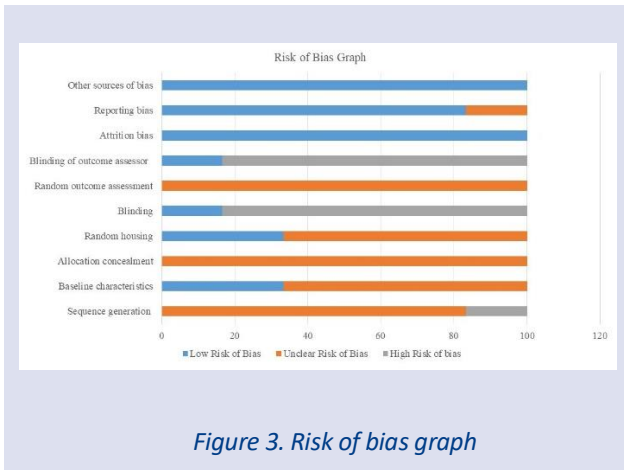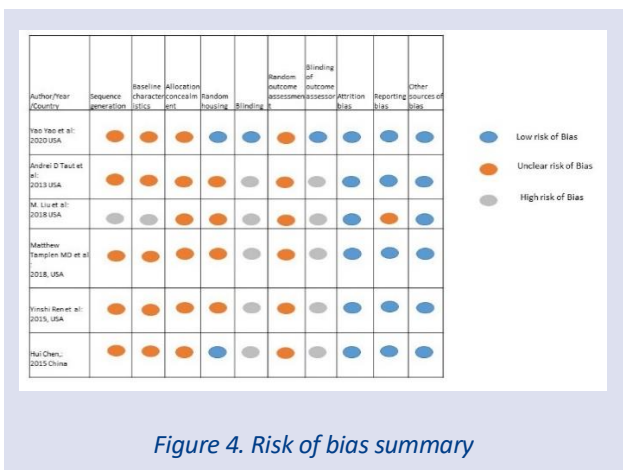

Figure 3. Risk of bias graph

Figure 4. Risk of bias summary

Discussion

Sclerostin is an inhibitor of the canonical Wnt pathway which is involved in bone remodelling. It is produced by several cells and tissues such as mature osteocytes, cementocytes, kidney, liver, heart, or carotid arteries. Due to its effect on bone formation and resorption, its potential

as a therapeutic agent for increasing bone formation has been explored.[26] Alveolar bone loss is a sign of periodontal disease which results in tooth loss. An agent that can modulate bone quality and quantity can have profound effects on treatment strategies for periodontal disease. The use of Scl-Ab in management of alveolar bone loss is a territory that requires further study. Hence this scoping has been performed to determine whether sclerostin antibody is an effective agent for alveolar bone regeneration. Scoping reviews facilitate a systematic identification and analysis of the existing data. It helps to chart out the literature on a particular topic and presents a descriptive overview of the research question[27] so that it would be possible to perform further studies to anwer the potential hypothesis that arise as a result of performing the scoping review.

All articles from January 2010 to February 2021 about animal studies regarding the efficacy of Scl-Ab in the treatment of alveolar defects were included because the research on sclerostin as a bone anabolic agent with a focus on alveolar bone regeneration has become robust only in the past 10 years.

Bone fill percentage, Bone volume, Bone fill, trabecular thickness, bone volume fraction, bone formation rate/bone surface, mineral apposition rate, percentage of bone volume/tissue volume, trabecular spacing are parameters indicative of bone quantity and quality.[28,29,30] An increase in these parameters are suggestive of increase in the amount of bone formed. The studies included in the review have attempted to identify the bone forming potential of Scl-Ab using these parameters and it was observed that the agent is capable for facilitating bone formation. The local and systemic administration of the drug were found to be equally effective in providing a positive effect on bone quantity[19] and osteogenesis is found to be increased with the use of Scl-Ab thereby enabling bone formation.[31]

