Official Publication of
Istanbul University
Faculty of Dentistry

European Oral Research

Volume 56 ▪ Issue 1 ▪ January 2022

ISSN print 2630-6158 ▪ ISSN online 2651-2823

EOR

eor.istanbul.edu.tr

Istanbul University Press

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2. Matbaacılar Sitesi 3NB 3 Topkapı/Zeytinburnu, İstanbul, Turkey
E-mail: www.ilbeymatbaa.com.tr
Certificate No: 17845

The publication languages of the journal is English.

This is a scholarly, international, peer-reviewed and open-access journal published triannually in January, May and September.

Publication Type: Periodical

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

Chapter in a book

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

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

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

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

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European Oral Research

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Eur Oral Res 2022; 56(1): 1-9

Original research

Changes in airway patency and sleep-breathing in healthy skeletal Class II children undergoing functional Activator therapy

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Purpose
Several studies agree that an abnormal maxilla-mandible relationship correlates better as an Obstructive Sleep Apnea (OSA) predictor, rather than obesity. One of the orthodontic therapies recommended for this kind of craniofacial deformity is to advance the mandible forward with an orthodontic activator, therefore, the aim of this study is to determine if healthy children that use this appliance experience a widening of the upper airway as well as an improvement in their sleep-breathing patterns.

Materials and Methods
39 healthy children, 20 for activator group (10 boys and 10 girls, 4 mean age 10.9 + 0.9; BMI 16.2 + 1.4), 19 for control group (13 boys and 6 girls, mean age 5 9.8 + 1.4; BMI 17.6 + 2.1) participated in this study. They were required to submit 2 lateral cephalometric radiographs both at initial and final stages of evaluation, and finally three at- home sleep-breathing monitoring results for the activator group and one for the control group.

Results
After radiographic evaluation, it was found that children in the activator group experienced an increase in all measured variables. After evaluation with the sleep monitor, an improvement of sleep-breathing was found in children from the activator group ($p<0.05$).

Conclusion
The activator not only provides a harmonious occlusion and proper development of the mandible, but it also helps improve the quality of sleep–breathing through widening of the upper airway and reducing the number of disordered breathing events in children that undergo this therapy.

Keywords: *Orthodontic activator, sleep-breathing, upper airway, retrognathic mandible, at-home sleep monitoring*

Introduction

Humans are born conditioned to eat by the mouth and breathe through the nostrils, an imbalance of this physiological pattern affects growth and development, not only on facial harmony but also in general health terms; when the child's breathing patterns are abnormal, they are then considered a multifaceted clinical entity, which produces alterations than can affect their physical and mental development (1,2). Many authors agree that obesity is a major risk factor for disordered sleep breathing which includes Obstructive Sleep Apnea (OSA) in children and adults, however, obesity levels are comparatively low in Japanese society, meaning that obesity may not be a leading cause of OSA in Japanese children and adult patients (3-7). Previous studies agree that an abnormal maxilla–mandible relationship correlates better than obesity as an OSA predictor, especially in oriental populations (8,9). According to a study in 2017, some

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Received: 9 October 2020
Revised: 20 January 2021
Accepted: 15 March 2021

DOI: 10.26650/eor.2022807392

How to cite: *Medina CC, Ueda H, Iwai K, Kunimatsu R, Tanimoto K. Changes in airway patency and sleep-breathing in healthy skeletal Class II children undergoing functional activator therapy. Eur Oral Res 2022; 56(1): 1-9.*

characteristics that are predominantly associated with OSA include retropositioning of the mandible, a smaller cranial base, an increase in the cranio-cervical angle as well as abnormal upper airway soft tissue morphology (10). Separate studies concur that the occlusion of the airway was highly associated to the anatomical structure of the upper airway and the mandible (e.g. hypertrophy of the pharyngeal and palatine tonsils) as one of the major causes of OSA or sleep disordered breathing in children (11-13).

One of the craniofacial deformities most frequently associated with OSA is maxillomandibular anteroposterior and vertical disproportion which is a result of poor mandibular growth, that can be ameliorated with simple orthopedic appliances during a relatively brief time; in children one of the treatment options include advancing the mandible forward by fixed or removable orthodontic functional appliances (10).

Hence, maxillomandibular correction represents an important and effective treatment to snoring and a preventive measure for OSA during childhood, as previously demonstrated in children of 4 to 10 years of age treated with removable oral appliances for 6 months (14,15). Therefore, we assessed the effects of one of those previously mentioned appliances, the functional activator, on healthy children, to determine if there actually is an improvement in said breathing patterns. By advancing the mandible forward during orthopedic therapy with the activator there is the possibility for the upper airway to widen, thus favoring a better sleep–breathing pattern for children undergoing this therapy (16,17). Therefore, the aim of this study is to confirm the hypothesis that besides the intended inducement of development of the mandible the activator may also help improving healthy sleep breathing patterns in skeletal Class II children even when there is an absence of sleep disordered breathing by increasing their upper airway' dimensions. To test this hypothesis, several evaluations, including radiographical assessment of the upper airway, sleep-breathing monitoring and questionnaires aimed both to the evaluated children and their parents were implemented.

Materials and Methods

Ethical statement

This research has been approved by the Ethics Review Committee of Hiroshima University (No. E – 56). Both groups needed to have provided informed consent from the parent or guardian prior all evaluations. This study has followed the guidelines stated in the Helsinki Declaration for clinical investigations.

Study sample

Subjects in this study consisted of 39 children, 20 for activator group (10 boys and 10 girls, mean age 10.9±0.9; BMI 16.2±1.4), 19 for control group (13 boys and 6 girls, mean age 9.8±1.4; BMI 17.6±2.1). This sample size was decided from the subjects that were indicated to wear an activator to treat a Class II skeletal discrepancy.

Anatomical features

The anatomical references that divide the skeletal pattern into Class I and II are SNA angle (anteroposterior position of the maxilla), SNB angle (anteroposterior position of the mandible) and ANB angle (relationship between the maxilla and the mandible), The skeletal pattern of the subjects in the control group at the beginning of the evaluation period was, SNA 81.3±0.3, SNB 76.4±2.3 and ANB 4.8±1.2, and for the subjects of the activator group were SNA 80.7±2.5, SNB 74.6±0.9 and ANB 6.8±1.7, from the average assessed values all our participants were divided accordingly with characteristics that the literature consensus agreed are skeletal Class I for the control group and Class II for the activator group respectively.

Inclusion and exclusion criteria

For the activator group, the specific inclusion/exclusion requirements included being skeletal Class II patients currently undergoing Andresen orthodontic activator and to have successfully cleared all screening tests. For the control group the specific inclusion/exclusion requirements, irrespectively of the orthodontic appliance used, that fulfill the following criteria: healthy skeletal Class I children who have successfully cleared all screening tests. Inclusion criteria for both groups included no previous history of sleep–related child breathing disorders, general good health, and agreement to be evaluated for the duration of this study, exclusion criteria included previous adenoidectomy, allergic rhinitis, muscular disorders, maxillofacial clefts or any systemic diseases.

Dental appliance

All patients in the activator group were treated using the acrylic–splint Andresen functional activator appliance to the point of mandibular advancement. It consisted of two anterior labial bows, one for the upper dentition and another for the lower dentition, both being straight 0.9 mm wires that embrace the labial surface of the anterior dentition from each left and right lateral tooth with two loops that go upwards to the buccal corridor from half point the labial surface of both left and right canines and end in the palatal surface immediately behind the canines. The average treatment time was 18.3±3.2 months. The mandible was initially advanced 6.0±1.7 mm on average and was opened by 4.0 + 1.0 mm vertically. Subsequent stepwise anterior activations were needed depending on the case.

For the control group, the orthodontic appliance used for their respective cases was irrelevant for this study, unless it impeded the use of a portable sleep monitor to evaluate their sleep-breathing patterns.

Questionnaires

As a method for screening if the child or the parents have noticed any abnormal sleep–related behavior, two different questionnaires were given to the patients and to the parents, respectively. First, an Epworth Daytime Sleepiness Scale (ESS) questionnaire was distributed among the children (Figure 1). This is a self–administered questionnaire that provides a scaled measure of a person's, in this case children, general level of daytime sleepiness. Because we are dealing with underage subjects, questions related to the consumption of alcohol included in the original questionnaire have been modified

(18). The second questionnaire used was the Sleep Related Breathing Disorder Subscale (SRBD) (Figure 1). In this case, this questionnaire is answered by the parents with information concerning what they have witnessed of their child's usual sleep behavior (19). Both surveys were given to the subjects and their parents or guardians at the initial stage and towards the end of active treatment to both study groups.

Cephalometric analysis

To confirm in a physical tangible way that the changes measured in this study were indeed happening, three lateral cephalometric radiographs were required from the patients. Lateral cephalometric radiographs were taken in the upright standard natural head position at two time points (T0', initial record, and T1' towards the end of active orthodontic treatment) for both groups; tracing was constructed on each lateral cephalography before performing the analysis. All radiographs were traced again after a month to check for inconsistencies. All cephalography radiographs were taken by an experienced technician in and manually traced by the first author, with the anatomical landmarks presented in Figure 2.

Upper airway linear width

The anteroposterior size of the upper airway has always been a topic of scrutiny when speaking about the effects of certain oral appliances, in this case we divided our analysis into linear and volumetric area of the upper airway. The anteroposterior lines of reference used to evaluate the width of the upper airway were (20): SPAS: The thickness of the airway behind the soft palate along a line parallel to Go–B. MAS: The thickness of the airway along a line parallel to Go–B through P. IAS: The thickness of the airway along a line extended through Go–B. (Figure 3t0') To measure the total volume or area of the remaining sections of the upper airway, the oropharynx (the part of the pharynx that lies between the soft palate and the hyoid bone) and the hypopharynx (part of the pharynx extending from the hyoid bone to the lower margin of the cricoid cartilage) (Figure 3)(20).

At-home monitoring of sleep-breathing

As a method of assessing the changes of breathing patterns during functional orthopedic therapy, the patients were asked to use a type 3 portable sleep monitor (BRIZZY NomicsÒ, Liege, Belgium) (Figure 4) three times, once without using the activator to check normal breathing parameters (T0); a second time with the child wearing the activator to confirm whether sleep–breathing improves (T1), data collection for this stage with the activator inserted was done once use of the oral appliance had been stable and continuous. A third time was asked for the subject patients in the activator group when activator therapy was finished or almost finished (T2). For control group, only one time was required to compare. This portable monitor (PM) works with midsagittal sensors which are positioned one in the chin and the other on the forehead, these must be positioned on the same axis and parallel to each other; these sensors measure the jaw movement by electromagnetism which is released in very low energy magnetic pulses and in short duration. By measuring the movements and behavior of the lower jaw during sleep, this monitor can determine which kind of respiratory event the patient is having.

From using this device many indicators of sleep disturbance can be measured, such as, Respiratory Disturbance Index (RDI), or apnea hypopnea index, is the number of obstructive, central, and mixed events per hour of sleep, Arousal Index, (ARL) or number of arousals or discontinuity per hour of sleep. This monitor also provides valuable information about a patient's obstructive, central and mixed respiratory events.

Statistical analysis

All statistical analyses were performed using MedCalc Statistical Software version 17.8.6 (MedCalc Software bvba,

Epworth Sleepiness Scale—Children

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently, think about how they would have affected you. Use the following scale to choose the most appropriate number for each situation.

0=would never doze or sleep
1=slight chance of dozing or sleeping
2=moderate chance of dozing or sleeping
3=high chance of dozing or sleeping.

Circle the most appropriate number for each situation:

1. Sitting and reading	0	1	2	3
2. Watching television	0	1	2	3
3. Sitting inactive in a public place (for example, a movie theater or classroom)	0	1	2	3
4. As a passenger in a car for an hour without a break	0	1	2	3
5. Lying down to rest in the afternoon when circumstances Permit	0	1	2	3
6. Sitting and talking to someone	0	1	2	3
7. Sitting quietly after lunch	0	1	2	3
8. Doing homework or taking a test	0	1	2	3

4. The SRBD questionnaire.

Child's Name: _____ Study ID #: _____
Person completing form: _____ Date: ____/____/____

Please answer these questions regarding the behavior of your child during sleep and wakefulness. The questions apply to how your child acts in general during the past month, not necessarily during the past few days since these may not have been typical if your child has not been well. You should circle the correct response or *print* your answers neatly in the space provided. A "Y" means "yes," "N" means "no," and "DK" means "don't know."

1. WHILE SLEEPING, DOES YOUR CHILD:				
Snore more than half the time?	Y	N	DK	A2
Always snore?	Y	N	DK	A3
Snore loudly?	Y	N	DK	A4
Have "heavy" or loud breathing?	Y	N	DK	A5
Have trouble breathing, or struggle to breathe?	Y	N	DK	A6
2. HAVE YOU EVER SEEN YOUR CHILD STOP BREATHING DURING THE NIGHT?	Y	N	DK	A7
3. DOES YOUR CHILD:				
Tend to breathe through the mouth during the day?	Y	N	DK	A24
Have a dry mouth on waking up in the morning?	Y	N	DK	A25
Occasionally wet the bed?	Y	N	DK	A32
4. DOES YOUR CHILD:				
Wake up feeling unrefreshed in the morning?	Y	N	DK	B1
Have a problem with sleepiness during the day?	Y	N	DK	B2
5. HAS A TEACHER OR OTHER SUPERVISOR COMMENTED THAT YOUR CHILD APPEARS SLEEPY DURING THE DAY?	Y	N	DK	B4
6. IS IT HARD TO WAKE YOUR CHILD UP IN THE MORNING?	Y	N	DK	B6
7. DOES YOUR CHILD WAKE UP WITH HEADACHES IN THE MORNING?	Y	N	DK	B7
8. DID YOUR CHILD STOP GROWING AT A NORMAL RATE AT ANY TIME SINCE BIRTH?	Y	N	DK	B9
9. IS YOUR CHILD OVERWEIGHT?	Y	N	DK	B22
0. THIS CHILD **OFTEN**:				
Does not seem to listen when spoken to directly.	Y	N	DK	C5
Has difficulty organizing tasks and activities.	Y	N	DK	C6
Is easily distracted by extraneous stimuli.	Y	N	DK	C8
Fidgets with hands or feet or squirms in seat.	Y	N	DK	C10
Is "on the go" or often acts as if "driven by a motor".	Y	N	DK	C14
Interrupts or intrudes on others (e.g., butts into conversations or games).	Y	N	DK	C18

Thank you!

Figure 1. *LEFT – The ESS questionnaire modified for children. RIGHT – The SRBD scale questionnaire.*

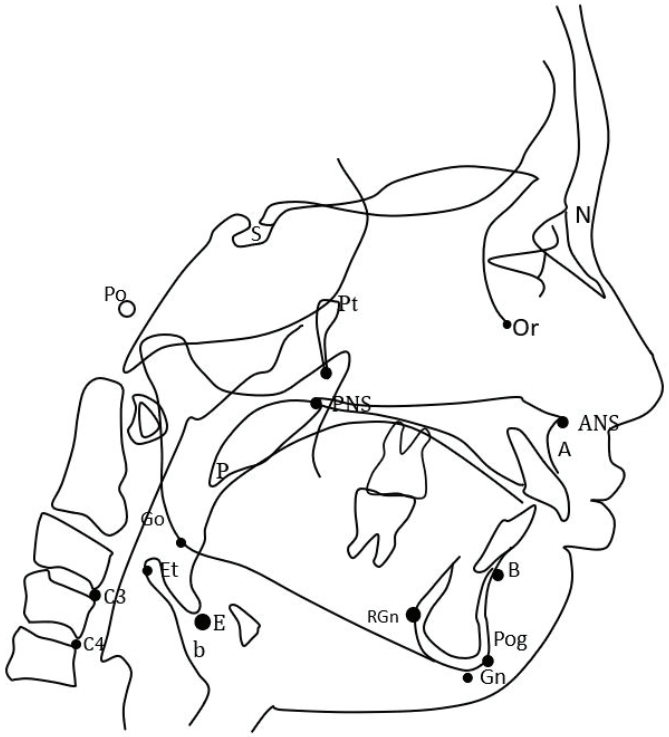

Figure 2. *Anatomical points of reference used for the cephalometric analysis in this study. They include: S: Center of Sella. N: Most anterior point of the frontonasal suture. Or: Lowest point on the average left and right inferior borders of the bony orbit. Po: Highest point on the superior surface of soft tissue of the external auditory meatus. Pt: Pterygoid point. ANS: Apex of the anterior nasal spine. PNS: Intersection between the nasal floor and the posterior contour of the maxilla. A: Most posterior point on the anterior contour of the upper alveolar process. B: Most posterior point on the anterior contour of the lower alveolar process. P: Lowest point of the soft palate. Pog: Most anterior point of the contour of the chin. Gn: Point on the chin determined by bisecting the angle formed by the facial and mandibular planes. RGn: Retrognathion, the most posterior point of the mandibular symphysis along the FH plane. Go: Most posterior–inferior point on the convexity of the angle of the mandible. Eb: Most anterior–inferior point of the epiglottic fold. Et: Tip of the epiglottis, the most superior point of the epiglottis. C3: third cervical vertebra. C4: fourth cervical vertebra.*

Ostend, Belgium; http://www.medcalc.org; 2019) and/or Microsoft Excel–based software unless otherwise stated. Data are presented as mean ± standard deviation (SD); ANOVA tests were used to compare the differences between the baseline and follow–up cephalometric and for the at home sleep monitoring values for each variable. Unpaired t–test was used to determine significance of the changes in the skeletal pattern of the children, as well as the upper airway size for both groups. A *P* value of < 0.05 was considered to indicate statistical significance.

Results

Questionnaires

The mean score of ESS administered to both groups are as follows; for the control group an average score of 4.7±1.5

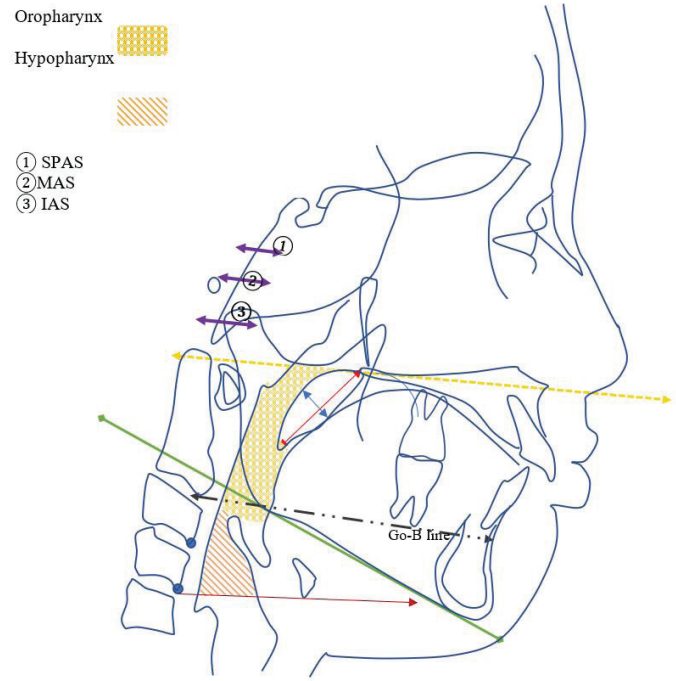

Figure 3. *The areas delimitating the anteroposterior width of the upper airways and the area of the oropharynx and the hypopharynx.*

Figure 4. *LEFT – The body of the PM used in this study. RIGHT – The facial sensors attached to the appliance.*

was obtained, whereas for the activator group an average score of 4.8±3.6 at the initial stage and of 4.5±3.0 towards the end of active treatment. Because all results are below 8 marks at all assessed points, we can say that these scores are within the normal range. The results yielded from the SRBD subscale were of 0.15±0.1 for the control group and of 0.22±0.2 for the activator group at the initial stage of treatment and of 0.14±0.1 towards the end of active treatment, revealing a slight betterment in the sleep breathing patterns of the children as perceived by their parents.

Cephalometric analysis

After tracing, digitizing, and determining the linear measurements to be assessed, it can be seen on the different tests that the airway space is generally wider in the test group than in the control group. The mean values for the measurement of the linear and volumetric size of the upper airway when comparing the activator (1.00±0.30) and control group (0.87±0.17) at T0', show that at baseline both groups have a very similar width and area size of the upper airway (Table 1).

The anteroposterior width of the airway is shown to have an increasing trend over time. A significant increment in the linear width of SPAS measurement can be observed at T1′ of the activator group (1.13±0.29). After one–year measurements potentially show that these changes are kept with continuous use of the activator. The volumetric area of the upper airway is shown to widen when the activator is in mouth, with the oropharyngeal space showing a significant increase when comparing T1′ of the control group (3.65±0.87) and T1′ of the activator group (4.37±0.97). Overall, the upper airway sees an increasing trend over time, and even more so at T1′ than at the starting point in T0′ (Table 1).

At–home monitoring of sleep breathing

All indicators of severity decrease significantly when the children wear the activator to sleep, which means the sleep quality is improved when the activator is in mouth during sleep time. RDI and ARL values show a statistical difference of P<0.05 when evaluating the statistical significance. All indicators of severity decrease significantly especially in T1, when the activator is inserted, RDI keeps the same decreasing trend after removal of the activator, in T2. Even though the number of arousals (ARL) is a little higher in T2 than in T1, it has the same levels as control group (Table 2). Because the subjects for this study were all generally healthy children, there is some sleep–breathing interruption to be expected. Respiratory events quantify how many times these interruptions happen and if they are less when wearing the activator as seen on Table 3, the total number of respiratory as well as obstructive events significantly decrease from T0 to T1 and T0 to T2 in the activator group.

***Table 1**. Mean values of the linear and volumetric area measurements of the upper airways. * p <0.05.*

Cephalometric variable	Control group (T0′)	Control Group (T1′)	Activator group (T0′)	Activator group (T1′)
SPAS (cm)	0.87±0.17	1.08±0.20	1.00±0.30*	1.13±0.29
		*		
MAS (cm)	1.20±0.29	1.22±0.18	1.18±0.36	1.26±0.29
IAS (cm)	1.00±0.33	1.02±0.35	0.92±0.31	1.01±0.22
Oropharynx (cm²)	3.61±0.82	3.65±0.87	3.89±1.05	4.37±0.97
		*		
Hypopharynx (cm²)	1.52±0.81	1.66±0.89	1.32±0.75*	1.65±0.72

***Table 2.** Changes in the values of the indicators of sleep breathing severity depending of the treatment stage for the Activator group comparing them to the values from the Control group. *p<0.05, **p<0.01*

	Control	Activator group (T0)	Activator group (T1)	Activator group (T2)
RDI (n/h)	1.03±1.37	2.25±3.6	0.84±1.1	1.93±2.3
		*		
ARL (n/h)	9.33±1.96	11.15±3.4	7.94±2.5	9.56±3.5
		*		
Total Respiratory events (n/h)	6.5±8.3	20.45±12.8	4.65±7.8	13±9.4
	*		**	
			**	
Obstructive events (n/h)	6.5±8.3	20.35±12.8	4.55±7.8	13±9.4
	*		**	
			**	
Central events (n/h)	0	0.05±0.22	0.05±0.22	0
Mixed events (n/h)	0	0.05±0.22	0.05±0.22	0

***Table 3.** Summary of the initial skeletal relationship of the subjects in this study. *P<0.05*

	SNA angle		SNB angle		ANB angle	
	Initial (T0′)	Final (T1′)	Initial (T0′)	Final (T1′)	Initial (T0′)	Final (T1′)
Control Group	81.3±0.3	**81.1±2.7**	76.4±2.3	**76.3±2.2**	4.8±1.2	**4.3±1.5**
Activator Group	80.7±2.5	**81.4±2.8**	74.6±0.9	**77.2±1.6**	6.8±1.7	**4.2±2.2**
			*		*	

Final skeletal pattern relationship

After a year on average of continuous functional therapy with the activator, the skeletal relationship of the activator group changed positively to a more harmonious craniofacial relationship (Table 3).

Discussion

The aim of this study was to determine if wearing a functional orthodontic activator, intended for improving a retrognathic mandible, would also help improve the child's sleep-breathing by widening of the patient's upper airway. The findings in this study demonstrate that after continuous functional therapy with the activator, not only does the upper airway widens, the changes are kept even at the end of active treatment (T2 results in the sleep monitor, T1′ of the cephalometric results). This is evident when assessing the radiographic findings which are in turn corroborated by the results obtained from the PM.

The results obtained from the questionnaires used showed that according to the ESS questionnaire, the children do not self–report levels of daytime sleepiness outside what would be considered normal for school age children. The average score obtained on both stages for the evaluated skeletal Class II children demonstrate that daytime sleepiness is not a concerning issue for the children evaluated in this study.

The final results obtained from the PSQ in both stages respectively for the activator group, shows that the parents' perceived sleep-breathing patterns of the children, improve over time and by the end of active treatment, the overall score improves after wearing the activator.

The consensus around the dental community is that skeletal Class II is a complex condition that may be corrected using different alternatives of treatment such as fixed, Andresen, Twin Block, Herbst, Bio bloc or headgear appliances (21-23). Concerning the present study, it was decided to evaluate the influence that the Andresen activator has not only physically on the upper airway, but also how these changes relate to sleep–breathing patterns as assessed with a PM.

The present study reveals that the anteroposterior width of the upper airway increases in size steadily over the course of the evaluation time, especially the superior airway space or SPAS. This may be due to the fact that the children assessed did not suffer from any condition that would constrict the upper airway and the linear size of the upper airway from the activator group was comparable to that of the control group.

In a study using the Faramand appliance, it was found that mandibular advancement has the potential to increase the dimensions of the upper airway during treatment and this increase in dimension remains stable over a long period (4 years + 2–8 years), which strongly agree with the results from this study, when analyzing the anteroposterior size of the upper airway it was found that not only are the changes kept in T1′, but there is also an increase in size of the evaluated measurements (24).

Regarding the dimension of the upper airway, especially on the areas delimited by the oropharynx and hypopharynx, there was a slight increase in the volume of both sites, especially the oropharynx experiences a larger increase than the hypopharynx which remains stable after a year of functional therapy at T1′. This may be explained by the fact that the activator when inserted the base of the tongue which is located in front of the anterior wall of the soft palate experiences an anterior displacement of the tongue induced by the activator which in turn reduces the tongue's gravitational effect on the soft palate, thus enlarging the oropharynx's measurements (25).

Changes of the dimension of the hypopharynx shown in the present study reveal a minor increase of this area of the upper airway that is kept stable after a year of continuous activator therapy at T1′, in spite of this no statistical significance could be found. The fact that following treatment with the activator, anterior displacement of the base of the tongue, by means of anterior repositioning of the mandible, can explain the increase in the hypopharyngeal dimension and its stability at T1′. This is in accordance with a study by Isono et al (26), which stated that there is an increase in the dimensions of the airway which result from mandibular anterior displacement even in obese individuals without failure.

Another study performed by Horihata et al, reported an increase in the anteroposterior width and total dimension of the upper airway after activator therapy when comparing to initial data, which is in accordance to the results from this study (27).

In the studies by Tsuiki et al, and Poon et al, when an oral appliance treatment was used in adult patients to treat mild OSA, they found an increase in the area behind the soft palate on the oropharynx, this conclusion agrees with this study which sees a similar result when assessing the same area (28,29).

There is a large amount of studies done to assess the changes of the upper airway post–functional therapy, despite this, the results are conflicting in that many of these studies research said changes evaluating the effects of a variety of functional appliances, and depending on the appliance being studied and the methodology the results also vary (27).

Shepard and Thawley proposed that most problems associated with respiration are present in the oropharynx, improvement of the size of this section with the use of the activator increases the importance of this functional appliance (30). However, studies done evaluating the upper airway changes following functional appliances agree that if the main mechanic is to move the lower jaw forward to achieve facial balance and correct occlusal posture, there is an added benefit when continuous use of the appliance is achieved, which is an increased size of the upper airway.

Overall, the activator produced positive changes in the skeletal pattern of children demonstrated a significant decrease in the difference of the various craniofacial angles evaluated in skeletal Class II children.

The SNA angle increased an average of ±1.2 degrees from T0 to T1 in the activator group, this result contrast with the ones by Mills, where he found a reduction of said angle (31). The SNB and ANB angles changed significantly, with an increase of the former and a decrease of the latter. Normal craniofacial growth and development, greatly assisted by the effects of the activator, propels the changes earlier demonstrated by the widening the upper airway. This shows that with proper compliance in the use of functional appliances, the changes from skeletal Class II to Class I are achieved, this is in accordance to the study by Santamaría–Villegas et al, but contrasting to the study by Koretsi et al, which affirms

that functional appliance mainly influence dento–alveolar changes rather than skeletal ones (32,33).

Concerning the sleep test done for this study, it must be noted that type 1 polysomnography (PSG) tests remain the gold standard for the diagnosis in patients suspected of having comorbid sleep disorders, unstable medical conditions, or complex sleep–disordered breathing. Type 3 PMs used in sleep studies are safe and convenient for diagnosing OSA in patients with a high pretest probability of moderate to severe forms of the condition without substantial comorbidities (34).

The results that the PM used in this study yielded, reveal that almost all indicators of respiratory severity decrease significantly when the activator is inserted, and the decrease is significantly stable at T2 after functional therapy. The indicator for RDI shows a positive decrease from T0 at the beginning of treatment with activator and a significant improvement at T1 when the appliance is inserted during sleep time. Even though no significance was found for the results obtained for T2, there is a positive trend of a decreased RDI when the activator is removed.

The number of arousals as determined by the ARL variable also show a significant reduction from T0 to T1 in the activator group. This is explained with the fact that as the activator is inserted throughout the night, the physical changes brought upon with the increased size of the upper airway, the children experience a more refreshed and sounder sleep.

A study done with the Herbst appliance and maxillary expansion by Schüts et al, showed that when the nasopharyngeal complex is enhanced by functional therapy, PSG results show an improvement in the sleep breathing patterns of the involved subjects (15). This agrees with the present research that a wider upper airway relates to better sleeping patterns. Even though the most observed respiratory event was the obstructive one, the number of obstructive events perceived by the PM was shown to experience a significant lowering from T0 to T1 when the activator is inserted to sleep, and even though there is a slightly higher number of obstructive events from T1 to T2, the number is significantly ($P<0.01$) lower than at T0, which is considered as a positive improvement in the sleep patterns of skeletal Class II children; due to the fact that the data collected from both T0 and T2 points are with no appliances inserted to sleep, the conditions are considered similar.

Bearing in mind that this research study was done using data from healthy children, there is a concern if the results presented thus far could also be applied to cases of childhood OSA, regarding this there is a clinical case of one subject who was supposed to be part of the activator cohort for this study, however, due to severe signs of childhood OSA exhibited by said case, it was deemed inappropriate to include in the present study. However, after continuous activator therapy with periodical checkups and follow–up appointments, the PM showed a considerable decrease in all indicators of severity, as well as less respiratory events, especially when the activator is inserted (35, 36). All of this can be translated into saying that functional appliances do in fact offer a positive impact in skeletal Class II children, that besides providing an improved facial pattern, they also benefit from a better sleep thanks to the physical changes brought upon by functional appliance treatment. Thus, the hypothesis for this study has been accepted.

Future studies validating the results from this kind of PM should be done with a larger and more varied cohort of subjects that may include children and adults, with sleep–breathing conditions ranging from healthy to OSA.

A limitation of this study as mentioned previously include the relatively small number of participants. In the future, a bigger cohort of subjects for both Control and activator group could be evaluated, including a longer evaluation period which might include the same number of data collection points for both groups, as well as an evaluation of the stability of changes brought upon by the activator in a more extended period.

Conclusion

It can be concluded that the activator not only provides a harmonious occlusion and proper development of the mandible, but it also helps improve the quality of sleep–breathing through widening of the upper airway and reducing the number of disordered breathing events in children that undergo this kind of orthopedic therapy. It could be said that the activator might be useful for preventing and/or diminishing the future risk of OSA of the children that receive orthodontic functional treatment with this appliance, so that when they become adults, they continue to experience improved sleep–breathing thanks to this activator, however, this is subject for a future study where this assertion could be confirmed or not.

Türkçe Özet: *Aktivatör tedavisi gören sağlıklı iskeletsel sınıf ıı çocuklarda hava yolu açıklığı ve uyku süresince solunumda oluşan değişiklikler. Amaç: Bazı çalışmalar, Obstrüktif Uyku Apnesi'nin (OSA) öngörülmesinde maksillo-mandibüler ilişkinin obesiteden daha yüksek korelasyon gösterdiği konusunda hemfikirdirler. Bu tip bir kraniyo-fasiyal deformitenin tedavisinde önerilen ortodontik tedavilerden biri aktivatör ile alt çenenin öne alınması olduğundan; bu çalışmanın amacı bu apareyi kullanan sağlıklı çocuklarda üst havayolunda genişleme ile birlikte uyku sırasındaki solunum şeklinde bir iyileşmenin gerçekleşip gerçekleşmediğinin incelenmesidir. Gereç ve Yöntem: 30 sağlıklı çocuk, 20 çocuk aktivatör grubu (10 erkek ve 10 kız; ortalama yaş 10.9 ± 0.9 yıl; BMI:16.2 ± 1.4); 19 çocuk kontrol grubu (13 erkek and 6 kız, ortalama yaş 9.8 ± 1.4 yıl; BMI 17.6 ± 2.1) olarak çalışmada yer almıştır. Aktivatör grubunda araştırmanın başında ve sonunda, kontrol grubunda ise bir kez alınan lateral sefalometrik filmler ve uyku sırasındaki solunum ölçümleri araştırma materyalini oluşturmuştur. Bulgular: Radyolojik inceleme sonrasında, aktivatör grubundaki çocuklarda incelenen tüm parametrelerde artış olduğu bulunmuştur. Uyku izleme sonrasında aktivatör grubundaki çocuklarda uyku sırasında solunum patternlerinde iyileşme olduğu bulunmuştur ($p<0.05$). Sonuç: Aktivatör apareyi ile tedavi gören çocuklarda tedavi sadece uyumlu bir oklüzyon ve alt çenenin uygun gelişimini sağlamakla kalmayıp, üst havayolunun genişlemesini sağlayarak uykudaki solunum kalitesinin artışını ve bazı solunum düzensizliklerinin azalmasını sağlamıştır. Anahtar Kelimeler: aktivatör, uykudaki solunum. üst havayolu, retrognatik alt çene, evde uyku izleme*

Ethics Committee Approval: : This research has been approved by the Ethics Review Committee of Hiroshima University (No. E – 56).

Informed Consent: : All subjects needed to have provided informed consent from the parent or guardian prior all evaluations. This study has followed the guidelines stated in the Helsinki Declaration for clinical investigations.

Peer-review: Externally peer-reviewed.

Author contributions: CCM, HU participated in designing the study. CCM participated in generating the data for the study. CCM, KI, RK participated in gathering the data for the study. CCM and KT participated in the analysis of the data. CCM wrote the majority of the original draft of the paper. CCM participated in writing the paper. CCM have had access to all of the raw data of the study. CCM have reviewed the pertinent raw data on which the results and conclusions of this study are based. CCM, HU, KI, RK, KT have approved the final version of this paper. HU guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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

Financial Disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2022; 56(1): 10-16

Original research

The effect of composite placement technique on the internal adaptation, gap formation and microshear bond strength

Cem Peskersoy[1], Duygu Recen[2], Hande Kemaloğlu[1]

Purpose
This study aimed to compare the efficiency of placement technique on internal adaptation, gap formation and microshear bond strength (≒SBS) of bulk-fill composite resin materials.

Materials and Methods
Standardized class V cavities were prepared for microcomputed tomography (mCT) test and divided into four groups (n=12) as follows: Group SDR: Smart Dentin Replacement system/bulk fill; Group SF2: Sonic-Fill system/bulk fill sonic-activated composite placement system; Group CHU: Herculite-XRV-Ultra composite resin inserted with Compothixo/sonic-vibrated composite resin placement system; Group HIT: Herculite-XRV-Ultra composite resin applied with incremental technique. Self-etch adhesive (Optibond-XTR) was used for bonding in all groups. After 10000 thermocycling, mCT scans were taken to reveal gap formation at the tooth-restoration interface and universal testing machine was used to test microshear bond strength (≒SBS) values (n=10). ANOVA, post-hoc Bonferroni and Tukey HSD tests were used for evaluating the gap formation and ≒SBS values (p=0.05).

Results
SF2 and CHU showed the best adaptability compared with both SDR and HIT. The difference between groups SDR and HIT was statistically significant ($p<0.05$). ≒SBS values were found to be the highest for SF2, and the lowest for HIT groups ($p>0.05$).

Conclusion
Bulk-fill composite resins placed either with sonic-activated or sonic-vibrated instrument demonstrated better adaptability, less gap formation and higher bond strength than both the bulk-fill flowable composite and conventional incremental techniques.

Keywords: *Bulk-fill composite, microcomputed tomography, gap formation, bond strength, sonic instrumentation*

Introduction

The use of resin-based composite (RBC) materials has been increasing enormously for nearly a decade. Due to the esthetic appearance and mechanically adequate properties, RBCs are the choice of materials. On the other hand, the performance of RBCs is influenced by several factors, including the design and size of the restoration, placement technique and material itself (1). Although most RBCs meet basic expectations of dentists in terms of adhesion, sealing, wear resistance, fracture toughness and biocompatibility, the main deficiencies of RBCs, such as polymerization shrinkage and internal stress, still exist (2,3). Polymerization shrinkage leads to voids, microleakage and debonding of RBCs, generates internal stresses due to contraction, and results in a series of challenges starting with voids, leading to gaps, contamination of composite layers, bond failures between increments and tooth-restoration surfaces (4). Moreover,

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Received: 17 March 2021
Revised: 10 April 2021
Accepted: 17 May 2021

DOI: 10.26650/eor.2022897456

How to cite: *Peskersoy C, Recen D, Kemaloglu H. The effect of composite placement technique on the internal adaptation, gap formation and microshear bond strength. Eur Oral Res 2022; 56(1):10-16.*

the presence of voids within the final restoration may cause drawbacks like discoloration due to marginal leakage (5,6). Furthermore, increased wear and microfractures can occur depending on the stress concentration around the voids (7-9). Ultimately, a decrease in bond strength leads to secondary caries and postoperative sensitivity, leading to failure in the final restoration (10).

Various attempts have been made to solve these problems, including increasing the percentage of the filler content in the composite matrix and reducing the size of filler particles (11,12). Several studies have shown that heavier filler loading or decreasing the viscosity of the material would result in an increased bond strength of the material, while others concluded that the percentage of the filler content plays a minor role in adhesion (13-16). However, most studies agreed on the effect of placement techniques, either incremental or bulk-fill, playing a major role in adaptation and polymerization (7,17,18). These techniques are faciolingual layering (vertical), gingiva-occlusal layering (horizontal), the three-site technique, wedge-shaped layering (oblique), the successive cusp build-up technique, the bulk technique, and centripetal build-up (19).

To reduce clinical steps by filling the cavity with a single increment, bulk-fill composite resins and sonic-activated/vibrated placement techniques were recently introduced (12,20). Bulk-fill composite resins can be used as a posterior restorative material, an underlining or a base material under suitable RBCs. These materials can be placed up to 4-mm bulk due to their high viscosity and reactivity to light curing, minimizing the voids, reducing porosity and improving the quality of the final restoration (6,21).

Several techniques have been used to evaluate the gap formation and microleakage at the composite-dentin interface, but most of the results vary due to semiquantitative scoring according to the degree of dye penetration, subjective determination of the visual documents or theoretical characterization of each composite that could not simulate the real shrinkage behavior of in vivo conditions (22,23). Additionally, it is almost impossible to determine and measure the voids within the bulk of the material using conventional methods (17,24). Therefore, a nondestructive and quantitative assessment method, X-ray microcomputed tomography (mCT), was used in this study to quantify and measure the interfacial gaps and voids and also reconstruct the materials and the surrounding tissues three dimensionally (3D), as well as to distinguish the different components into a range of grayscale values, based on the ability to absorb the X-ray to evaluate properly (25).

The aims of this study were to: 1) compare the placement techniques' efficiency in internal adaptation and gap formation using mCT; 2) test the microshear bond strength (mSBS) of bulk-fill composite resin materials; 3) compare the effect of gaps and mSBS values and reveal whether any positive correlations exist. Although there are former studies focused on these factors, none evaluated the gaps in the RBCs and investigated the correlation between the rate of adaptation and mSBS of the bulk-fill and conventional RBCs.

The hypotheses of the study were as follows: 1) the shear bond strength will be higher in the sonic-activated/vibrated groups than the incremental placement technique groups, 2) the adaptability and subsequent gap formation will be a statistically different between the bulk-fill and incremental placement technique.

Materials and Methods

Ethical statement

Gap formation and shear bond strength were assessed in human molars that were extracted for periodontal reasons; The study protocol has been reviewed and approved by the Ege University Medical Research Ethics Committee (21-5T/66). 96 extracted caries-free human third molars (48 for mSBS & 48 for mCT test) were selected to create a pool of teeth for randomization. The teeth were stored in a 2% chloramine solution for a month.

Specimen preparation for the micro-ct test

Forty-eight double-sided standardized class V cavities were prepared using a cylindrical medium-grit diamond bur (835 314 010; Komet, Lemgo, Germany) mounted in a high-speed air turbine (650; KaVo, Biberach, Germany) under water cooling. Cavities were prepared in the approximal surfaces to achieve similar enamel and dentin thickness at occlusal and gingival margins of the cavity using these dimensions: width, 3 mm; length, 3 mm; depth, 4 mm. A two-step self-etch adhesive (Optibond-XTR, Kerr Co, CA, USA) was used for bonding in all the groups.The primer was scrubbed with a brushing motion for 20 s on dentine, air thinned for 5 s, subjected to adhesive application for 15 s and was light-cured for 10 s (Demi Ultra; Kerr Corp, Orange Co, CA, USA). The teeth were randomly divided into four groups (n=12): three experimental and one control. The placement techniques were explained below:

Group - I (SDR)

Smart Dentin Replacement (Dentsply, Konstanz, Germany), is a bulk-fill resin composite placed using the bulk-fill method.

Group - II (SF2)

SonicFill2 (Kerr, Sybron Endo, CA, USA), is a bulk-fill resin composite placed using the sonic-activated composite resin placement method. For this group, the SonicFill compule (compatible with the SonicFill Handpiece) was used.

Group - III (CHU)

Herculite Ultra XRV (Kerr, CA, USA) is a conventional resin composite inserted using Compothixo (a portable sonic vibration instrument).

Group - IV (HIT)

Herculite XRV Ultra (Kerr, CA, USA) is inserted using the Incremental Technique (Control group).

The materials used in the study, their composition, mode of application and manufacturer information are shown in Table 1. The specimens were subjected to a thermocycling

regimen of 10000 cycles, at 5-55 ± 8°C with a dwell time of 2 min using a thermocycling machine (Delta Tpo2, Nemo, Mashhad, Iran). High-resolution *m-CT* (mCT 40; SCANCO Medical, Switzerland) was used to evaluate the adaptation by measuring the overall gap formation percentage and void dimensions at the cavity floors (occlusal, pulpal, cervical & overall) as well as in the bulk. The imaging settings were as follows: dual-source at 70 kVp; resolution, 8 µm; nominal isotropic pixel area, 72 µm.

To minimize the changes in the specimen's position during repeated processes, each specimen was mounted on a special template. 2D sagittal images of each specimen were obtained from the mesial to distal surfaces of the tooth (Figure 1). Nine hundred sagittal cross-sectional images with an interval of 20 mm from the coronal part of the specimens were taken. One hundred slices corresponding to the restoration area (composite-tooth interface) for each specimen at an interval of 30 µm were chosen for the measurements.