High bone mineral density has been observed in patients with high serum sclerostin levels and an inverse relation was noticed between PTH and sclerostin[32]. In the studies included in this review, Scl-Ab has been shown to have the ability to increase the bone mineral density as well as increase the osteocyte length, number and volume which is indicative of an improvement in the bone quality.[23] The enhancement in bone mass parameters after Scl-Ab treatment can be ascribed to the increase in osteoblastic activity.[33]

Serum osteocalcin levels, TRAP 5b, P1NP levels, CTX-1 are markers of bone metabolism which have been assessed in the included studies. The increase in osteocalcin, P1NP levels are indicative of increase in bone formation whereas CTX-1and TRAP are associated with bone resorptive activities.[34,35,36] It has been observed from the studies included in the review that Scl-Ab favours an increase in the bone formative markers and a reduction in bone resorptive markers.

A detailed review of the Scl-Ab indicated its beneficial effect in restoring alveolar bone. Existing literature has shown that Scl-Ab can improve the quality of bone in conditions that predispose towards impaired trabecular

quality and density such as osteoporosis.[37,38] Clinical trials using Scl-Ab therapy have resulted in increased bone mineral density and reduced fracture risk.[39] In this review, a similar beneficial effect was observed in the alveolar bone of OVX rats. In the case of the Ts65 Down syndrome mice, the balance between the osteoblast and osteoclast activity results in bone resorption which may be reversed using Scl-Ab as it facilitates a pro-osteoclastogenic cell signaling between osteocytes and osteoclasts.[40] Thus Scl-Ab can be beneficial for the low alveolar bone mass seen in Down syndrome.

In addition to the above findings, this review also showed that a combination of DAB along with Scl-Ab had a better bone anabolic activity as compared to Scl-Ab alone. DKK1 acts as an endogenous factor that limits jaw bone volume and density[41]. Thus inhibition of both sclerostin and DKK1 resulted in better alveolar bone regeneration as compared to either one alone. Systemic administration of the drug had better bone regenerative potential than the local application of Scl-Ab microspheres. PLGA biodegradation in vivo in osseous defects might limit the efficacy of Scl-Ab MS[42] apart from the different dosing levels between local (125 µg per defect) and systemic groups (31.25 mg in total per animal). Application of a higher dose locally using the local drug delivery system has potential disadvantages such as insufficient loading capacity, unsatisfactory polymer biodegradation, and material-mediated inflammation. However, local application of the drug can reduce the potential systemic side effects.

Disadvantages of Scl-Ab may be the possibility that inhibition of sclerostin may be deleterious to cartilage and may facilitate acceleration of disease in a rheumatoid arthritis mouse model as shown in studies on Sclerostin-deficient mice.[43,44]

This review is limited by the diverse methodology utilized in each of the articles and the limited number of articles satisfying the inclusion criteria. A systematic review has not been made due to the wide clinical and methodological heterogeneity.

Conclusions

This scoping review highlights the potential benefits of Scl-Ab in improving Bone Mineral Density (g/cm^2), bone volume fraction (BVF). It can bring about favourable changes in bone biomarkers such as Serum Osteocalcin (ng/ml), N-terminal propeptide of procollagen type I, tartrate-resistant acid phosphatase 5b, and improve the quality of bone by improving trabecular thickness in alveolar bone, Percentage of bone volume/tissue volume (BV/TV). Another valuable observation was that systemic administration of Scl-Ab might be more beneficial than local administration. The osteoanabolic effects of Scl-Ab may also be beneficial in conditions like Down syndrome, osteoradionecrosis which is characterized by a reduction in osteoblast activity.

The research for alveolar bone regeneration has resulted in different non-bone and bone graft materials. Bone anti-resorptive agents like Scl-antibody are one of the components of the wide-arsenal available to preserve and regenerate bone which is still under research. Additionally, Scl-Ab promoted the vertical restoration of the atrophic edentulous maxillary ridge of rats without surgical interventions suggesting its possible use in vertical alveolar bone augmentation. The conclusions drawn from this review may be applied to design clinical trials to test the effectiveness of Scl-Ab in alveolar bone preservation as well as augmentation/ regeneration.

Data availability statement

As this is a scoping review, the data availability is not applicable.

Funding

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