To analyze the distribution of gaps at the composite-tooth interface at the occlusal margin (Figure 1a-d), gingival margin (Figure 1e-h) and voids within the bulk of the material surface-rendered 3D volumetric models (Figure 1i-l) of each specimen obtained. The 3D images were analyzed using image analysis software (ImageJ ver. 1.46; National Institutes of Health, Bethesda, MD, USA). The percentage, distribution, cluster form and volumetric quantity of the dark pixels (voids & gaps) as well as the light pixels (tested material and dental hard tissues) were calculated using image analysis software.

Specimen preparation for msbs test and analysis

Twelve teeth for each group were sectioned parallel to the occlusal surface from the upper middle coronal region (~ 1.0-mm thick) and perpendicular to the long axis of the root at one side of the specimen to reach both dentin surfaces, using a water-cooled diamond saw (Isomet, Buehler, IL, USA). The resulting dentin specimens were embedded with the coronal surface exposed in polyvinyl chloride tubes using epoxy resin, both occlusal and polished using wet 400-600-800 grit silicon carbide paper for 60 seconds to create standardized surfaces.

Twenty resin composite cylinders (1.5 mm. diameter & 6.0 mm. length) were constructed on the dentin surfaces (two for each teeth) targeting the center of the dentin while avoiding the pulpal horns and enamel residues. After applying the same adhesive system used in mCT tests, composite cyclinders were created with a bonding jig (Ultradent Inc., UT, USA) and cured for 20 s using the same light-emitting diode unit. One of the cyclinders on each specimen was constructed parallel to long axis of the tooth to measure the occlusal dentin shear bond strength, while the other cyclinder was constructed perpendicular to the long axis of the tooth to measure the axial-pulpal dentin shear bond strength. Two random specimens were excluded prior to the mSBS test due to the technical reasons during composite cylinder construction phase. After thermocycling procedure, cross-sectional radiographs and mCT images were taken from each sample prior to the mSBS test to ensure the adaptation was optimal with no gaps (Figure 2). mSBS was tested using the Universal Testing Machine (Instron Inc., MA, USA) at a crosshead speed of 1.0 mm/min and 50 kg of load cell using a 0.5-mm-wide chisel. mSBS values were calculated in MPa by dividing the peak load at failure to the specimen's surface area (10).

Statistical analysis

mSBS (Mpa), gap formation (%) and maximum void (mm^2) scores obtained from mCT scans were statistically analyzed using SPSS version 23.0 (IBM Corp, IL, USA). One-way analysis of variance and post hoc Bonferroni test were used for evaluating the gap formation scores and post hoc Tukey HSD test was used for comparing the mSBS values amongst the groups. Spearman rank correlation test was chosen to determine the correlation relationship between gap formation and mSBS results (p=0.05).

Results

The mean mSBS for each group is presented in Table 2. The highest mean mSBS value was observed in the SF2 group (17.62 Mpa). A statistically significant difference in mSBS was found between the test groups and control group (p=0.035). The results of the post hoc Tukey test showed that SDR and CHU demonstrated similar results (p=0.872). Therefore, first hypothesis related to shear bond strength is confirmed. Adhesive failures were observed in all samples in the SF2 and SDR groups, while 2 specimens in each CHU and HIT groups exhibited cohesive failures (p=0.677) (Figure-2g and 2h).

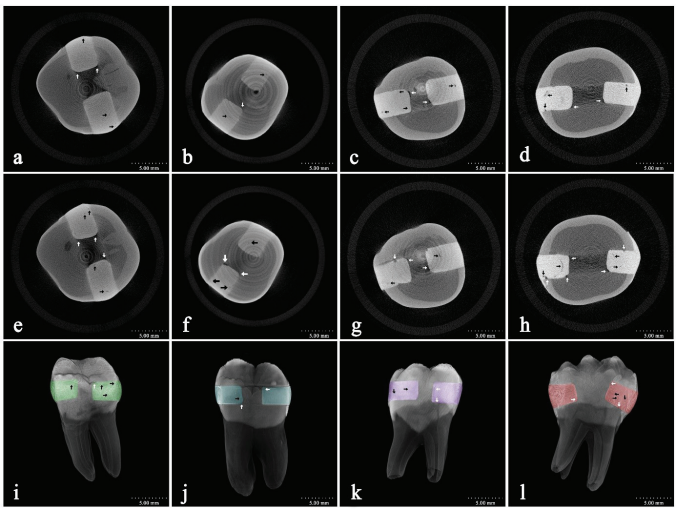

Figure 1. *2D micro-CT images and 3D volumetric display for all the groups showing maximum voids within the material and gap formations are as follows: (a,e,i) SDR, (b,f,j), SonicFill2.*

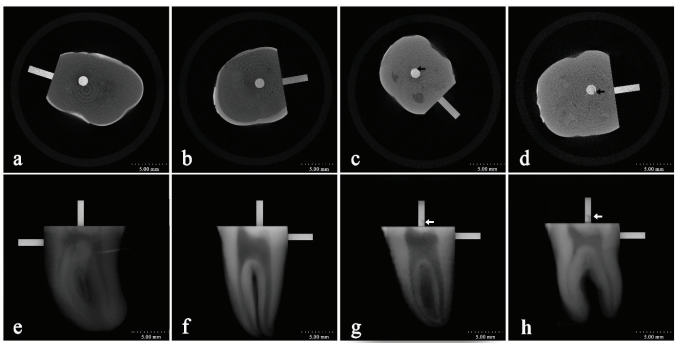

Figure 2. *Cross-sectional radiographs and mCT images prior to the bond strength test. (Arrows represent voids within the material).*

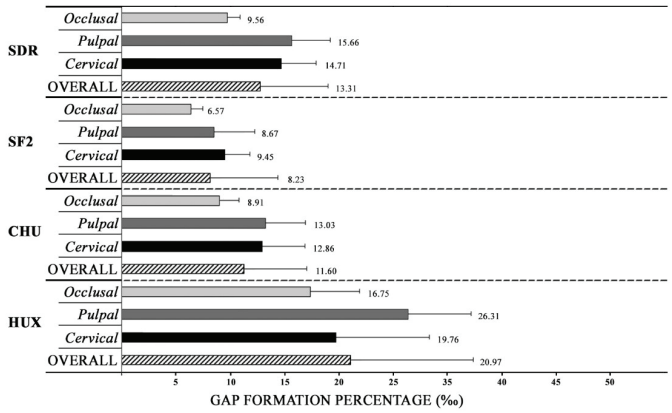

Figure 3. *Gap formation percentage (‰) observed in occlusal, cervical and pulpal walls.*

Total restoration volume for each group were similar (~35.75 mm^3), void and gap percentages (‰10^7) were be calculated for each group separately according to the bulk volume of the composites. Occlusal, cervical, pulpal and overall mean values (SD) of the maximum void dimensions ($mm^3 \times 10^{-8}$) and overall gap formation percentages observed under mCT for each group are presented in Table 3 and Figure 3. mCT images showed that voids within the bulk of the material were observed commonly close to the pulpal walls in all groups (Fig: 1), but the distribution of the voids in the bulk of the material between SDR, SF2, CHU groups was statistically insignificant (p=0.632). Additionally, most of the voids occurred close to the pulpal walls (p=0.044), and gap formation occurred at pulpal regions (p=0.039), with the exception of the SF2 group (p=0.231).

The largest void dimensions were observed in "the bulk" of the material for all of the groups, followed by the pulpal and cervical interfaces (p=0.003). The pulpal cavity walls exhibited the largest gap formations, but the differences were statistical-

Table 1. *Materials used in the study, composition and manufacturer information.*

Material Category	Brand Name	Manufacturer	Filler Concentration	Composition
Nanohybrid Composite	SonicFill2 ™	Kerr Co. USA Kavo, Germany	*81 wt%; 63 vol%*	2,2'-ethylenedioxydiethyl dimethacrylate, silicon dioxide, titanium dioxide, zinc-oxide, kaolin
Flowable Composite	Surefill SDR	Dentsply. Konstanz. Germany	*78 wt%; 45 vol%.*	Urethane di-methacrylate resin, di-methacrylate, di-functional diluents, stronsium & barium alumino-fluoro-silicate
Microhybrid Composite	Herculite Ultra XRV	Kerr Co. USA	*79 wt%; 59 vol%.*	Hexamethylene diacrylate, hexane-1,6-diol diacrylate, titanium dioxide, methacrylate ester monomer, zinc oxide,
Self-Etch Adhesive	Optibond XTR	Kerr Co. USA	*None*	Ethanol, esters with acrylic acid, propylidynetrimethanol, 2-hydroxyethyl methacrylate ethoxylated, trimethylsilyl, propanediylbismethacrylate silanamine, alkalifluorosilicate

Table 2*. Mean microshear bond strength values (MPa) (with SD*) of each group. Standard deviation values presented in parenthesis. Significant differences between group were given on table using different superscript letters.*

Groups	Minimum µmSBS Value	Maximum µmSBS Values	Occlusal Dentin µmSBS Values	Axio-Pulpal Dentin µmSBS Values	Mean µmSBS Values
Gr-1: SDR	11.902[a]	23.351[a]	19.785 (2.42)	13.150 (4.51)[a]	16.468 (3.41)[a]
Gr-2: SF2	9.556[b]	24.575[a]	19.982 (3.15)	15.247 (3.99)[a]	17.615 (3.83)[a]
Gr-3: CHU	8.326[b]	22.821[a]	17.878 (2.78)	13.084 (4.05)[a]	15.481 (4.50)[a]
Gr-4: HIT	4.855[c]	16.426[b]	15.142 (3.02)	6.376 (4.21)[b]	10.759 (3.42)[b]

Table 3*. Total restoration volume (mean - mm^3), mean void dimensions (mm^3) observed in occlusal, cervical, pulpal, in the bulk of the material and the percentage of voids (% 10^{-8}) acquired from mCT data. Significant differences between group were given on table using different superscript letters.*

Groups	TOTAL (Mean)	Occl.	Cerv.	Pulp.	Bulk	Overall
Gr-1: SDR	35.62 mm^3	2.06[a]	2.21[a]	2.28[a]	2.85[a]	7.41 μm^3 [2.08%][a]
Gr-2: SF2	35.87 mm^3	1.17[b]	1.32[b]	1.34[b]	2.08[a]	4.92 μm^3 [1.37%][a]
Gr-3: CHU	35.39 mm^3	1.45[b]	1.44[b]	1.76[b]	2.43[a]	5.76 μm^3 [1.62%][a]
Gr-4: HIT	36.21 mm^3	4.39[c]	4.47[b]	4.90[c]	6.48[b]	14.85 μm^3 [4.11%][b]

ly insignificant compared with the other interfaces (p=0.462). The mCT images showed that SF2 has the best adaptability, smallest void dimensions (1.17 μm^3) and least gap formation (% 1,37 $x10^{-8}$) (Figure 3). Although CHU showed similar void dimensions in the bulk of material (2,43 mm^3) as SF2 (2,08 mm^3), the differences in the overall void dimensions between CHU vs. SF2 (p=0.125) and CHU vs. HIT (p=0.348) were statistically insignificant (p>0.05). Therefore, second hypothesis related to gap formation is partially accepted.

The correlation significance scores between mSBS and gap formation obtained from Spearman's rho test showed that sonically placed composites SF2 (r=0.020), CHU (r=0.045) and microhybrid RBC (HIT) (r=0.010) presented a negative correlation between gap formation and the mSBS scores (p=0.001). On the other hand, no correlation was found between mSBS and gap formation for the SDR group (r=0.127).

Discussion

Although many RBC placement techniques are described, success was not achieved in certain cases, especially in class V cavities, which are susceptible to abfraction forces and leakage of gingival sulcus fluid (4,26). Bulk-fill materials, which have self-levelling features, decreased viscosity and polymerization stress, have been developed to enhance the quality of adaptability to the cavity walls (27). However, the filler content and concentration of the bulk-fill materials are associated with some limitations such as microgaps and internal stresses within the material. Therefore, sonic vibrational placement techniques have been developed as an alternative to overcome these challenges (28). In this study, both the microtomographic images and bond strength test results of four materials from each category were investigated to explain the challenges and outcomes of these methods.

Restoration with an RBC involves a rigorous and time-consuming technique that is susceptible to the mechanical properties and chemical composition of the material, as well as the experience of the operator. Both the Bulk-fill SDR and Sonicfill techniques allow condensation of resin composites that can be cured up to a depth of 4-5 mm and at least 20% reduction of the working time compared with the traditional techniques (29,30). Sampaio *et al*., (31) suggested that SDR is a type of flowable composite, which can be used as a bulk-fill base material and provide better shrinkage and microleakage scores than other flowable RBCs. According to Kapoor *et al*., (19) SDR is a specially designed RBC and can be used as dentine replacement. Moreover, it showed comparable results to SonicFill when packed in shallow Class I cavities. Contrary to these results, Benetti *et al*., (8) showed that in "deep" Class II cavities with a gingival cavosurface margin below the cement-enamel junction, high microleakage values were recorded for SDR when compared with SF2. In our case, SDR showed significantly comparable mSBS results (16.47 Mpa) to SF2, but lesser adaptability and increased gap formation (13.31%) compared with Sonicfill2. Decreased filler concentration (both weight and volume) and faster application time of the bulk-fill material SDR could be the main reasons for these differences. Even if all precautions have been taken in the placement stage of this study, increased flowability of the SDR may have caused the application to be performed rapidly, resulting in residual air bubbles in the material and close to the internal surfaces.

The restoration technique and precision in application are the most critical factors in determining the mechanical and physical properties of in vitro conditions because all restorative materials contain defects. Voids in RBCs are unavoidable flaws that contribute to make them prone to wear and fracture (32). To minimize the voids within the restorations, every effort was made to place the RBCs as close as possible to the cavity walls by one operator.

In this study, SF2 presented the least gap formation (8.23%) and mean void dimension of 4.92 mm^3, which corroborate with the study of Furness (33) *et al*., in which the SF2 system achieved a mean value of 10% in gap formation and SDR and conventional RBC presented similar results. Additionally, the results of a study conducted by Swapna *et al*., (34) demonstrated that most of the gap formation occurred at the cervical and pulpal cavity walls, followed by the occlusal walls, a finding that was consistent with our findings. This study also proved that the voids within the bulk of RBCs comprised most of the defects. These results can be explained by the working principle of the SF2 system. The composite resin used in this system is a combination of both flowable and universal composite resin that incorporates a highly filled proprietary resin with special modifiers that react to sonic energy. Because sonic energy is applied through the hand piece, the modifier causes the viscosity to drop (up to 87%), increasing the flowability of the composite resin until the sonic energy is stopped and then the viscosity turns into a solid and nonslumping phase (35). In accordance with these results, a study conducted by Rengo *et al*., (26) showed that low-viscosity restorative materials provide satisfactory bond strengths and reasonable microleakage scores compared with conventional RBCs when "deep" Class V cavities were restored.

Another sonically placed composite resin technique evaluated in this study was Compothixo, which is a vibration condensation instrument. The main difference between the two sonically activated placement techniques (SF2 vs. CHU) lies in the incrementation and bulk-filling of the RBCs that change the polymerization stress by slowing the radical polymerization rate (36). In this study, CHU showed comparable mSBS results (15.481 Mpa) with SDR and SF2, while gap formation and void dimensions were relatively better than SDR (11.6%). According to Tolidis *et al*., (37) conventional RBC packed with the Compothixo System presented better microleakage scores than Sonicfill2 and bulk-fill composites. By contrast, Ortiz *et al*., (28) compared the adaptation of a fluidized composite resin with a system that modifies its viscosity (Compothixo and SonicFill2) and found a statistically significant difference, showing susceptibility to the formation of the voids at the margins. However, they only measured the cavo-superficial margin using adhesion capability of the selected materials that did not allow evaluation of the degree of total adaptation of a composite resin. Therefore, it is not feasible to evaluate the possible faults that can occur both in the internal margins and within the bulk of the material.

According to our observations, voids commonly occur at internal angles and within material close to the external surfaces of the cavity. In both sonically placed RBCs, an average of 8% (SF2) to 11% (CHU) gap formation was observed; in the

bulk-fill placement technique, 13.31% (SDR) gap formation was found. In all groups, the least gap formation was found in the occlusal margins and most of the occlusal gaps were observed at the internal angles. The formation of gaps in the pulpal walls was found to be highest among all groups except for SF2 with a high probability that the unpolymerized RBCs remained in these deeper areas, lost its flowability fast and caused dimensional changes and debonding of the material.

In our study, we evaluated the adaptability of the internal walls as 91% in both sonic-activated placement techniques, a value that is considered satisfactory according to Orlowski *et al*. (27). Regarding the localization of the gaps, it is important to elucidate whether these are the consequences of the placement technique's shortage or polymerization shrinkage generated by viscosity changes or poor adaptation of the RBC. mCT analysis of our results demonstrated that the sonic-activated-placed high-viscosity bulk-fill resin composite with reduced polymerization contraction (SF2) and sonic-vibrated high-viscosity microhybrid RBC (CHU) resulted in similar void formation and mSBS to the conventional RBC. In Group CHU and HIT, voids in the transversal mCT images were consistent with lower mSBS values.

We acknowledge that polymerization shrinkage is the major factor involved in the development of contraction stresses and gap formation around cavity margins. In this study, a positive correlation was observed between voids and gap formation for all tested materials consonant to the literature (17,38). The most likely causes are polymerization shrinkage and less likely air bubbles trapped in the material would be the relevant factors affecting the debonding of the material from the cavity walls and decreases the bond strength (33,39). This fact was also confirmed when a negative correlation was observed between gap formation and mSBS values. Bonding to different dentin regions proved to be an important effect factor on mSBS values on bond strength and gap formation values indicating that the internal adaptation performance may not be similar inside the cavity walls (40). The negative correlation between bond strength and gap formation may be explained by the influence of tubule orientation and depth of dentin, positively affecting monomeric infiltration when the tubules are wider, as on the depth dentin and perpendicularly configured, as on the pulpal wall (41). In this study, the occlusal surface showed higher bond strength and lower gap formation than the proximal surface in consisted with the literature (39-41).

Conclusion

Sonic-activated insertion technique of bulk-fill composites can serve as an alternative choice in restoration of deep cavities to minimize voids because they have enhanced flowability, leading to good adaptation and increased bond strength.

Türkçe Özet: *Kompozit yerleştirme tekniğinin adaptasyon, boşluk oluşumu ve mikro makaslama bağ dayanımı üzerindeki etkisi. Amaç: Bu çalışmanın amacı; bulk-fill rezin kompozitlerin farklı uygulama tekniklerinin, bu materyallerin kavite içine adaptasyon, boşluk oluşumu ve mikro-makaslama bağ dayanımı (mSBS) üzerindeki etkinliklerini karşılaştırmaktır. Yöntem ve Materyaller: Bilgisayarlı mikro tomografi (mCT) kontrolleri için standardize edilmiş 48 adet Sınıf V kavite hazırlanmış ve dört gruba şu şekilde ayrılmışlardır (n=12): Grup SDR: Smart Dentin Replacement sistemi (bulk fill); Grup SF2: Sonic-Fill sistemi (bulk-fill: sonik enerji ile aktive edilmiş kompozit yerleştirme tekniği); Grup CHU: Herculite-XRV-Ultra rezin kompozitin Compothixo (sonik titreşime sahip el aleti) ile uygulandığı sistem; Grup HIT: Herculite-XRV-Ultra rezin kompozitin geleneksel inkremental teknikle uygulanmasıdır. Kendinden asitli bir adeziv sistem (Optibond-XTR) tüm gruplarda kompozit restorasyonlar öncesi uygulanmıştır. 10000 seferlik termo-siklus işlemi sonrasında tüm örneklerden mCT taramaları alınmış, diş ve dolgu ara yüzünde oluşan boşluklar incelenmiş ve üniversal bir test cihazı ile yapılan mikro makaslama testi ile bağ dayanımları (≒SBS) ölçülmüştür (n=10). İstatistiksel değerlendirmede; boşluk oluşumlarının ve mSBS değerlerinin analizi ve gruplar arası kıyaslanması için tek yönlü varyans ANOVA ve sonrasında post-hoc Bonferroni ve Tukey HSD testleri yapılmıştır (p=0.05). Bulgular: SF2 ve CHU grupları, tüm gruplar ile kıyaslandığında en iyi kavite içerisine adaptasyon değerlerini vermiştir. SDR ve HIT grupları arasındaki fark ise istatistiksel olarak anlamlı değildir (p<0.05). SF2 grubunda en yüksek ≒SBS değerlerine ulaşılırken, en düşük değerler ise HIT grubunda gözlenmiştir (p>0.05). Sonuçlar: Bulk-fill kompozitler geleneksel inkremental teknikle yerleştirilen rezin kompozitler ile kıyaslandıklarında, hem sonik enerji ile aktive edilen sistemler hem de sonik titreşim yapan el aletleri ile kaviteye yerleştirildiğinde, kavite duvarlarına daha iyi adaptasyon, daha az boşluk oluşumu ve daha yüksek bağlanma dayanımı sergilemektedirler. Anahtar Kelimeler: Bulk-fill kompozit; Bilgisayarlı mikro-tomografi; Boşluk oluşumu; Bağ dayanımı; Sonik enstrümantasyon.*

Ethics Committee Approval: The study protocol has been reviewed and approved by the Ege University Medical Research Ethics Committee (21-5T/66).

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: CP, DR participated in designing the study. CP, DR participated in generating the data for the study. CP, DR participated in gathering the data for the study. DR, HK participated in the analysis of the data. CP, DR wrote the majority of the original draft of the paper. HK participated in writing the paper. CP has had access to all of the raw data of the study. CP has reviewed the pertinent raw data on which the results and conclusions of this study are based. CP, DR, HK have approved the final version of this paper. CP guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2022; 56(1): 17-22

Original research

Expression of BMP-4 in dentigerous cyst and ameloblastoma: Is it a differentiation measure?

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Purpose

This study aimed to determine the expression of Bone Morphogenic Protein-4 (BMP-4) in dentigerous cyst (DC), unicystic-ameloblastoma (UA), and Multicystic-ameloblastoma (MA), and assess whether this marker can be a differentiation measure.

Materials and Methods

This study included 30 DC, 30 UA, and 30 MA blocks if the histopathologic diagnosis of the lesion was definitive, the clinical information and medical records were complete, and the microscopic slides and the paraffin block were available. Age, gender, and location of the lesion were recorded. The samples were analyzed after the immunohistochemical staining (Envision technique). BMP-4 marker was evaluated and reported using Intensity Score (IS), Proportional Score (PS), and Total score (TS). The data were analyzed using SPSS version 21.0. Kruskal-Wallis and Mann-Whitney U tests were applied at the significance level of 0.05.

Results

In this study, DCs, UA, and MA had a significant tendency to occur in males compared to females ($p<0.001$, $p<0.001$, and $p<0.001$ respectively), and in the mandible compared to the maxilla ($p=0.02$, $p=0.024$, and $p=0.02$ respectively). The epithelial IS was significantly different among three lesions ($p<0.001$). IS was higher in MA than UA and DC ($p<0.001$ and $p=0.006$, respectively). The IS was not significantly different among the three lesions in connective tissue and around micro-vessels ($p=0.3$ and $p=0.26$ respectively). The PS in the epithelium and connective tissue of DC, UA, and MA had no statistical difference ($p=0.549$ and $p=0.540$ respectively). The epithelial TS was statistically different among DC, UA, and MA ($p<0.001$). The TS was higher in UA than MA and DC ($p=0.004$ and $p<0.001$ respectively).

Conclusion

The expression of BMP-4 in the epithelium was higher in ameloblastoma compared to DCs. BMP-4 is a potential measure to differentiate different types of ameloblastoma and dentigerous cyst. The differentiation of these lesions is important as the right treatment plan changes according to the diagnosis.

Keywords: *Ameloblastoma, dentigerous cyst, BMP4, Histopathological assessment, staining*

Introduction

With a prevalence of 14 to 24%, DC is the second most common odontogenic cyst, assumed to originate from the dental follicle, however, its pathogenesis is still unclear (1–3). This cyst is frequently associated with the crown of an impacted, embedded, or an unerupted permanent tooth and is rarely associated with an odontoma, a developing tooth, or a deciduous tooth (2,3). DC mostly involve third molars followed by maxillary canines, mandibular premolars, and mandibular canines (3). DCs may lead to displacement and root resorption of associated or adjacent teeth if larger than

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Received: 27 March 2021
Revised: 30 May 2021
Accepted: 13 June 2021

DOI: 10.26650/eor.2022903844

How to cite: *Sargolzaei S, Maleki D, Zohary M. Expression of BMP-4 in dentigerous cyst and ameloblastoma: Is it a differentiation measure? Eur Oral Res 2022; 56(1): 17-22.*

2 cm or getting infectious (1). Generally, DCs are asymptomatic and are detected during routine radiographic examination with the appearance of a well-defined unilocular lesion (2, 3).

Ameloblastoma represents 19% of all odontogenic tumors (4). Ameloblastoma is a locally invasive benign odontogenic tumor derived from remaining tooth-forming components and the epithelial lining of dentigerous cysts (3, 4). The tumor presents as desmoplastic, peripheral, uni-cystic, or multi-cystic forms (4). UA with a prevalence of 5 - 15% of all reported cases, occurs in the young population in their 2nd or 3rd decade and may appear as a unilocular radiolucency with scalloped or lobulated border in radiographic images (4, 5). MA is more common in a wider age range compared to UA and is more aggressive (4).

The histopathological confusion between DC and UA is possible and has long been known. However, the diagnosis is important because of different treatment plans. DCs and UAs can be treated with enucleation or curettage, while other types of ameloblastoma require resection with an adequate margin of normal tissue (6, 7).

Bone Morphogenic Protein (BMP) is a growth factor and participates in cellular proliferation, extracellular matrix production, and differentiation of neoplastic tissues (8, 9). This study aimed to determine if BMP-4 can be used as a factor to differentiate odontogenic cysts such as DC from odontogenic tumors like UA and MA. The null hypothesis was that there are no differences in the BMP-4 expressions of DC, UA and MA.

Materials and Methods

This was a retrospective cross-sectional descriptive-analytic study.

Ethical statement

This project has been reviewed and approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. (IR.SBMU.REC.1395.330)

Specimen selection

The microscopic slides were included in the study if the histopathologic diagnosis of the lesion was definitive, the clinical information and medical records were complete, and the microscopic slides and the paraffin block were available. The samples were excluded if the diagnosis after re-examination did not confirm the previous diagnosis and if the tissue sample was not sufficient for immunohistochemical (IHC) analysis.

The biopsies of DC and ameloblastoma from 2010 to 2020 were obtained from the archive of the oral pathology department of the dentistry faculty of Shahid Beheshti University of Medical Sciences. 30 DC, 30 UA, and 30 MA blocks were selected. The samples were re-evaluated by an expert pathologist and the diagnoses were confirmed. Information such as age, gender, and location of the lesion were extracted from the medical records.

Sample size estimation

To calculate the sample size, information about MP4 marker expression by immunohistochemistry was used in the two groups of dentigerous cyst and odontogenic keratocyst related to the article by Kim *et al.* To do this, a formula suitable for comparing ratios in two independent societies and generalizing it to three groups has been used. Considering the statistical power of 0.975%, the error level of 0.05 and the expression ratio of 30.3 in the Dentigerous group and 97.1 in the Odontogenic keratocyst group, the minimum sample size of 23 was obtained. Which was considered for each group of 30 to achieve higher power (20) Formula is presented in Figure 1.

$$n_0 = \frac{\left[z_{1-\frac{\alpha}{2}} \cdot \sqrt{2\bar{p}(1-\bar{p})} + z_{1-\beta} \cdot \sqrt{p_1(1-p_1) + p_2(1-p_2)} \right]^2}{(p_1 - p_2)^2} = 15.08 \cong 16$$

$$\bar{p} = \frac{p_1 + p_2}{2}$$

$$n = n_0 \times \sqrt{g-1} = 16 \times \sqrt{2} = 22.63 \cong 23$$

Figure 1. *Formula used for sample size calculation.*

Specimen preparation and IHC staining

Following these steps, the samples were prepared for the IHC analysis of the BMP-4 marker. 5-micrometer-thick sections were cut from paraffin-embedded tissue blocks and IHC staining of BMP-4 was performed using the Envision technique. To deparaffinize the samples, the tissues were respectively placed in 100% Xylen, 99% alcohol, 96% alcohol, and 25% alcohol for 2-3 minutes. The samples were rinsed with distilled water for 2-3 minutes to remove the alcohol. To deactivate endogen peroxidase, the tissue was covered with a drop of Peroxidase Blocking Reagent (USA thermo TA-262-H222Q) for 10 minutes at room temperature. Then the tissue was immersed in a solution with a PH of 7.6 for 5 minutes. The remaining peroxidase blocking reagent was rinsed twice with distilled water for 2-3 minutes each time. Antigen retrieval was carried out by placing the tissue sample in a plastic Coplin jar containing retrieval solution at PH 6. The jars were then macro-waved for 7 minutes (power of 450 W), and cooled down for 5 minutes, and macro-waved for 15 minutes again (Power of 800 W). The samples were kept at room temperature and were rinsed with Tris Buffered Saline (TBS) solution with a PH of 7.6. Later, the samples were incubated by the primary anti-bodies for 1-hour and were then rinsed with TBS solution with a PH of 7.6 for 5 minutes. Afterward, the samples were incubated by Primary Antibody Amplifier Quanto (USA Thermo TL-060-QPB) for 10 minutes and rinsed again for 5 minutes with the same solution as the previous step. The samples were incubated with HRP Polymer Quanto (USA Thermo TL-060-QPH) for 10 minutes and rinsed with TBS solution (PH=7.6) for 5 minutes. The samples were immersed in diaminobenzidine (DAB USA Termo TA-222-QHCX) for 5 minutes until a brownish reaction was observed. The samples were washed with tap water for 5 minutes and placed in distilled water for 5 minutes. Finally, the sections were counterstained with hematoxylin for 30 seconds, and with lithium carbonate for 5 minutes, then rinsed with tap water. The samples were mounted afterward.

Specimens assessment

Squamous Cell Carcinoma (SCC) sections were used as a positive control and tumoral sections which were incubated with Tris-buffered saline (TBS) instead of primary antibodies were used as a negative control.

The immune-expression of BMP-4 in microscopic slides were evaluated under a light microscope at x100 magnification. The staining was considered positive if the cytoplasm of tumoral cells were brown stained (Figure 1-3).

Intensity score (IS) was recorded as score 0 (No staining or <10% staining), score 1 (incomplete membranous staining of weak to moderate intensity in 10%< of the cells), score 2 (complete membranous staining of moderate intensity in 10%< of the cells), and score 3 (complete membranous staining of strong intensity in 10%< of the cells) for the assessment of BMP-4 marker in the epithelium, connective tissue, and around micro-vessels. Proportional Score (PS) of BMP-4 marker was measured as score 0 (no stained cell), score 1 (<20% stained cells), score 2 (20-80% stained cells), and score 3 (80%< stained cells) in the epithelium and connective tissue. Total score (TS) was reported for epithelium and connective tissue by summing the IS and PS of the BMP-4 marker.

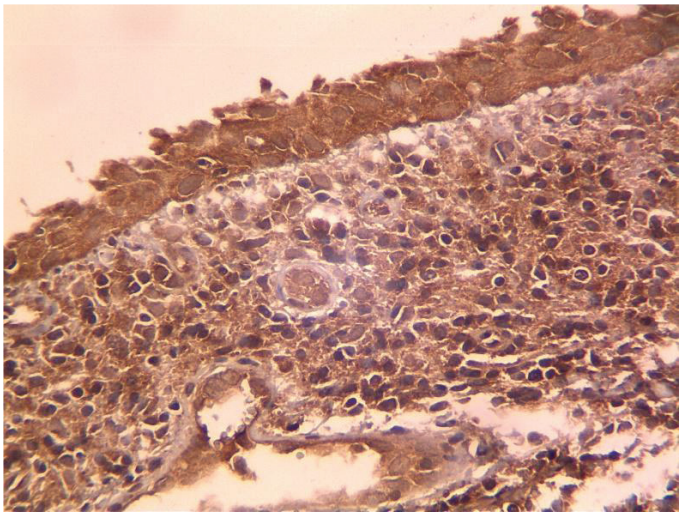

Figure 1. *The microscopic view of dentigerous cyst after immunohistochemical staining indicate BMP-4 marker. (×400).*

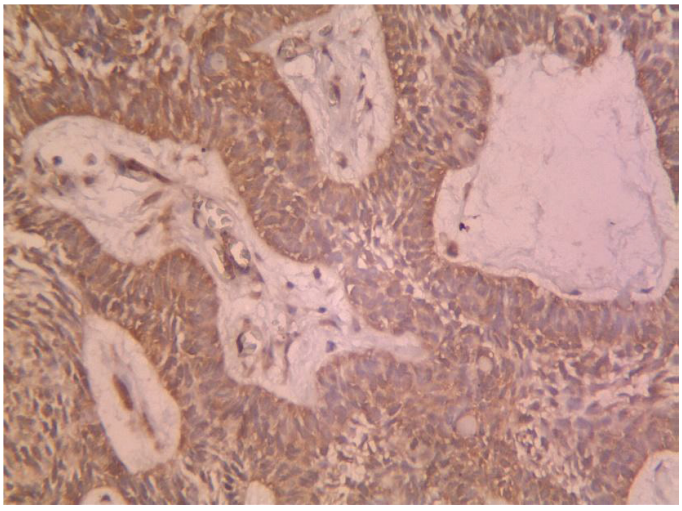

Figure 2. *The microscopic view of Unicystic-ameloblastoma after immunohistochemical staining indicate BMP-4 marker. (×400).*

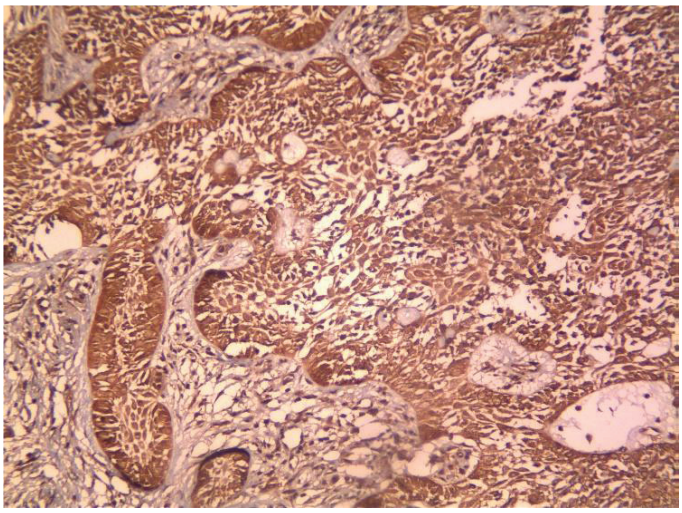

Figure 3. *The microscopic view of Multicystic-ameloblastoma after immunohistochemical staining to indicate BMP-4 marker. (×200).*

Statistical analysis

The data were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 21.0 (IBM Corp, Armonk, NY, USA). Frequency was applied to determine the characteristics of the sample. The chi-square test was used to compare the demographic distribution among DC, UA, and MA. Since the data distribution was not normal, the nonparametric Kruskal-Wallis one-way analysis of variance by ranks and Mann-Whitney U tests were used for the multiple and pairwise comparisons, respectively. The confidence interval was set to 95% and the significance level was set at 0.05.

Results

In this study, the expression of BMP-4 was assessed in 30 DC, 30 UA, and 30 MA microscopic slides using IHC analysis. The data distribution is presented in table1. According to intra-group comparison, no statistical difference was found among the three study groups in terms of age, gender, and the location of the lesions (P=0.1, P=0.1 and P=0.1 respectively). The mean age of patients was 31.9 ±17.8, 30.7 ±17.9, and 39.6 ±16.9 in DC, UA, and MA groups, respectively.

The prevalance of the pathology was higher in males than females in DC, UA, and MA study groups (P<0.001, P<0.001, and P<0.001 respectively), and higher in the mandible than themaxilla (P=0.02, P=0.024, and P=0.02 respectively). The IHC analysis of BMP-4 markers in the epithelium, connective tissue, and micro-vessels of 3 lesions is reported in Table2-4.

The IS of BMP-4 marker in the epithelium was significantly different among the three lesions (P<0.001, df=2, x2=40.947). The Mean of IS was 26.72 in DC, 42.18 in UA, and 67.6 in MA. MA had a significantly higher IS compared to UA and DC (P<0.001 and P=0.006, respectively). Also, the IS in UA lesions was significantly higher than in DC lesions (P<0.001). The differences of IS in the connective tissue and the micro-vessels of DC, UA, and MA lesions were not statistically significant (P=0.389 and P=0.26, respectively).

The PS of the BMP-4 marker in the epithelium and connective tissue was not statistically different among the three lesions (P=0.549 and P=0.540, respectively).

The TS of BMP-4 marker in the epithelium was statistically different in DC, UA, and MA lesions (P<0.001, df=2, x2=23.857). The results showed that UA had a significantly higher TS compared to MA and DC (P=0.004 and P<0.001 respectively), and MA had a significant higher TS compared to DC (P<0.001). The difference of TS was not significant between DC, UA, and MA lesions in connective tissue (P=0.424).

Table 1. *The data distribution of patients with Dentigerous Cyst, Unicystic Ameloblastoma and Multicystic Ameloblastoma according to gender and the location of the lesion in percent (number).*

The type of the lesions	Gender		The location of the lesions	
	Female	Male	Maxilla	Mandible
Dentigerous cyst	36.7% (11)	63.3% (19)	30% (9)	70% (21)
Unicystic Ameloblastoma	23.3% (7)	76.7% (23)	16.7% (5)	83.3% (25)
Multicystic Ameloblastoma	13.3% (4)	87.6% (26)	3.3% (1)	96.7% (29)
Total	24.4% (22)	75.6% (68)	16.7% (15)	83.3% (75)

Table 2. *The IS, PS and TS of BMP-4 marker in the epithelium of Dentigerous Cyst, Unicystic Ameloblastoma and Multicystic Ameloblastoma.*

The type of the lesions	Intensity Score				Proportional Score			Total Score
	Score 0	Score 1	Score 2	Score 3	Score 1	Score 2	Score 3	
Dentigerous cyst	63.3% (19)	33.3% (10)	0 (0)	3.3% (1)	33.3% (10)	20% (6)	46.7% (14)	30.6
Unicystic Ameloblastoma	0 (0)	20% (6)	60% (18)	20% (6)	16.7% (5)	46.7% (14)	36.7% (11)	62.45
Multicystic Ameloblastoma	23.3% (10)	36.7% (11)	26.7% (8)	3.3% (1)	43.3% (13)	13.3% (4)	43.3% (13)	43.5

Table 3. *The IS, PS and TS of BMP-4 marker in the connective tissue of Dentigerous Cyst, Unicystic Ameloblastoma and Multicystic Ameloblastoma.*

The type of the lesions	Intensity Score				Proportional Score			Total Score
	Score 0	Score 1	Score 2	Score 3	Score 1	Score 2	Score 3	
Dentigerous cyst	66.7% (20)	23% (7)	10% (3)	0 (0)	80% (24)	20% (6)	0 (0)	43.06
Unicystic Ameloblastoma	53.6% (16)	23% (7)	20% (6)	3.3% (1)	70% (21)	30% (9)	0 (0)	49.92
Multicystic Ameloblastoma	66.7% (20)	20% (6)	10% (3)	3.3% (1)	76.7% (23)	23.3% (7)	0 (0)	43.52

Table 4. *The IS of BMP-4 marker around the micro-vessels of Dentigerous Cyst, Unicystic Ameloblastoma and Multicystic Ameloblastoma.*

The type of the lesions	Intensity Score			
	Score 0	Score 1	Score 2	Score 3
Dentigerous cyst	70% (21)	16.7% (5)	13.3% (4)	3.3% (1)
Unicystic Ameloblastoma	56.7% (17)	23% (7)	13.3% (4)	6.7% (2)
Multicystic Ameloblastoma	73.3% (22)	20% (6)	6.7% (10)	0 (0)

Discussion

This study assessed the expression of BMP-4 in DC, UA, and MA microscopic slides. In this study, the occurrence of DC, UA, and MA was significantly higher in males and was more common in the mandible. In terms of gender, Açikgöz *et al.* (10) found the same results in the Turkish population. Açikgöz *et al.* (10) investigated the distribution and number of odontogenic and non-odontogenic cysts in a 9year study. In their study, out of 122 patients with DCs, 56 were women (45.6%) and 66 (54.1%) were men. Similarly, Ramachandra *et al.* (11) studied the prevalence of odontogenic cysts over 5 years in India. In their study, 24 of the 45 DCs samples were male (53.33%) and 21 were female (46.67%). In agreement, Meningaud *et al.* (12) reported that among 154 samples of DCs 108 were male and 46 were female. Also, Alvelar *et al.* (13) assessed the prevalence of odontogenic tumors in the Brazilian population and reported that 30 of 57 patients were male and 27 were female. Sah *et al.* (14) reported that 5 and 13 of 18 subjects diagnosed with UA were respectively female and male. In contrary to the results of the above-mentioned studies, Olgac *et al.* (15) carried out a study to examine odontogenic tumors over 32 years in Turkey in 2006 and their results showed that out of 133 sam-

ples of ameloblastoma tumors, 58 samples were men and 75 were women. This difference may be associated with the different study populations.

The results of the current study showed that the prevalence of DC, UA, and MA was higher in the mandible compared to the maxilla. Açikgöz *et al.* (10) also found that 34 samples of DCs were in the maxilla while 88 samples were observed in the mandible. Ramachandra *et al.* (11) reported that 20 of the 45 DCs were located in the maxilla and 25 of them were found in the mandible. Núñez-Urrutia *et al.* (16) evaluated the prevalence of odontogenic cysts in Spain over 10 years and found that among 91 subjects, 63 cysts were located in the mandible and 28 cysts were in the maxilla. Alvelar *et al.* (13) showed that 48 lesions were located in the mandible and 9 lesions were found in the maxilla. Sah *et al.* (14) claimed that 17 samples of 18 UA were located in the mandible and one sample was found in the maxilla. Olgac *et al.* (15) reported that 15 samples of odontogenic tumors were seen in the upper jaw and 118 samples in the lower jaw. Considering the above-mentioned studies, DC and Ameloblastoma are located preferably in the mandible which is confirmed in this study.

BMP-4 is a bone morphogenetic protein belonging to the TGF-B family (17). The absence of BMP-4 leads to severe defects in osteogenesis (17). BMP-4 affects normal epithelial cell differentiation and odontogenic epithelium malignancies through epithelial-mesenchymal reaction, also, results in cytodifferentiation of ameloblastoma histologic subtypes (18). Some studies have evaluated the expression of BMP-4 markers in odontogenic cysts and tumors but no study, up to the authors' knowledge, has compared the expression of BMP-4 marker in DCs and ameloblastoma as a possible measure to differentiate these lesions (19–22).

The study of Kumamoto *et al.* (19) was performed to assess the role of BMPs in the differentiation of odontogenic tumors. 37 samples of ameloblastoma, 6 samples of Adenomatoid odontogenic tumor (AOT), and 5 samples of malignant ameloblastoma were compared to 10 third mandibular molar follicles using RT-PCR, IHC to determine BMP-2, BMP-4, BMP-7 markers, and Osterix, CBFAI, BMPRII, and BMPRI receptors. The results showed that mRNA expression resulting from BMPs and other related molecules are found in all odontogenic tumors.

Kim *et al.* (20) conducted a study to compare the expression of BMP-4 in Odontogenic kerato-cyst (OKC) and DC using IHC and in situ hybridization. The results indicated that BMP-4 had higher expression in OKC compared to DC especially in the recurrent form of OKC. In the current study, also, BMP-4 had higher expression in UA and MA compared to DC.

Ruhin-Poncet *et al.* (21) evaluated the expression of BMPs, Dlx, and Msx in odontogenic epithelial tumors (recurrent ameloblastoma and central calcifying odontogenic cyst (CCOC)) They stated that Dlx, and Msx were found in ameloblastoma unlike CCOC. In contrast, BMP-2 was only found in CCOC while BMP-4 was expressed in both tumors. The findings of Ruhin-Poncet study were in agreement with the current study reporting the expression of BMP-4 in ameloblastoma.

Also, Nascimento *et al.* (9) studied the expression of BMP-2, BMP-4, and their receptors in AOT and ameloblastoma using IHC. In MA, positive correlations were observed between the stromal and parenchymal expression of BMP-2 and between the stromal expression of BMP-2 and BMP-4, as well as between the stromal expression of BMPR-II and BMP-4 and the stromal and parenchymal expression of BMPR-II. In UAs, a correlation was detected between the stromal and parenchymal expression of BMP-4 and between the stromal expression of BMP-4 and BMPR-IA. In AOTs, analysis of immune-expression in the parenchyma revealed positive correlations between all proteins. BMPs and their receptors play an important role in the differentiation and development of ameloblastoma and AOTs, but may not explain the different biological behaviors of these lesions. The positive correlation observed in AOTs might be related to the formation of mineralized material in this tumor.

Not assessing the relation of clinical behaviors of the lesions and the expressions of BMP-4 is one of the limitations of this study. Also, the expression of BMP-4 was not compared among different types of ameloblastoma.

Conclusion

Considering the limitation of this study, it was found that the expression of BMP-4 in the epithelium was significantly higher in ameloblastoma compared to DCs. However, the difference was not significant in the connective tissue and around the micro-vessels. The IS of the BMP-4 marker was higher in MA compared to UA, while the TS of the BMP-4 marker was higher in the UA compared to MA. According to the findings of this study, it can be concluded that BMP-4 is a potential measure to differentiate different types of ameloblastoma and dentigerous cyst. The differentiation of these lesions is of importance as the right treatment plan changes according to the diagnosis.

Türkçe Özet: *Dentigeröz kist ve ameloblastomada bmp-4 ekspresyonu ayırıcı bir yöntem midir?. Giriş: Bu çalışmanın amacı dentigeröz kist (DK), unikistik ameloblastoma (UA) ve Multi-kistik ameloblastoma (MA) olgularında kemik morfogenik protein 4 ekpresyonunu belirlemek ve bu değerlerin lezyonların ayrılmasında yardımcı olup olmayacağının incelenmesidir. Gereç ve yöntem: Bu çalışma, lezyonun histopatolojik tanısı kesinleşmiş, klinik bilgileri ve tıbbi kayıtları tam olan ve mikroskobik slaytları ve parafin blokları bulunan 30 DK, 30 UA ve 30 MK bloklarını içeriyordu. Lezyonun yaşı, cinsiyeti ve yeri kaydedildi. Örnekler immünohistokimyasal boyamadan (Envision tekniği) sonra analiz edildi. BMP-4 belirteci Yoğunluk Skoru (IS), Orantılı Skor (PS) ve Toplam Skor (TS) kullanılarak değerlendirildi ve rapor edildi. Veriler SPSS 21.0 versiyonu kullanılarak analiz edildi. Kruskal-Wallis ve Mann-Whitney U testleri 0.05 anlamlılık düzeyinde uygulandı. Bulgular: Bu çalışmada DK'ler, UA ve MA, erkeklerde kadınlara göre (sırasıyla P<0,001, P<0,001 ve P<0,001) ve maksillaya kıyasla mandibulada (P= 0.02, P=0.024 ve P=0.02 sırasıyla) daha fazlaydı. Epitelyal IS, üç lezyon arasında önemli ölçüde farklıydı (P<0.001). IS, MA'da UA ve DK'den daha yüksekti (sırasıyla P<0,001 ve P=0,006). Bağ dokusunda ve mikrodamarların çevresindeki üç lezyon arasında IS anlamlı şekilde farklı değildi (sırasıyla P=0.3 ve P=0.26). DC, UA ve MA'nın epitelindeki ve bağ dokusundaki PS'de istatistiksel bir fark yoktu (sırasıyla P=0.549 ve P=0.540). Epitelyal TS, DK, UA ve MA arasında istatistiksel olarak farklıydı (P<0.001). TS, UA'da MA ve DKden daha yüksekti (sırasıyla P=0,004 ve P<0,001). Sonuç: Epitelde BMP-4 ekspresyonu DK'lere kıyasla ameloblastomda daha yüksekti. BMP-4, farklı ameloblastom ve dentigeröz kist türlerini ayırt etmek için potansiyel bir ölçüttür. Tanıya göre doğru tedavi planı değiştiği için bu lezyonların ayrımı önemlidir. Anahtar Kelimeler: Ameloblastoma, dentigeröz kist, BMP4, Histopatolojik Değerlendirme, Boyama*

Ethics Committee Approval: This project has been reviewed and approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. (IR.SBMU.REC.1395.330)

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: SS, MZ participated in designing the study. SS, MZ participated in generating the data for the study. SS, MZ participated in gathering the data for the study. DM, MZ participated in the analysis of the data. DM wrote the majority of the original draft of the paper. SS, DM, MZ participated in writing the paper. SS, DM, MZ have had access to all of the raw data of the study. SS, DM, MZ have reviewed the pertinent raw data on which the results and conclusions of this study are based. SS, DM, MZ have approved the final version of this paper. SS, DM, MZ guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2022; 56(1): 23-27

Original research

Analysis of gingival display during static and dynamic smiles in a Turkish sample: A clinical study*

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Purpose
The aim of this study is to determine the prevalence of smile types in spontaneous smiles among a Turkish population aged 18–23 and to compare it with the prevalence of static smiles.

Materials and Methods
This study was carried out with 150 undergraduate students at Başkent University Faculty of Dentistry (75 females, 75 males). For this purpose, photo recordings for static smiles and 20-second video recordings for dynamic smiles were taken 40 cm from the participant's nose. Measurements were made with an electronic ruler.

Results
High smile line was found to be the highest prevalence in both static and dynamic smiles ($p<0.001$). The average soft tissue display is higher in dynamic smiles ($p<0.05$). In both static and dynamic smiles, the average amount of gingival display was higher in females than in males ($p<0.05$).

Conclusion
When the smile line was evaluated on the photograph recordings while the patient was posing, it was found to be lower than the natural spontaneous smile line obtained from the video recordings. Since the gingival display increases when patients smile naturally instead of posing, clinical evaluations and restorative considerations should be planned according to the dynamic smile.

Keywords: *Smile line, dynamic smile, static smile, gingiva, esthetic*

Introduction

Evaluating dental esthetic parameters has become a routine procedure for patients who need esthetic dental treatment. Performing a detailed esthetic evaluation, by integrating it with biological and functional parameters, will allow the clinician to accurately diagnose and select the most appropriate treatment plan for the patient (1).

The smile is an important facial expression and communication parameter (2). An esthetic smile is related to the color, shape, and size of the teeth as well as the amount of gingival display (3). Gingival display is defined as the amount of gingival visibility during a smile or the distance between the gingiva and upper lip (4). The amount of soft tissue displayed is related to the position of the smile line (5). During the smile, the relationship between the lower border of the upper lip and the upper teeth and/or gingival display forms the smile line. In previous years, smile line types were categorized in three groups. According to this classification, cases with enamel-cement junction visibility and gingival display above this junction were described as Class 1 (gummy smile), cases where only gingival embrasures were seen as Class 2, and cases where less than 75%

** This study was presented at ERDİŞ 2020, 26-29th Feb 2020, Kayseri, Turkey*

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Received: 1 March 2021
Revised: 10 May 2021
Accepted: 16 July 2021

DOI: 10.26650/eor.2022888850

How to cite: *Arifagaoglu O, Yilmaz U. Analysis of gingival display during static and dynamic smiles in a Turkish sample: a clinical study. Eur Oral Res 2022; 56(1): 23-27.*

of the anterior teeth were visible were Class 3 (6). In 2004, Liebart et al. (7) expanded the types of smile lines into four groups: Class 1, with a very high smile line (more than 2 mm gingival display); Class 2, with a high smile line (0–2 mm gingival display); Class 3, with an average smile line (display of gingival embrasures only); and Class 4, with a low smile line (gingival embrasures and enamel-cement junction not visible) (Figure 1).

Figure 1. *Smile line types (Liebart et al., 2004) (A: Class 1; B: Class 2; C: Class 3; D: Class 4).*

Clinicians often base their diagnosis, treatment planning, and research on patients' static smiles at a single moment of a posed smile. For all those purposes, this static analysis can lead to misdiagnosis and non-ideal treatment because patients' natural smiles may be significantly different from their posed smiles, displaying more teeth and/or gingiva. Dynamic smile assessments should be used to determine the entire range of a spontaneous smile (8).

In addition, the prevalence of a smile line is determined according to a static (posed) smile. It is thought that obtaining dynamic smile records spontaneously can change the prevalence of smiling lines and give more accurate results.

The aim of this study was to determine the prevalence of smile types in dynamic (spontaneous) smiles in a young Turkish population between the ages of 18 and 23 and to compare it with the prevalence in static smiles. The first null hypothesis was that gingival display would not change between static and dynamic smiles. The second null hypothesis was that the smile line types would not be different between males and females.

Materials and Methods

Ethical statement

This study was approved by the Başkent University Institutional Review Board (project no.: D-KA20/10) and Ethics Committee with support from Başkent University Research Fund. Informed consent was obtained from all participants by asking them to sign a consent form containing details about the study.

Sample size estimation

This study was carried out with 150 students (75 females, 75 males). The sample size was calculated for the chi-square test, which was used to test the primary hypothesis of our study. As a result of the sample size analysis performed using Cohen's effect size value of 0.29, a minimum of 150 individuals should be included in the study ($1 - \beta = 0.80$) to reveal significant differences between the groups with 80% power and $\alpha = 0.05$ error (95% confidence interval).

Study protocol

Exclusion criteria were orthodontic treatment in process, a missing anterior tooth, restoration in the anterior region, and periodontal disease. For static and dynamic recordings, photographs and video recordings were taken at a distance of 40 cm between the lens and the tip of the nose by using a camera (Canon EOS 750D, Canon Inc., Japan), a lens (100 mm, Canon Inc., Japan), a ring flash (MR-14EX II, Canon Inc., Japan), and a tripod (HAMA HM.4605 FlexProS, China). All photographs were taken under the same conditions by the same photographer. Participants were positioned standing in front of a white background. Before the recordings were taken, 13.1 cm wide archless flat glasses were fitted to each participant to calibrate the gingival distance measurement in the photos (Figure 2). For the static recording, the participants were asked to look across and smile with their mouths open while the photo was taken. In order to record the dynamic smile correctly, the participants were engaged in relaxed conversations and asked to laugh more comfortably, and the video of these moments was recorded for 20 seconds while maintaining the same distance. Then, the video recordings were evaluated, and the screenshot taken of the moment when the participant smiled most comfortably. Thus, the highest smile line was recorded. This moment was determined as a dynamic smile line. Gingival display in the static and dynamic records was measured with an electronic ruler by using the Keynote (v6.6.1; Apple Inc., USA) program (Figure 2). All photographs were adjusted, and black bars were placed on the eyes of the participants' photographs by using Adobe Photoshop (version 21.2, USA). Measurements were made between the enamel-cement junction of the maxillary central teeth and the lower border of the upper lip. In cases of asymmetry, the amount of gingival display of

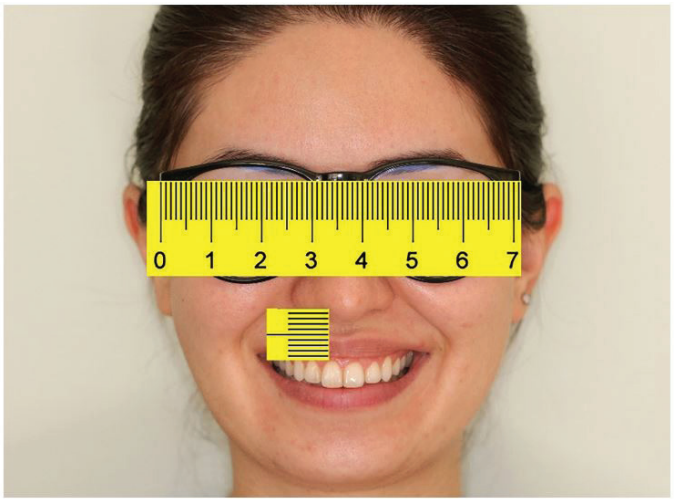

Figure 2. *Gingival display measurement and calibration.*

both central teeth was measured, and the arithmetic mean was calculated. Then, smile lines were classified according to the classification of Liebart et al. (7).

Statistical analysis

Post hoc power analysis was conducted to determine the power of the study, with a type-1 error value of 0.05 for the primary hypotheses that were found to be statistically significant. The G*power software (version 3.1.9.7, Heinrich Heine University Düsseldorf, Germany) was used for sample size estimation and post hoc power analysis. Statistical analysis of the data collected in our study was performed using the SPSS package program (Version 22.0, SPSS Inc., USA). Frequency distributions of categorical variables were presented as number and percentage (%). Descriptive statistics of continuous variables were reported with mean ± standard deviation, in accordance with the data normality distribution. The normal distribution of the data was tested with the Shapiro–Wilks test. Relationships and ratio comparisons between categorical variables were evaluated using the chi-square test. Gingival display measurements were compared by Student's *t*-test between two independent groups. The statistical significance level was considered as $p<0.05$.

Results

A statistically significant difference was found between the ratios of smile line types in static and dynamic smiles in young individuals between the ages of 18 and 23 (*chi square*^{2}(3)= 47.87, $p<0.001$). The statistical post hoc power for this comparison was calculated to be 100%. Class 2 was found to be most prevalent in both static and dynamic smiles at 36% and 44%, respectively. The prevalence of Class 2 in dynamic smiles is higher than in static smiles ($p<0.001$). While the least common type in static smiles is a very high smile line, it was the second most frequent type in dynamic smiles ($p<0.001$). Average smile line was the second most frequent smile type in posed smiles, while it was the second least frequent in spontaneous smiles ($p<0.001$) (Table 1).

Figure 3 shows the change in smile types of individuals from static smile to dynamic smile. As a descriptive analysis, none of the participants with a low smile line in the static smile showed a very high smile line in the dynamic smile (Figure 3). All of the participants with an average smile line in a static smile showed high or very high smile lines in a dynamic smile. There was no decrease in the amount of gingival display in any of the participants from static smile to dynamic smile.

The ratios of the smile line types in static and dynamic smile by gender are given in Table 2. The ratios of smile line types were statistically different between males and females in both static and dynamic smiles among young individuals aged 18–23 (*chi square*(3)= 17.33, $p=0.001$; *chi square*(3)= 22.12, $p<0.001$, respectively). For this comparison performed by gender, statistical post hoc power was calculated to be 100% for static smiles and 100% for dynamic smiles. While the prevalence of high smile lines in static smiles and very high smile lines in dynamic smiles is highest in females, the prevalence of average smile lines in static smiles and high smile lines in dynamic smiles was highest in males ($p = 0.001$ and $p < 0.001$, respectively).

The arithmetic mean gingival display was 0.35 ± SD mm in static smiles and 1.44 ± SD mm in dynamic smiles. The average soft tissue display is higher in dynamic smiles ($p<0.05$). The arithmetic mean gingival display of females and males in static smiles was 0.47 ± SD mm and 0.20 ± SD mm, respectively; in dynamic smiles, it was 1.92 ± SD mm and 0.87 ± SD mm, respectively. In both static and dynamic smiles, the average amount of gingival display was higher in females than in males ($p<0.05$ and $p<0.05$, respectively).

The average gingival display in participants with a Class 1 smile line is 2.41 ± SD mm in static smiles and 3.34 ± SD mm in dynamic smiles. On the other hand, the average gingival display in static and dynamic smiles was 0.52 ± SD mm and 0.62 ± SD mm, respectively.

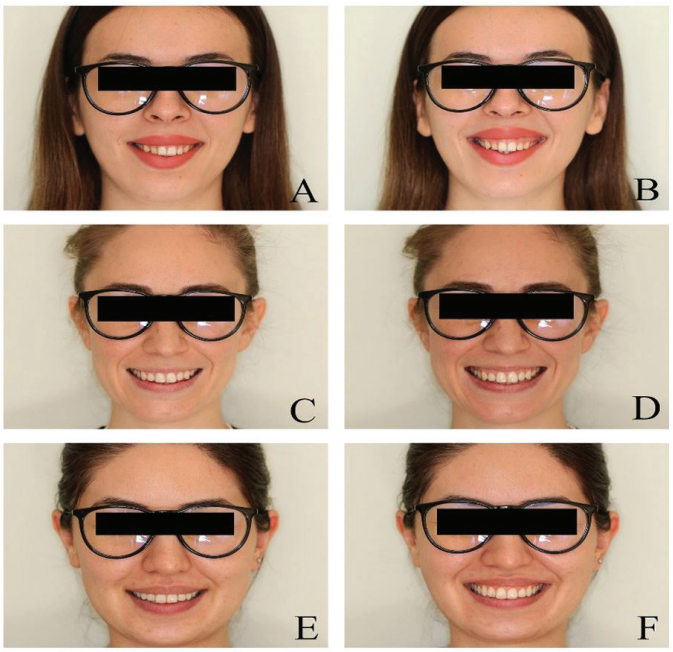

Figure 3. *Change in smile types from static to dynamic smile. A, C, E show posed (static) smiles, B, D, F show spontaneous (dynamic) smiles. (A: High smile line; B: Very high smile line; C: Average smile line; D: High smile line; E: Low smile line; F: High smile line.).*

Table 1. *Prevalence of smile line types in static and dynamic smile (*Statistically significant with Chi-square test).*

Smile Line Types	Static Smile n (%)	Dynamic Smile n (%)	*P* value	Power
Class 1 (Very high smile line)	12 (8)	51 (34)	**<0.001***	**100%**
Class 2 (High smile line)	54 (36)	66 (44)		
Class 3 (Average smile line)	51 (34)	18 (12)		
Class 4 (Low smile line)	33 (22)	15 (10)		

Table 2. *The prevalence of static and dynamic smile lines by gender (*Statistically significant with Chi-square test).*

Smile Line Types	Smile Types							
	Static (Posed)				Dynamic (Spontaneous)			
	Female (%)	Male (%)	*P* value	Power	Female (%)	Male (%)	*P* value	Power
Very high	8	8	**0.001***	**100%**	48	20	**<0.001***	**100%**
High	48	24			40	48		
Average	32	36			8	16		
Low	12	32			4	16		
Total	100	100			100	100		

Discussion

Regardless of gender and age, there are parameters for the evaluation of smile esthetics, such as midline, incisor width/height ratio, buccal corridor, incisor crown inclination, and smile arch appearance. Among these parameters, the amount of gingival display is one of the most important keys to smile beauty (9–12). Both null hypotheses of the presented study were rejected; gingival display changed during static and dynamic smiles within both male and female populations.

In this research, static photographs were evaluated to measure gingival display. Capturing dynamic smiles using a video camera recording can change the display of soft tissues and teeth compared to static images produced by a camera (13–15). Recording a video for dynamic smiles can allow a proper analysis of esthetics and function. Usually, when the patient is asked to smile broadly during the clinical evaluation, the patient poses with a lower smile than usual (16, 17). In this study, the frequency of smile types in static and dynamic smiles in a young Turkish population aged 18–23 was compared. This age range was preferred because the gingival display changes as the age increases (14, 18).

Maxillary central incisors are the key determinant in the evaluation of smile type and esthetics. Therefore, in this study, the smile type was evaluated by measuring the gingival display of the maxillary central teeth (19–21).

There are a few studies from different ethnic communities that show that the smile line may be found to be different in a patient's comfortable position, since the smile line assessment is mostly based on the patient's photographs and the patient is asked to pose (8, 22). Also in this study, the prevalence of the smile line types changed between the posed smile and the spontaneous smile.

In a study performed by Mahn et al. (8), while the prevalence of the smile line changed in dynamic smiles, the most common smile line was the low smile line in static smiles, while the average smile line was found in dynamic smiles. However, in this study, although the most common smile line type is the high smile line in both static and dynamic smiles, the rate of high smile lines is higher in dynamic smiles. This is thought to be related to the conduct of studies in different ethnic communities.

The prevalence order of smile types in static smiles and dynamic smiles in both females and males has changed. While the most common smile line in females in static smiles is Class 2, it is Class 1 in dynamic smiles. The rate of very high smiles line in dynamic smiles is six times higher (48%) than in static smiles (8%) in the female population. While the lowest two smile lines (Class 3 and Class 4) are most prevalent in static smiles in males, the highest two smile lines (Class 1 and Class 2) are most prevalent in dynamic smiles. Based on these findings, it can be said that the posed smiles of the majority of the Turkish population do not reflect their natural smiles.

In a study by Jensen et al. (23), it was reported that the average amount of gingival display of females was higher than that of males in posed smiles. Similarly, in this study, the amount of gingival display is higher in both posed and spontaneous smiles of females compared to males.

This study obtains specific frames for the desired purpose, using videographic imaging technology. The fact that video recordings are as easy, reproducible and reliable as photographic recordings makes them valuable in clinical practice. With the help of a software program, the desired analyses and measurements can be performed on the images obtained from video recordings.

This study showed that the use of digital photography alone is insufficient for evaluating gingival display and planning the esthetic restorations to be decided accordingly, because most of the participants showed a change in the type of smile between posed and spontaneous records. Moreover, treatments should be planned individually, as females usually present higher gingival display than males in both posed and spontaneous smiles.

The reason for the limitation of this study to the young age group is the decrease in gingival display due to age. Further studies should be investigated on types of smiles and changes in gingival display during static and dynamic smiles in different age ranges.

Considering that the amount of gingival display is an important parameter that directly affects esthetics, it is critical to accurately determine the amount of gingival display in the planning and performing stages of certain clinical applications, such as esthetic restorations, prosthetic material decision, orthognathic surgeries, philtrum and lip repositioning operations, and periodontal crown lengthening.

Conclusion

The smile line is usually found lower than its natural position since the smile line assessment is based on the patient's photographs and the patient is asked to pose. The smile

line in spontaneous smiles is higher than in static evaluations. Clinical evaluations and restorative thoughts should be planned according to the dynamic smile, as the gingival appearance increases when patients are smiling naturally rather than posing.

Türkçe Özet: *Türk popülasyonunda statik ve dinamik gülüş sırasında gingival görünüm analizi: klinik çalışma. Amaç: Gülüş tipi en çok hasta statik durumda iken, fotoğraflarda poz verirken sınıflandırılır ve buna göre popülasyondaki gülüş tiplerinin yaygınlığı belirlenir. Ancak spontane gülümsemede prevalansın farklılık gösterebileceği düşünülmektedir. Bu çalışmanın amacı, 18-23 yaş arasındaki Türk popülasyonunda spontan gülüşte gülme hattı tiplerinin yaygınlığını belirlemek ve statik gülüş prevalansı ile karşılaştırmaktır. Gereç ve Yöntem: Bu çalışma Başkent Üniversitesi Diş Hekimliği Fakültesi'nden 150 lisans öğrencisi (75 kız, 75 erkek) dahil edilerek yapıldı. Bu amaçla katılımcının burun ucundan 40 cm mesafede, statik gülüş için fotoğraf kayıtları, dinamik gülüş için ise 20 saniyelik video kayıtları alındı. Ölçümler elektronik cetvelle yapıldı. Bulgular: Hem pozlanmış hem de spontan gülüşte yüksek gülme hattı prevalansı en fazla bulunmuştur. (p<0.001). Ortalama dişeti yüksekliği dinamik gülüşte daha fazladır (p<0.05). Kadınlarda hem statik hem de dinamik gülmede erkeklere göre ortalama dişeti görünme miktarı daha fazladır (p<0.05). Sonuç: Gülme hattı, hastanın poz vererek fotoğraflandığı kayıtlar üzerinde değerlendirildiğinde, video kayıtlar yoluyla elde edilen doğal gülme hattına göre daha düşük bulunmuştur. Hastalar poz vermek yerine doğal şekilde güldüklerinde gingival görünüm arttığından, klinik değerlendirmeler ve restoratif düşünceler dinamik gülüşe göre planlanmalıdır. Anahtar kelimeler: Gülme hattı, statik gülüş, dinamik gülüş, dişeti, estetik.*

Ethics Committee Approval: This study was approved by Baskent University Institutional Review Board (Project no: D-KA20/10).

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

Peer-review: Externally peer-reviewed.

Author contributions: OA and UY participated in designing the study. OA participated in generating the data for the study. UY participated in gathering the data for the study. OA, UY participated in the analysis of the data. OA wrote the majority of the original draft of the paper. UY participated in writing the paper. OA, UY have had access to all of the raw data of the study. OA and UY have reviewed the pertinent raw data on which the results and conclusions of this study are based. OA and UY have approved the final version of this paper. OA and UY guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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

Financial Disclosure: The authors declared that this study received financial support Başken University Research Fund.

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2022; 56(1): 28-34

Original research

Oral health experiences of Turkish children with acute rheumatic fever or rheumatic heart disease

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Purpose
Children with acute rheumatic fever (ARF) or using depot-penicillin because of rheumatic heart disease (RHD) are prone to the risk of infective endocarditis (IE) and poor oral hygiene. This cross-sectional study aimed to investigate oral health experiences of a group of healthy children and a group of children with ARF or who were using depot-penicillin because of RHD (study group).

Materials and Methods
Medical and dental data of 86 children aged between 5-12 years were investigated in this study. Medical histories, decayed, missing, and filled teeth, plaque index, gingival index, toothbrushing frequencies, and the study and healthy groups' socioeconomic levels were recruited and examined.

Results
The 'dmft' of the study and healthy groups were found to be 5.51±3.81 and 2.37±2.31, respectively, while the 'DMFT' of the study and healthy groups were 1.71±2.28 and 1.06±1.59, respectively. There was no significant difference between the gingival indexes of the study group 0.89±0.39 and the healthy group 0.62± 1.03 (p=0.112). Nevertheless, the groups significantly differed regarding the plaque index, which were 0.87±0.40 and 0.45±0.41, respectively (p<0.001). The tooth brushing frequencies in the study and healthy groups being twice a day or more were 23.3% and 46.5%, respectively.

Conclusion
The children with ARF or using depot-penicillin because of RHD had more permanent and primary tooth caries and poorer oral health than the healthy group in this study.

Keywords: *Acute rheumatic fever, dental caries, cardiac disease, rheumatic heart disease, oral health*

Introduction

The acute rheumatic fever (ARF) is an inflammatory disorder mainly involving the heart and joints, but rarely the central nervous system, skin, and subcutaneous tissues as a late-stage finding, due to Group A beta-hemolytic streptococcal (GABHS) infections (*Streptococcus Pyogenes (SP))* during childhood (1). This disease manifests the peak ratio in school-age children aged 5-15 years (2). While it has nearly evanesced in the affluent countries (North America and Western Europe), it is still a significant problem in developing countries (2,3). Although there has been no factual statistical data about this disease in Turkey, it has been suggested to be seen with a frequency similar to the Middle Eastern and Mediterranean countries (25-100 per 100.000) (4,5). The status of both ARF and rheumatic heart disease (RHD) have changed due to improved living conditions, good nutrition, advanced medical nursing, penicillin, oral and medical hygiene (2,3).

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Received: 15 January 2021
Revised: 2 April 2021
Accepted: 6 August 2021

DOI: 10.26650/eor.2022868100

How to cite: *Sogukpinar Onsuren A, Gullu UU, Ipek S. Oral health experiences of Turkish children with acute rheumatic fever or rheumatic heart disease. Eur Oral Res 2022; 56(1): 28-34.*

ARF diagnosis is made with either two major criteria or one major and two minor criteria (1). The 2015 revision of the Jones Criteria for ARF indicates only two groups regarding this illness: moderate-high and low-risk groups (6). The critical symptom of ARF (60%) with long-term sequelae is carditis (1,2). While RHD may be seen after known ARF, the disease might be formerly asymptomatic, or patients may not recall ARF history (7). The minor complications of ARF are arthralgia, fever, first-degree heart block, and the high ratio of acute phase reactants (1).

If ARF patients do not have intramuscular injections of benzathine penicillin G (BPG) every 21 days, they will likely have more cardiac problems, heart failure risk, palsy, and early demise (3,7). The prophylaxis may avoid an initial ARF attack following a sore throat (Table 1) (3). The American Heart Association has suggested that children with a complaint of ARF or RHD should receive secondary penicillin prophylaxis (SPP) as either oral diurnal penicillin (penicillin V) or BPG every 21 or 28 days (3). Patients might have infective endocarditis (IE) in the presence of three major components: a cardiac lesion and endocardial harm, significant mucosal lesions responsive to bacteremia and the microbial inoculant capacity, and the bacterial factor's virulence. IE might be diagnosed and treated early; however, the mortality rate varies between 10-15%. IE, which has complicated relationships with invasive dental therapies and poor oral health, occurs in ARF patients' mitral or aortic valves since they are most frequently disrupted (7). Thorney *et al.* (8) claimed that dental caries is associated with sugar, and ARF is fed by sugar. Thus, if the patient has untreated caries, ARF's prognosis might worsen. Untreated dental caries has adverse impacts on children's well-being and public health status, and may create pain, abscess, and fever, together with chewing, growing, and sleeping problems.

Oral health is a crucial part of public health, with a two-sided correlation (9). The oral cavity is the most prevalent origin for bacteremia due to periodontal pockets, dental pulp, and periradicular bone. Tooth brushing, flossing, chewing, and breathing are the daily activities for spreading bacteremia (7). Administration of drugs with sucrose is a risk factor for caries and periodontal diseases in children (10). A dental therapy, such as performing an extraction, periodontal surgery, scaling, and root planning, are risk factors for bacteremia (7). Ultimately, a team involving a pediatric cardiologist and a pediatric dentist should collaborate for their patients' oral health (11). If the cardiac patient is in the high-risk group for IE, a pediatric cardiologist may recommend dental therapy for the patient (Table 2) (7).

If an invasive dental procedure is indicated, patients under IE risk should have proper oral health. In the literature, children with congenital heart disease (CHD) have been more susceptible to caries lesions when compared to healthy control groups (10,12). To our best knowledge, only a few studies have assessed the impacts of interference with oral health in children with ARF (8,13-15). Moreover, there is no such previously conducted study in Turkey.

Hence, this cross-sectional study aimed to investigate oral health experiences in healthy children and a group of children with ARF or using depot-penicillin because of RHD. The null hypothesis tested in the present study is that there would be no difference between the children with ARF or using depot-penicillin for RHD and healthy children.

Materials and Methods

Ethical approval

The ethical approval of this study was obtained from the Kahramanmaras Sutcu Imam University Faculty of Medicine

Table 2. *The high risk of the patients with CHD (7).*

- Prosthetic cardiac valve or prosthetic material used for cardiac valve repair
- Prior IE
- Unrepaired cyanotic CHD, inclusive of palliative shunts and conduits
- In patients who have cyanotic CHD with residual defect or palliative residual defect
- In patients who have full-repaired CHD without residual defects up to 6 months after surgery
- In patients who have undergone transplantation and whose valve insufficiency continues

Table 1. *Methods for prevention from streptococcal infections in acute rheumatic fever (3).*

Medicine	Application criteria	Dose/Method of administration	Primary protection time	Secondary protection time
Benzathine penicillin G	>27 kg ≤27 kg	1.200.000 unit, IM 600.000 unit, IM	Single-dose Single-dose	Every 21 days*
Penicillin V	Primary protection Secondary protection	3x250 mg/day, PO 2x250 mg/day, PO	10 days	Everyday
Sulfadiazine or sulfisoxazole	>27 kg ≤27 kg	1x1 gr/day, PO 1x0.5 gr/day, PO	-	Everyday
Erythromycin**	Primary protection Secondary protection	20-40 mg/kg/day, 2-4 dose, PO 2x250 mg/day, PO	10 days	Everyday
1st generation cephalosporin***	Primary protection	Variable by drug	10 days	-

** In regions where rheumatic fever is not endemic, this period can be extended to 28 days. ** Given to those with penicillin allergies*** 15% of people with penicillin allergy also develop an allergic reaction to cephalosporins. IM: Intramuscular, PO: Peroral.*

Clinical Investigations Ethics Board (2019/18-294). Written informed consent was obtained from the parents of the children included in the study.

Study design and participants

The present study was a cross-sectional one, conducted with all children admitted to the Kahramanmaras Sutcu Imam University Faculty of Medicine of Pediatric Cardiology Department between August 2019 and March 2020 and diagnosed with cardiac disease. A group of children with ARF or using depot-penicillin because of RHD (study group) was compared to a randomized group of healthy children (control group; no medical history and systemic disease).

A total of 86 children aged between 5-12 years were included in the study. The participants were divided into two groups: study group (n=43) and healthy group (n=43). Fourteen males and 29 females with a mean age of 10.3±1.81 years were in the study group, whereas the healthy group comprised 22 males and 21 females with a mean age of 9.65 ±2.64 years.

Inclusion criteria

Children with ARF or using depot-penicillin because of RHD and referred to the Pediatric Dentistry Department during the study period were included.

Exclusion criteria

Children with a medical history of cardiac or other disorder (e.g., cancer, chronic renal disease, chronic liver disease, autoimmune disease, congenital heart disease, aplastic or different types of anemia, immune disorder, cardiomyopathy, congenital malformation, neoplasm, metabolic syndrome, nervous system disorder, chronic lower respiratory disease, neonatal cerebral disorder) were excluded from the study (8,16). Inputs of the study were realized by a single pediatric cardiologist (U.U.G).

Data collection

The study was conducted on data related to gender, age, socioeconomic status, toothbrushing habits, cardiac status detection, and dental health.

Clinical oral investigation

The caries experiences of the patients were noted according to the World Health Organization (WHO) criteria for primary (dmft) and permanent teeth (DMFT) indexes (i.e., the sum of decayed, missing (due to caries), and filled teeth). The Gingival and Plaque Indexes were measured at the Kahramanmaras Sutcu Imam University of Faculty of Dentistry of Pediatric Dentistry Department. A pediatric dentist (A.S.O) examined the dried teeth with a probe and mount mirror in a dental unit.

Gingival Index was measured for six surfaces using a probe pressed into the gingival margin parallel to the buccal surfaces (55/16, 52/12, 64/24, 75/36, 72/32, and 84/44). The scoring was recorded as follows: 0 – no inflammation and no bleeding; 1- bleeding on at least one surface or mild inflammation, such as a slight color change; 2- moderate inflammation, such as moderate glazing, redness, edema, and hypertrophy; 3- progressive inflammation, such as advanced redness, edema, and spontaneous glazing (17).

Plaque Index was measured for six surfaces using a probe pressed into the gingival margin parallel to the buccal surfaces (55/16, 52/12, 64/24, 75/36, 72/32, and 84/44). The scoring was recorded as follows: 0- no plaque; 1- a thin film of plaque along the free gingival margin; 2- moderate accumulation with a plaque in the sulcus; 3- a large amount of plaque in the sulcus or pocket along the free gingival margin (17).

The panoramic radiographs and clinical photographs of children were obtained when possible and necessary. The children in the study group were offered prophylaxis involving dental examination and treatment (extracting and periodontal scaling) by their pediatric cardiologist. The European Society of Cardiology's recommendations were considered while deciding on the need for IE prophylaxis (18).

Statistical analysis

The Jamovi (Version 1.0.4) Computer Software was used for all statistical analyses. Descriptive statistics and Chi-square tests were utilized for participants' demographic characteristics. The normality of data distribution was checked using the Shapiro–Wilk test. Since the assumptions for normal distribution were met, one-way analysis of variance (ANOVA) was used for the dmft/DMFT scores, plaque index, and gingival index. The pairwise comparisons were done using Tukey's post hoc test (p=0.05).

Results

Demographic data

A total of 86 children, 43 with ARF or using depot-penicillin because of RHD [14 males (32.6%), 29 females (67.4%)], and 43 healthy children [22 males (51.2%), 21 females (48.8%)] participated in the study. The demographic characteristics of the participants were presented in Table 3. The children's ages were between 5-12 years; the mean age of children in the study group was 10.3±1.81 years, whereas it was 9.65±2.64 years for children in the healthy control group. The

Table 3. *Demographic information of the study participants.*

Variables	Study Group n= 43	Healthy Group n= 43	p value
Age (years ±SD)	10.3±1.81	9.65±2.64	0.072
Sex			
Female	29 (67.4%)	21 (48.8%)	0.080
Male	14 (32.6%)	22 (51.2%)	
Toothbrushing frequency			
≥2 times a day	10 (23.3%)	20 (46.5%)	0.024*
<2 times a day	33 (76.7%)	23 (53.5%)	
Socioeconomic status			
Low	13 (30.2%)	16 (37.2%)	
Medium	25 (58.1%)	22 (51.2%)	0.778
High	5 (11.6%)	5 (11.6%)	

study and healthy groups' socioeconomic status were low in 13 (30.2%) and 16 (37.2%) children, moderate in 25 (58.1%) and 22 (51.2%) children, and high in 5 (11.6%) and 5 (11.6%) children, respectively. There was no statistically significant difference between the two groups regarding age, gender, and socioeconomic status (p=0.072, p=0.80, p=0.778, respectively). The study and healthy groups' toothbrushing frequencies being two or more a day were 23.3% and 46.5%, respectively. A significant difference was present between the two groups regarding the toothbrushing frequency (p=0.024).

Medical diagnosis

Thirty-four patients (79.1%) had mild mitral insufficiency, and two patients (4.7%) had severe mitral insufficiency in our study. Twenty-three patients (53.5%) had mild aortic regurgitation and, only one patient (2.3%) had severe aortic regurgitation. The numbers of patients with first-degree, second-degree, and third-degree carditis were 37 (86%), 4 (9.3%), and 2 (4.7%), respectively (Table 4).

Oral health experiences: prevalence and severity

The children's data relevant to caries, gingival index, and plaque index were presented in Table 5. While the 'dmft' index is used for primary teeth of children aged 5-12 years, the 'DMTF' index refers to permanent teeth of children aged 12-15 years. The 'dmft' indexes of the study and healthy groups were found to be 5.51±3.81 and 2.37±2.31, respectively, and these values significantly differed between the groups (p<0.001). The patients in the study group had more teeth with caries than the healthy group; the difference was statistically significant (p<0.001). Forty-one children in the study group and 35 children in the healthy group had permanent teeth. The 'DMFT' indexes of the groups were 1.71±2.28 and 1.06±1.59, respectively. However, these values did not significantly differ between the groups (p=0.150). There were more permanent teeth with caries in the study group, and the difference was statistically significant (p=0.029).

It was found that there was no significant difference between the gingival indexes of the study group (0.89±0.39) and the healthy group (0.62±1.03) (p=0.112). On the other hand, the groups significantly differed regarding the plaque index, which were (0.87±0.40) and (0.45±0.41), respectively (p<0.001).

Table 4. *The medical diagnoses of the patients in the study group.*

Mitral Insufficiency (MI)	
Absent	3 (7.0%)
Mild	34 (79.1%)
Moderate	4 (9.3%)
Severe	2 (4.7%)
Aortic Regurgitation (AR)	
Absent	19 (44.2%)
Mild	23 (53.5%)
Moderate	0 (0.0%)
Severe	1 (2.3%)
Degree of Carditis	
First	37 (86.0%)
Second	4 (9.3%)
Third	2 (4.7%)

Table 5. *The oral health experiences of children with acute rheumatic fever or using depot-penicillin because of rheumatic heart disease and the healthy group.*

Caries index	Group	N	Mean ± SD	p-value
D	Study group	43	5.05±3.61	<0.001*
	Healthy group	43	1.14±1.92	
M	Study group	43	0.33±0.78	0.028*
	Healthy group	43	0.05±0.21	
F	Study group	43	0.14±0.52	<0.001*
	Healthy group	43	1.19±1.40	
Dmft	Study group	43	5.51±3.81	<0.001*
	Healthy group	43	2.37±2.31	
D	Study group	41	1.32±1.71	0.029*
	Healthy group	35	0.57±1.19	
M	Study group	41	0.22±0.82	0.154
	Healthy group	35	0.03±0.17	
F	Study group	41	0.17±0.77	0.158
	Healthy group	35	0.46±0.95	
DMFT	Study group	41	1.71±2.28	0.150
	Healthy group	35	1.06±1.59	
Gingival index	Study group	43	0.89±0.39	0.112
	Healthy group	43	0.62±1.03	
Plaque index	Study group	43	0.87±0.40	<0.001*
	Healthy group	43	0.45±0.41	

Discussion

ARF and RHD are estimated to affect almost 20 million children in developing countries and constitute the leading cause of cardiovascular death during the first five years of life (2). The incidence of ARF has decreased with improvement in living conditions and common antibiotics for treating streptococcal transmission (7).

To our best knowledge, this study is the first dental study conducted on Turkish children with ARF or using depot-penicillin because of RHD. This study had some restrictions, such as that the examined study group was small, no power analysis was performed due to unclear prevalence of ARF in the Turkish population, and some patients with ARF but no cardiac involvement were excluded. This study aimed to emphasize the importance of oral health in these diseases. The study group diagnosed by an experienced pediatric dentist disclosed some significant results for their previous dental therapies. The oral health examinations relied on clinical and radiographic diagnoses. The children were first examined when they were admitted to the Pediatric Cardiology Department of Medical Faculty to receive intramuscular injections of BPG.

The literature embodies several studies comparing children with ARF and healthy children regarding their caries experiences (8,13-15). Thornley *et al.* (8) claimed that there was a positive correlation between caries and ARF. Intake of sugar leads to dental caries; therefore, the relationship between sugar intake and ARF might be significant. *Streptococcus Mutans (MS)*

is a bacterium associated with dental caries; therefore, there may be a correlation between this bacterium and ARF (19). *SP,* which is related to ARF, ferments sugar (especially glucose and fructose), and the sugar is proposed to be a fundamental source of ARF owing to the growing number of *SP* in sugar (20). Nevertheless, in their study, Thornley *et al.* (8) used the hospital records for the participants' medical histories. In our study, the children were diagnosed by a pediatric dentist when they presented to the Department of Pediatric Cardiology.

The dmft/DMFT outcomes in our study suggest that the null hypothesis should not be accepted. The dmft index of primary teeth in the study group was found to be significantly higher than that of the healthy group ($p<0.001$), similar to various studies conducted with cardiac patients in the literature (10,12). One study concluded that the patient group with ARF had significantly more caries than the control group (14). Such a result in this study may suggest that using depot-penicillin because of RHD accelerates caries in primary teeth (21). Dental treatments may cause significant problems, such as monetary, psychological, somatic, and educational problems, in small-age children. A team consisting of a pediatric dentist and a pediatric cardiologist might inform patients about the importance of optimal oral health. A general dentist might consider that his/her knowledge may be insufficient for children with special needs and may direct such patients to a pediatric dentist.

This study group's missing primary teeth were significantly more than that of the healthy group ($p=0,028$). When the pulp tissue is involved in caries lesions, the pediatric dentist uses radical treatment such as extraction instead of root endodontic treatment or vital pulp therapy (22). The extraction might be performed under antibiotic prophylaxis (18).

The study group's filled primary teeth were significantly less than that of the healthy group ($p<0.001$). It is known that if dental caries of a patient with ARF or who is receiving depot-penicillin for RHD is not treated, the patient will have an increased risk of dental sepsis. The sepsis outcome may be lethal or critical complications may develop in surgically undermined children (7). Optimal oral health restrains bacteremia and IE. When the patient's dental therapy was tooth extraction or a periodontal operation, it was performed by the pediatric dentist (A.S.O) under antibiotic prophylaxis. The guideline claims that dental procedures should be performed under antibiotic prophylaxis when oral mucosal perforation involving the tooth's periapical area and manipulation of gingival tissue are present (18). As formerly reported, caries in primary teeth increases the possibility of developing caries in permanent teeth, and plaque and gingivitis are risk factors for periodontitis in the future (23,24).

The DMFT index of permanent teeth was found to be 1.71 ± 2.28 in the study group, and there was no significant difference between the groups ($p=0.150$). Similar to our study, Stecksen-Blicks *et al.* (10) reported no significant differences between patient and control groups regarding permanent teeth and suggested that such a result might have been related to the participants' age. Our findings were also parallel with what was previously reported for cardiac patients in the literature (10,25). Moreover, the study group's permanent tooth caries was significantly more than that of the healthy group in our study ($p=0.029$), similar to Franco *et al.'s* study (26). On the other hand, the number of filled permanent teeth in the study group was less than that of the healthy group, but it did not significantly differentiate them ($p=0.158$). In a study, the children with CHD were more worried than their healthy peers due to their former therapy experience and hospitalization; thus, such children might delay visiting their dentists due to their anxiety (27). The missing permanent teeth in the study group were higher in number than that of the healthy group, and there was no statistical difference between the groups ($p=0.154$). This result may be related to the the patients' age. In this age group, children are more aware of the importance of tooth brushing.

In our study, the plaque index of the study group was significantly higher than that of the healthy group ($p<0.001$), which shows a similarity with the study of Ali *et al.* (28). Poor oral health among Turkish children might be related to the typical attitude towards dental treatments and less awareness of dental treatments' importance.

It has been strongly recommended that patients brush their teeth twice a day, use dental floss, and visit their dentists regularly, thus protecting their oral health and quality of life (7). In our study, 23.3 % of the children in the study group brushed their teeth more than twice a day. There was a significant difference between the groups in this study regarding tooth brushing ($p=0.024$). Berger (29) claimed that pediatric cardiac patients with more caries lesions might have had improper tooth brushing habits.

There was no statistical difference between the groups in our study regarding socioeconomic status ($p=0.778$). Previous studies investigating the relationship between socioeconomic status and dental caries lesions have suggested precise results (29,30). Like our study, Balmer *et al.* (22) claimed no association between socioeconomic status and dental caries lesions in children with CHD. The result in the present study may be expounded upon the free-of-charge dental therapy in Turkey.

Moreover, patients with systemic disease and their parents should be more aware of preventive dental treatments and required measures to prevent dental caries because it is acknowledged in the literature that oral health and general health are interrelated (9). Some daily practices, such as tooth brushing, dental flossing, and chewing, tend to decrease oral bacteremia risks (7).

In almost half of the patients with this disease, the valvular endocardium may be impaired due to cardiac inflammation (31). The patients' medical histories were taken to investigate their cardiac conditions and suitability for dental operations (Table 4). A further limitation of this study was that the study group's caries could not be fully correlated with the patients' medical histories. All patients with ARF or using depot-penicillin because of RHD started regularly visit our dental clinic after relevant information pertinent to this study's results was provided.

Poor oral hygiene due to caries lesions is a significant problem for IE, and a pediatric dentist and cardiologist should perform proper dental therapy. This study's team recommended that patients visit the dentist regularly, take good care of their oral health (tooth brushing, dental flossing, dental fluoride treatment), and feed with less sugar. If caries is diagnosed initially, dental therapy will be easier, cheaper, and quicker. The parents of children with ARF or using depot-penicillin for RHD should be warned about the infective endocarditis risk.

Conclusion

The children with ARF or using depot-penicillin because of RHD had worse oral health experience than the healthy group in this study.

Türkçe Özet: *Akut romatizmal ateş veya romatizmal kalp hastaliği olan türk çocuklarinin ağiz sağliği deneyimleri. Amaç: Akut romatizmal ateş (ARA) veya romatizmal kalp hastalığına (RKH) bağlı depo penicillin kullanan çocuklar enfektif endokardit (EE) riskine ve kötü ağız hijyenine yatkındır. Bu kesitsel çalışmanın amacı bir sağlıklı çocuk grubu (kontrol grubu) ile ARA veya RKH nedeniyle depo penicillin kullanan çocukların (çalışma grubu) oral bulgularını karşılaştırmaktır. Gereç ve yöntem: Bu çalışmada yaşları 5-12 arasında toplam 86 çocuğun tıbbi ve diş verileri incelenmiştir. Çalışma ve kontrol gruplarının tıbbi öyküleri, çürük, çekim ve dolgulu diş, plak indeksi, gingival indeks, diş fırçalama sıklıkları, sosyoekonomik durumları kaydedildi ve incelendi. Bulgular: Çalışma ve kontrol gruplarında 'dmft' sırasıyla 5,51 ± 3,81 ve 2,37 ± 2,31 ve bu gruplarda 'DMFT' sırasıyla 1,71 ± 2,28 ve 1,06 ± 1,59 olarak bulunmuştur. Çalışma ve kontrol gruplarının gingival indeksleri sırasıyla 0,89 ± 0,39 ve 0,62 ± 1,03 olup, istatistiksel olarak aralarında anlamlı fark yoktur (p=0,112). Bununla birlikte, grupların plak indekleri sırasıyla 0,87 ± 0,40 ve 0,45 ± 0,41 dir ve aralarında anlamlı fark vardır (p<0,001). Çalışma ve kontrol gruplarının günde iki kere veya daha fazla diş fırçalama sıklığı %23,3 ve %46,5'dir. Sonuç: ARA veya RKH nedeniyle depo penicillin kullanan çocukların sağlıklı gruba göre süt dişlerinde ve daimi dişlerinde çürükleri daha fazladır ve ağız hijyenleri daha kötüdür. Anahtar Kelimeler: Akut romatizmal ateş; diş çürüğü; kalp hastalığı; romatizmal kalp hastalığı; ağız sağlığı.*

Ethics Committee Approval: The ethical approval of this study was obtained from the Kahramanmaras Sutcu Imam University Faculty of Medicine Clinical Investigations Ethics Board (2019/18-294).

Informed Consent: The informed consents were obtained from the parents of the children included in the study.

Peer-review: Externally peer-reviewed.

Author contributions: ASO participated in designing the study. ASO participated in generating the data for the study. ASO, UUG, SI participated in gathering the data for the study. ASO, UUG, SI participated in the analysis of the data. ASO wrote the majority of the original draft of the paper. ASO participated in writing the paper. ASO has had access to all of the raw data of the study. ASO, UUG, SI have reviewed the pertinent raw data on which the results and conclusions of this study are based. ASO, UUG, SI have approved the final version of this paper. ASO, UUG, SI guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2022; 56(1): 35-41

Original research

Which is the most effective biomaterial in indirect pulp capping? 4- year comparative randomized clinical trial

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Purpose
The aim of this study is to compare the clinical outcomes of Mineral Trioxide Aggregate (MTA) and calcium hydroxide pulp capping after complete caries removal.

Materials and Methods
In 73 regular patients (47 women, 26 men; age 20.65±3.02 years), having at least one deep carious lesion was recruited. Following complete caries removal, the pulp was indirectly capped with either MTA(n=51) or calcium hydroxide (n =49), randomly. Final restoration with a resin-based composite in a single session was performed. Clinical parameters including pulp vitality, sensitivity to cold or heat stimulants, percussion tests and discomfort during chewing and color were recorded after 6 months, 1 year, 2- year, 3- year and 4- year. Data were analysed statistically ($p < 0.05$).

Results
After 4- year, the survival rates were 86% (for MTA), and 82.9% (for calcium hydroxide). Totally, 8 teeth from calcium hydroxide group and 7 teeth from MTA group were endodontically treated. No significant difference was detected between the groups in terms of pulp vitality (p=0.613). Grey discoloration rate was 63% in MTA group.

Conclusion
Both pulp-capping materials, MTA and calcium hydroxide showed similar clinically successful performance in terms of pulp vitality in the treatment of deep dentin caries lesions after 4- year.

Keywords: *Mineral trioxide aggregate, calcium hydroxide, indirect pulp capping, complete caries excavation, randomised clinical trial*

Introduction

The goal of indirect pulp capping treatment of the vital teeth must be the protection of tooth vitality by regenerating reparative dentin at the materio - pulpal complex, that works as a biological seal before causing to the need of more expensive and invasive treatment option such as root-canal treatment (1).

For the stimulation of the self-remineralizing properties of dentin, bacteriostatic and / or bactericidal and biocompatible agents can be applied on deep dentin neighbouring vital pulp before the placement of permanent restoration. Calcium hydroxide ($Ca(OH)_2$) is widely used in the treatment of deep caries lesions. It has a series of reparative properties arising from thermoelectric stimulus blockage, initial low-grade irritation of pulp tissue, and anorganic calcium ion precipitation, which are demonstrated to be associated with the differentiation of dental pulpal cells (2,3). On the other hand, some disadvantages were reported such as gradual degradation, tunnel defects, an increased number of inflammatory cells and localized pulp necrosis, over time (4-7). Hence, the treatment outcomes

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Received: 15 May 2021
Revised: 22 July 2021
Accepted: 8 August 2021

DOI: 10.26650/eor.2022895748

How to cite: *Koc Vural U, Kiremitci A, Gokalp S. Which is the most effective biomaterial in indirect pulp capping? 4- year comparative randomized clinical trial. Eur Oral Res 2022; 56(1): 35-41.*

of calcium hydroxide pulp-capping can be considered as unpredictable since different success rates ranged between 13-96% were reported, previously (8-10).

Considering the disadvantages of calcium hydroxide, bioactive silicate cements, such as mineral trioxide aggregate (MTA) was introduced with many indications including treatment of deep caries lesions. Although, pulp exposure cannot be seen directly in the absence of bleeding, an unpredictable micro-level pulp damage may occur during deep dentin caries excavation.

MTA has been introduced as a successful pulp-capping material owing to its small sized particles providing tight barrier against the migration of microorganisms and high pH level leading minimal inflammatory reaction during healing. Besides, MTA can stimulate cell differentiation/activation, which may contribute to hard tissue matrix formation/mineralization (11). Ford, et al. (12) investigated the MTA pulp-capping in monkey teeth and found that the clinical performance of MTA was higher than calcium hydroxide in terms of pulpal inflammation and dentin bridge formation. MTA showed better hard tissue formation and less pulpal inflammation compared to calcium hydroxide (13).

Deep caries lesions can be treated by complete, partial or stepwise caries excavation techniques. Complete caries excavation technique suggests to remove whole carious lesion from dentin at one appointment. In partial caries excavation technique, only the caries in peripheral areas is removed. Stepwise technique suggests the removal of whole deep caries lesion at several appointments gradually (14). In complete caries excavation technique, decayed dentin is fully removed followed by final restoration. Nowadays, although complete caries excavation or removal of whole carious dentin regarded as over-treatment, several surveys carried out in different countries indicated that approximately 70% of the responding dentists continue to use complete caries removal technique thinking complete caries removal has an increased pulp exposure risk but the fear about the progression of the carious lesion is eliminated (1,15-25). Thus, the caries removal technique must be the technique of choice depending on the time, patient's attendance to the second appointment or specific requirements of the patients. In this study, complete caries removal technique was applied as it is mostly preferred by dental practitioners. There is a limited number of mid-term clinical trials comparing the clinical performance of MTA and calcium hydroxide after complete caries removal, which allows clear recommendations for daily dental practice. As the survival rates of the treated teeth tend to drop with time, short - term findings may overestimate the treatment outcome (8,26). Therefore, the aim of this mid-term clinical study was to compare the outcomes of MTA and calcium hydroxide indirect pulp-capping regarding conservation of pulp vitality in a 4-year follow-up period. The tested null hypothesis was there would be no difference between the materials in terms of treatment outcome at 4-year follow-up period.

Materials and Methods

Ethical statement

This article contains studies with human participants. The local ethical committee approved this prospective randomized clinical trial and registered to clinicaltrials.gov. All procedures were performed in line with the ethical standards of the institutional / national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent of each participant was obtained after the study procedures clearly explained.

Sample size calculation

Before the recruitment, the n number was calculated using the G-power program. The parameters were as follows: Effect size = 0.4, type I error = 0.05, power = 0.85, Df = 5. Although obtained sample size was n=90, the n number was increased to 100, to considering possible drop-outs.

Study participants

The candidates, presented with at least 1 permanent premolar / molar tooth characterized by deep caries lesions without pulp exposure were recruited to study population.

Inclusion and exclusion criteria

The inclusion criteria were as follows: the teeth judged by the clinician that; removal of the deep caries lesion after direct complete caries excavation would not cause to exposure of the pulp (radiograph depth reaching 3/4 of the dentin), absence of any signs of irreversible pulpitis at radiographic examination, including widening of the periodontal ligament or absence of periapical lesions, absence of spontaneous and heavy pain, abscesses, or sinus tract, functional permanent posterior teeth, where the direct restoration is indicated and the teeth, positively responded to the electric pulp-testing and negatively responded to thermal-testing. Exclusion criteria were; two or more cuspal loss, caries beneath the gingival margin, spontaneous pain history, presence of any periapical pathology, immature teeth with open apex and pathologic mobility

A hundred and ten patients were assessed for eligibility. The radiographs were used to predict the distance between the caries and the pulp. Seventeen candidates refused to participate (n= 26), 9 candidates with deep caries lesion had spontaneous pain history (n= 12), 11 teeth with deep caries lesion were unrestorable without crown restoration (n=14). Finally, 100 teeth in 73 patients, meeting the inclusion criteria were recruited.

Study groups

The recruited teeth were randomly assigned as MTA or calcium hydroxide groups. After complete caries removal, the control group received the calcium hydroxide (Dycal, Dentsply/Caulk, Dentsply International Inc, Milford, DE, USA) while the test group received MTA (Dentsply, Tulsa Dental, Johnson City, TN, USA) pulp-capping followed by final restoration in the same session in both groups.

Randomization and blinding

The randomization unit was the tooth. A simple randomization technique was used for the selection of pulp-cap-

ping material. Blinding of patients was also possible. To avoid possible influence in the process, the clinician was not aware about the indirect capping material until randomization during removal of the carious lesion. If more than one case is included into the study in one patient, after allocation of the first tooth, the second case was always treated using other pulp-capping material. Thus, neither the investigators nor the participants were not aware which treatment was administered. Blinding at the follow-ups were not possible in all cases due to the grey discoloration of MTA over time.

Calibration

Only one researcher managed the operative procedures for standardization. Kappa statistics were performed to reveal inter / intra examiner agreement at follow-ups. Inter and intra examiner agreement in the detection of success or failure was determined as perfect ($k > 0.98$).

Interventions

Description of the materials were presented in Table 1. All teeth were treated under consistent, standardized conditions using a minimal invasive standardized operative intervention procedure. Single operator conducted clinical treatments to standardize the procedures. A local anaesthetic was administered before the operation procedure, if needed (severe sensitivity or pain during the preparation, rinsing, or drying and dental phobia). Carious lesion was revealed using sterile diamond burs at high-speed under copious water-cooling. Removal of superficial and soft dentin was started from lateral walls and dentino-enamel junction using a sterilized sharp and spoon-shaped hand excavator. Then, a slow-speed handpiece with sterilized stainless-steel burs were used to complete caries excavation. The cavities were rinsed with water and dried with mild water.

Hardness, color and the unique sound of the dentine while probing were used to assess the caries free diagnosis of the dentine. The isolation was achieved with a well-performing saliva ejector and cotton rolls. Subsequently, the teeth were then randomly divided into MTA or calcium hydroxide groups. The materials were applied according to the manufacturer's instructions. A detailed application procedure, the names, compositions and manufacturers of the materials used are presented in previous report of the present study (27).

Following pulp capping, a resin modified glass ionomer cement (RMGIC), (SDI, Riva Light Cure LC, Southern Dental Industries, Bayswater, Australia) was mixed and placed according to manufacturers' instructions. Then, an etch&rinse adhesive (Prime and Bond NT, Dentsply DeTrey, Konstanz, Germany) was applied. The cavities were eventually restored with composite resin (Gradia Direct Posterior, GC, Tokyo, Japan) using incremental layering technique. Composite finishing burs, discs and rubber cones were used for finishing and polishing of the final restoration.

Data collection

Two calibrated and experienced researches other than the operator were trained to ensure standardization of the evaluating process at recalls. They collected the demographic and clinical data from the patients. Demographic data included age, gender and number of interfered teeth at pre-treatment time, while clinical data were collected before the intervention (baseline) and at 6- month, 1- year, 2- year, 3- year and 4- year follow ups, which included electric pulp testing (Kerr Vitality Scanner 2006, SybronEndo, Orange, CA, USA), thermal testing (air-water syringe) tactile tests (palpation and percussion), as well as the predictors of inflammation and self-reported patient history.

The treatment outcome was decided as "clinically successful" when the following criteria were met: positive answer to electric pulp testing, negative response to cold stimuli or triggered but not lingering by air-water shrinkage, no general pain, normal response to tactile tests or triggered pain not lingering, absence of abscess, sinus tract and non-physiological tooth mobility. In case of persistent severe pain, negative response to electric pulp testing or triggered pain by tactile or cold tests, the patient was referred for root canal treatment and the treatment outcome was considered to be "clinically unsuccessful". The presence of discoloration reflecting from under the restoration also recorded.

Table 1. *Description of the materials.*

Material	Composition	Manufacturer
Dycal®	Base paste: 1,3-Butylene glycol disalicylate, Zinc oxide, Calciumphosphate, Calcium tungstate, Iron oxide pigments Catalyst paste: Calciumhydroxide, N-ethyl-o/p-toluene sulfonamide, Zinc oxide, Titanium dioxide, Zinc stearate, Iron oxide pigments (dentine shade only)	Dentsply/ Caulk, Dentsply International Inc, Milford, DE, USA
ProRoot® MTA	Oxides: Lime(CaO), Silica(SiO_2), Aluminum oxide(Al_2O_3), Ferric oxide(Fe_2O_3), and Bismuth trioxide Tricalcium aluminate, Tetracalcium aluminoferrite, Tricalcium aluminate	Dentsply Tulsa Dental, Johnson City, TN, USA
Riva Light Cure	Compartment 1: Polyacrylic Acid, Tartaric Acid, 2-Hydroxyethyl Methacrylate, Dimethacrylate Cross-linker, Acidic Monomer Compartment 2: Fluoroaluminosilicate glass powder	LC/Southern Dental Industries – SDI, Bayswater, Australia
Prime and Bond NT	Di and Trimethacrylate resins, PENTA (dipentaerythritol penta acrylate monophosphate), Nanofillers — Amorphous Silicon Dioxide, Photoinitiators, Stabilizers, Cetylamine hydrofluoride, Acetone	Dentsply Detrey, Konstanz, Germany
Gradia Direct Posterior	Methacrylate monomers, Silica, Fluoro-Alumino-Silicate Glass, Prepolymerised filler, Pigments, Catalysts	GC, Tokyo, Japan

Statistical analysis

IBM SPSS package version 19 (IBM, Armonk, NY,USA) was used for statistical analysis. The data were analysed using descriptive statistics including standard error, min. max. and mean values. The Kaplan-Meier survival analysis was conducted to reveal the survival rate. The qualitative variables were compared using Pearson chi-square, Fisher exact and logrank statistics tests. P<0.05 was determined as statistically significant.

Results

The study population consisted of 47 women (64.4%) and 26 men (35.6%). The mean age was 20.65 ± 3.02 for the calcium hydroxide group and 21.18 ± 3.85 years for the MTA group. The most commonly recorded preoperative tooth symptom was sensitivity to cold air. The patient characteristics, tooth distribution and preoperative symptoms observed are shown in Table 2. The recall rate was 72.1% at 4- year post-treatment. One patient at 24 months, 8- at 36 months, 5- at 48 months did not attend to follow up. At 48 months, out of 100 analysed treatments, 14 teeth were lost to follow-up (6 Pertaining to MTA group, and 8 pertaining to calcium hydroxide group) (Figure 1). At 4-year, 15 teeth were treated endodontically due to the irreversible pulpitis (2- at six months, 3- at 1 year, 1- at 2- year, 6- at 3- year, 3- at 4- year) (Figure 1). The Kaplan-Meier function allowed the analysis of the cumulative survival rate of the pulp at 48 months. This function revealed that the survival rate 82.9% for calcium hydroxide and, 86% for MTA after 48 months. Survival

Table 2. *The patient characteristics, tooth distribution and preoperative symptoms. *Mineral trioxide aggregate*

	Calcium hydroxide	MTA*
	N %	N %
Sex		
Female	20 (27.4)	27 (37.0)
Male	14 (19.2)	12 (16.4)
Age range		
18-20	23 (31.5)	24 (32.9)
21-25	8 (11.0)	9 (12.3)
26-30	3 (4.1)	6 (8.2)
Tooth distribution		
Premolar	23 (23.0)	15 (15.0)
Molar	26 (26.0)	36 (36.0)
Arch distribution		
Upper	28 (28.3)	23 (23.2)
Lower	20 (20.2)	28 (28.3)
Preoperative symptoms		
Cold	17 (17.0)	24 (24.0)
Heat	6 (6.0)	16 (16.0)
Chewing	4 (4.0)	7 (7.0)
Percussion	10 (10.0)	16 (16.0)

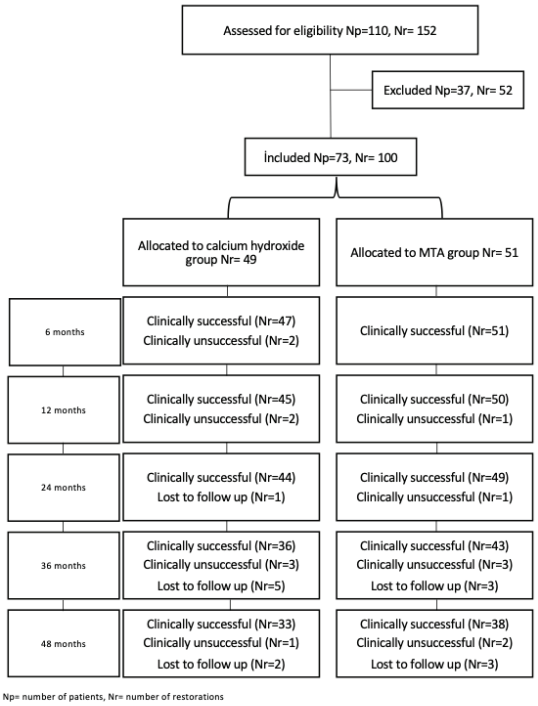

Figure 1. *Flow diagram.*

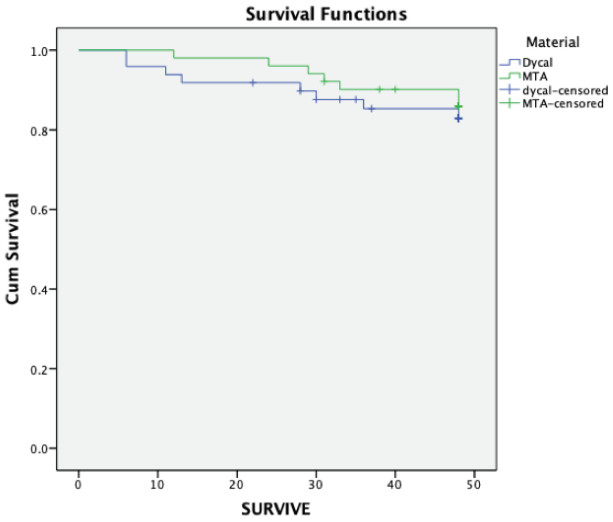

Figure 2. *Survival after indirect pulp capping by materials (Kaplan-Meier curves).*

Table 3. *Distribution of symptoms at baseline, and 6, 12, 24, 36- and 48-months post-treatment. *Mineral trioxide aggregate*

	MTA* (n)				Calcium hydroxide (n)			
	Cold	Heat	Chewing	Percussion	Cold	Heat	Chewing	Percussion
Baseline	7	1	-	1	6	2	2	2
6- mo	5	-	-	2	2	-	1	2
1 year	3	-	-	1	7	1	1	1
2- year	2	-	-	-	3	1	-	-
3- year	-	-	-	1	-	-	-	-
4- year	-	-	1	-	1	-	-	1

graphic showed that, the treatment success rate decreased over time (Figure 2). The longrank test showed no significant difference between the groups regarding the maintenance of pulp vitality at 4- year (p=0.613).

The majority of the teeth included in the study were molars (62%), whereas 38% were premolars (Table 2). No significant relationship was found between upper and lower arches (p = 0.186) or premolar and molar teeth (p = 0.154) in terms of treatment outcome.

None of the teeth showed neither newly developed nor recurrent caries lesion. Only one tooth presented restoration failure at 4- year and received restoration repair. Since the tooth vitality was preserved, this tooth was not removed from the follow-ups. During the 4-year follow-up periods, symptoms were continued to reduce in both groups (Table 3).

At 4-year 1 tooth was symptomatic during the chewing in MTA group while 1-tooth were sensitive to cold-air and 1 tooth sensitive to percussion tests in calcium hydroxide group. Sixty-three percent of the MTA treated teeth showed grey discoloration.

Discussion

The null hypothesis was accepted as no difference was found between the indirect pulp capping materials in terms of success of the treatment outcome at 4- year follow-up period. An ideal pulp-capping material must have the ability to eliminate bacteria, to create an adequate seal and induce mineralization and normal root development (28). At present, despite their well-known drawbacks, calcium hydroxide and MTA are the materials of choice for direct or indirect pulp-capping treatments (28-33). Thus, these two materials were investigated in this randomized controlled study.

Studies showed that, MTA is one of the most investigated material as direct and indirect pulp-capping material (7,34-36). In direct pulp-capping studies, MTA showed superior results compared to calcium hydroxide (37-39). In histologic point of view, better results for MTA were reported compared to calcium hydroxide in terms of osteogenic, odontogenic and angiogenic effects, and inflammatory pulpal cell response (40,41). Besides, in a recently released review article, it was reported that, MTA was found superior than calcium hydroxide for maintaining pulp vitality in direct pulp capping treatment at long-term (42). In terms of indirect pulp-capping of permanent teeth, no statistically significant difference between the materials were reported. The results of the present study are in line with the current literature as no significant differences were found between materials.

Marchi, et al. (43) conducted a 4- year follow-up study and reported 88% success rate for calcium hydroxide after indirect pulp-capping in primary teeth. The result of the current study showed that calcium hydroxide and MTA survival rates were 82.9% and 86%, respectively in human posterior teeth at 4-year follow-up period in parallel with the current literature.

Although, MTA has a series of positive properties, its major disadvantage is an unsightly tooth-discoloration. Discoloration of the tooth after the MTA application can be very important especially in anterior region (44). In the present study 63% of the MTA group showed for grey discoloration, which was in consistent with the current reports (45-47). It is well known that to achieve a successful restoration at long-term relies on durable marginal integrity of the restoration (48). Leakage and bacterial migration from the restoration interface can cause a series of problem such as post-operative hypersensitivity, secondary caries and eventually failure of the restoration (49). In the present study building a well-sealed restoration was the main goal at every stage of the study. Therefore, less clinical symptoms and no secondary caries development during the course of the study was not surprising. Slight tooth sensitivity recorded in a few patients might be related with the preoperative condition of the pulp.

One of the limitations of this study was that blinding at the follow-ups. Some MTA treated cases showed grey discoloration, which makes the blinding impossible. Another limitation is study design. Present study planned as randomized only since to find a patient having at least two deep dentin caries lesions, meeting the inclusion criteria is very difficult. This specific study group did not allow to conduct the study in split-mouth study design. Thus, future studies must be designed considering these limitations.

Conclusion

Indirect pulp-capping with MTA was found clinically more successful than calcium hydroxide at 4- year post-treatment in the treatment of deep caries lesions. However, discoloration risk of MTA treated teeth must be taken into consideration. Prospective clinical trials comparing different indirect pulp capping materials in mid-term and long-term are needed.

Türkçe Özet: *İndirekt pulpa kaplamasında en etkili biyomateryal hangisidir? 4 yıllık karşılaştırmalı randomize bir klinik çalışma. Amaç: Bu çalışmanın amacı, total çürük ekskavasyonu sonrası Mineral Trioksit Agregat (MTA) ve kalsiyum hidroksit pulpa kaplamasının klinik sonuçlarını karşılaştırmaktır. Gereç ve Yöntem: Çalışmaya en az bir derin çürük lezyonu olan 73 hasta (26 erkek; 47 kadın; ortalama yaş 20,65±3,02) dahil edildi. Total çürük ekskavasyonu sonrası, MTA(n=51) veya kalsiyum hidroksit (n=49) materyallerinden biri ile indirek pulpa kaplaması ve takiben tek seansta rezin bazlı bir kompozit ile daimi restorasyon yapıldı. Vitalite, soğuk veya sıcak uyaranlara duyarlılık, perküsyon testleri, çiğneme esnasında rahatsızlık ve renk gibi klinik parametreler 6 ay, 1 yıl, 2 yıl, 3 yıl ve 4 yıl kontrollerinde kaydedildi. Veriler istatistiksel olarak analiz edildi (p < 0.05). Bulgular: 4 yılda ağızda kalma oranları MTA için %86 ve kalsiyum hidroksit için %82.9 bulundu. Kalsiyum hidroksit grubundan 8, MTA grubundan 7 dişe kök-kanal tedavisi uygulandı. Pulpa vitalitesi açısından gruplar arasında anlamlı bir fark bulunmadı (p=0,613). MTA grubunda gri renklenme oranı %63 bulundu. Sonuç: 4 yılda, derin dentin çürüğü lezyonlarının tedavisinde kullanılan MTA ve kalsiyum hidroksit materyalleri, pulpa vitalitesi açısından benzer klinik performans göstermiştir. Anahtar kelimeler: Mineral trioksit agregat, kalsiyum hidroksit, indirek pulpa kaplaması, total çürük ekskavasyonu, randomize klinik çalışma.*

Ethics Committee Approval: The local ethical committee approved this prospective randomized clinical trial.

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: UKV, AK, SG participated in designing the study. UKV, AK, SG participated in generating the data for the study. UKV, AK, SG participated in gathering the data for the study. UKV, AK, SG participated in the analysis of the data. UKV wrote the majority of the original draft of the paper. UKV, SG participated in writing

the paper. UKV, AK, SG have had access to all of the raw data of the study. UKV, AK, SG have reviewed the pertinent raw data on which the results and conclusions of this study are based. UKV, AK, SG have approved the final version of this paper. UKV, AK, SG guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: This study was supported by Hacettepe University Scientific Research Projects Coordination Unit.

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2022; 56(1): 42-48

Original research

The effects of pediatric dentifrices with different types of fluoride on the color change of restorative materials

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Purpose
This study aimed to evaluate the effects of dentifrices with different fluoride content on color change of restorative materials commonly used in pediatric dentistry.

Materials and Methods
Three restorative materials (glass hybrid [Equia Forte (EF)], glass carbomer [GCP Glass Fill (GCP)] and compomer [Dyract XP (DXP)]) were used to prepare 120 disc shaped specimens by using a Teflon ring. Four dentifrice groups were created as Sodium Fluoride (NaF), Amine Fluoride (AmF), Stannous Fluoride (SnF_2) and no-fluoride (n=40). Simulated tooth brushing was performed for each specimen by applying 6720 strokes for 6 months. Color changes [CIEDE2000 ($\Delta E00$)] were calculated by using generalized linear model procedure and the data were subjected to two-way analysis of variance.

Results
The highest color changes for NaF and AmF dentifrice groups were observed in the GCP restorative material ($p<0.05$). The color changes of restorative materials tested with SnF_2 dentifrice group were statistically different ($p<0.05$) in each restorative material and ΔE_{00} values were observed as GCP> EF>DXP. SnF_2 dentifrice provided better color stability for all restorative materials when compared to NaF and AmF dentifrices; although, this was not statistically significant. GCP underwent significant discoloration values when brushed with all types of dentifrices.

Conclusion
Although the glass carbomers caused significant color change, the compomers seem to be more resistant to the color change when brushed with all types of dentifrices. The fluoride content of dentifrices is crucial for the color change of restorative materials.

Keywords: *Pediatric dentifrices, fluorides, color change, discoloration, restorative materials*

Introduction

The concept of dental aesthetics is a crucial issue for children as well as adults, since their beauty perception is affected by today's appearance-oriented culture. Maintenance of dental and oral health with better aesthetic appearance is also important for the physiological and psychological development of children (1). Various hybrid restorative materials have been developed for aesthetic and restorative purposes in pediatric dentistry including polyacid-modified composite resins (compomers), resin modified glass ionomer cements (RMGICs) and glass carbomer cement (GCP) to combine the superior properties of conventional glass ionomer cements with aesthetic advantages of composites (2). Among these restorative materials, compomers and RMGIs are widely used for restorations in pediatric dentistry due to advantageous features like fluoride release and adhesion ability (3). To improve the mechanical properties of

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Received: 16 February 2021
Revised: 22 March 2021
Accepted: 10 August 2021

DOI: 10.26650/eor.2022881264

How to cite: *Kaya E, Yildirim S. The effects of pediatric dentifrices with different types of fluoride on the color change of restorative materials. Eur Oral Res 2022; 56(1): 42-48.*

these restorative materials, most recently glass carbomers were developed as a restorative material containing a polydialkylsiloxane component and nanofluoride hydroxyapatite particles (4).

For the success of all restorative materials, the most important criterion is the color stability after long term use. Discoloration of restorative materials can be caused by various factors as intrinsic and/or extrinsic factors (2). Intrinsic discoloration comprises staining of the restorative material itself due to the matrix type, polymer quality, amount of inorganic filler and the type of accelerator. Extrinsic discoloration arises from water absorption, water-soluble colorants adsorption, insufficient polymerization or poor oral hygiene (5). Deficient oral hygiene accelerates discoloration, since it causes accumulation of stained pellicle and colored residues. Tooth-brushing with dentifrices is widely used in home dental care to provide healthy oral hygiene. Today, there are many commercial dentifrices on the market each with a special function. Fluorides, considered the gold standard for control and prevention of caries, have been added in dentifrices as an active ingredient in general (6).

Dentifrices have been produced with various fluoride contents such as amine fluoride (AmF), stannous fluoride (SnF_2), sodium fluoride (NaF) and sodium monofluorophosphate (SMFP) (7). Although the effects of various dentifrices with different fluoride formulations on the surface roughness of teeth and restorative materials or the effects of dentifrices on removal of tooth staining have been evaluated in several studies, the number of studies investigating the color change of restorative materials caused by the dentifrices themselves is insufficient (3, 6, 8 -13). In the present study, the following null hypotheses; 1) color change is not affected by the restorative material type, 2) there are no difference in the color stability of restorative materials after being exposed to dentifrices with different types of fluoride content were tested.

Materials and Methods

Table 1 presents the characteristics of the materials evaluated in this study. In Table 2, the pediatric dentifrices with different fluoride content employed in the current study are presented.

Specimen preparation

120 specimens (8 mm in diameter × 2 mm thick) were prepared by using a Teflon ring (n = 40). The Teflon ring was covered with a strip of cellulose acetate matrix and held between two 1 mm thick glass slides to eliminate air entrapment and voids. A2 color was used in all materials to ensure standardization. The specimens were randomly divided into four dentifrices groups (n=10). G*Power software program (version 3.1.9.2; power 0.95, α = 0.05, β = 0.05) was used to calculate the minimum sample size (10 specimens per group, n=10), based on a previous study in the literature (5).

Polymerization protocol

Polymerization of DXP specimens was carried out by using a light-emitting diode (LED) polymerization light (Elipar Free light 2, 1,200 mW/cm2, 3M ESPE, Ireland) for 20 seconds to each surface, with the tip of the light on the glass slide for 40 seconds. EF restorative material was applied to each capsule with a 10-second mixer, molded with a carrier, and left at room temperature for 5 minutes to complete the hardening. According with the manufacturer's recommendation, the EF coating was applied to the surface of the specimens and cured for 20 seconds using the LED unit. GCP restorative material was applied to each capsule for 15 seconds with a mixer, molded with a carrier, and the GCP Gloss surface coating was applied following the manufacturer's guide. Curing was performed with GCP CarboLED (1,400 mW/cm2 (max 60° C), GCP-Dental, Elmshorn, Germany) for 90 seconds.

Polishing and storage conditions

After polymerization, aluminum oxide discs (Sof-Lex, 3M ESPE, St. Paul, MN, USA) were used to polish each specimen sequentially with an electric hand piece, at 15,000 rpm. All specimens were numbered to identify each one and preserved in distilled water at 37° C for 24 hours.

Color change measurement and brushing cycles

The specimens were lightly rinsed and dried with tissue paper, before performing color measurement. After calibration of the clinical spectrophotometer (Vita EasyShade Advance 4.0, Ivoclar Vivadent, Liechtenstein), the color of each specimen was measured with the CIEDE2000 color system relative to D65 standard illumination against a standard white background. All measurements were repeated three times for each specimen and the mean value was calculated.

A tooth brushing simulator (Willytec, Munich, Germany) was used to mimic tooth brushing procedure. An electronic toothbrush (Oral-B Junior Kids, Procter & Gamble, USA) and soft toothbrush heads (Oral-B Sensi Ultra-thin, Procter & Gamble, USA) were used by fixing on a holder. Each specimen was fixed on the sample holder with a standardized force of 2 N (14). Each dentifrice was diluted in distilled water in a proportion of 1:1 by weight to mimic the oral environment during tooth brushing. Considering that tooth brushing is performed twice a day, this means that each specimen will be submitted to 40 strokes in a two-minute tooth brushing, resulting total 6720 strokes (1120 strokes in a month) for 6 months. After each 1200 strokes, tooth brushes and dentifrices were renewed and this procedure was repeated for each dentifrice group (15). Specimens were removed from the sample holders, cleaned for 1 minute with an air/water spray and they were wiped with tissue paper for the final measurement. Previous studies that used water as the control group have shown that water caused no visible color change; therefore, dentifrice with no fluoride content was used instead as the control group in this study (16).

Measurement of each specimen was performed three times (L*, c*, h*) with the measuring head of the spectrophotometer in accordance with the CIEDE2000 (ΔE_{00}) system. ΔE_{00} was calculated using the following formula (17) depicted in Figure 1 :

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L} \right)^2 + \left(\frac{\Delta C'}{K_C S_C} \right)^2 + \left(\frac{\Delta H'}{K_H S_H} \right)^2 + R_T \left(\frac{\Delta C'}{K_C S_C} \right) \left(\frac{\Delta H'}{K_H S_H} \right) \right]^{1/2}$$

Figure 1. *Formula used in the present study.*

Evaluation of color differences was carried out ultimately via comparison with 50:50% perceptibility (PT) and 50:50% acceptability (AT) thresholds. The PT (0.81 units) and AT (1.77 units) values for CIEDE2000 (1:1:1) were obtained from a study published recently (18).

Scanning electron microscope (SEM)

After completing final measurement, randomly four specimens were selected from each group to evaluate the micro-morphology of the restorative materials by using SEM. 1,000, 2,000 and 6,000× magnifications were used to photograph the most representative areas with an accelerating voltage of 20 kV while scanning the entire surfaces.

Statistical analysis

For each variable descriptive statistics were calculated and shown as "Mean ± standard deviation (SD). Two-way ANOVA (analysis of variance) with generalized linear model procedure was used. The model included "Dentifrice", "Restorative Material" as the main effects with their two-way interaction term (Dentifrice*Restorative Material). Simple effect analysis with Bonferroni adjustment was used to break down the significant interaction effect term as post hoc analysis. A probability value of less than 0.05 was considered significant. SPSS 14.01 (SPSS Inc. Chicago, IL, USA) was used for statistical analysis.

Results

The mean color change (ΔE_{00}) and standard deviation values of all restorative materials exposed to simulated tooth brushing with four dentifrices are showed in Table 3. Statistically significant differences were indicated with superscript letters in the Table 3. The highest color change for the Brand A and B dentifrice groups was observed in GCP restorative material and this was statistically significant ($p<0.05$). The color change for the Brand C dentifrice group was statistically different in each restorative material and ΔE_{00} values were observed as GCP>EF>DXP ($p<0.05$). For the Brand D dentifrice group, the lowest color change was observed in the DXP material which was statistically significant ($p<0.05$).

No significant differences were found in ΔE_{00} values of DXP restorative material brushed with different dentifrices. For EF restorative material, the highest color change was observed in the Brand D dentifrice group ($p < 0.05$). The highest color change of GCP restorative material was observed in the Brand B dentifrice group and the lowest color change was seen in the Brand D dentifrice group ($p < 0.05$).

Evaluating the data of color change for DXP brushed with different dentifrices, it was determined that the ΔE_{00} values were lower than 1.8 (50:50% acceptability threshold value for CIEDE2000 (1:1:1) according to a recent study (18). The EF and GCP did not yield clinically acceptable ΔE_{00} values in

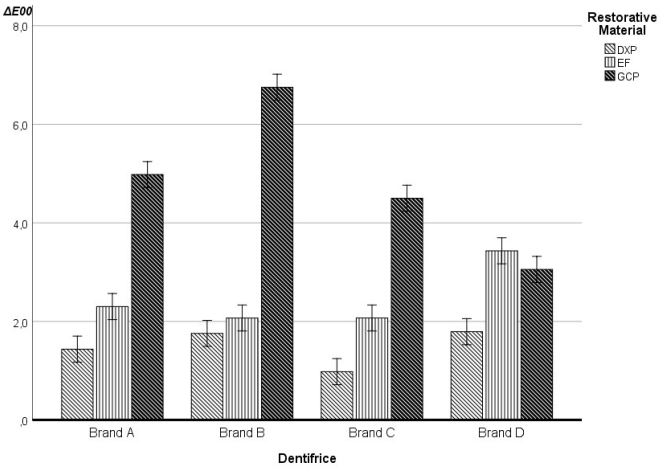

Figure 2. *Color changes of the restorative materials with the dentifrices.*

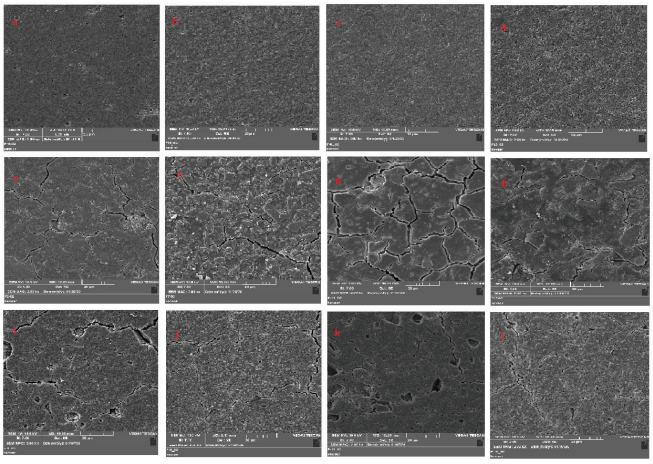

Figure 1. *SEM images of restorative materials after simulated tooth brushing. * a= DXP/Brand A, b=DXP/Brand B, c=DXP/Brand C, d=DXP/Brand D; e=EF/Brand A, f=EF/Brand B, g=EF/Brand C, h= EF/Brand D; i=GCP/Brand A, j=GCP/Brand B, k=GCP/Brand C, l= GCP/Brand D.*

Table 1. *The restorative materials used in the study and their compositions.*

Restorative material	Code	Type	Material composition	Manufacturer
Dyract XP	DXP	Poly acid-modified composite resin (Compomer)	UDMA, TEGDMA, TCB, Strontium-alumino-sodium-fluorophosphor-silicate glass SrF2, SiO2 fillers, Filler: 73% (wt),47% (vol), 0.8 µm	Dentsply, DeTrey, Konstanz, Germany
EQUIA Forte	EF	Glass Hybrid	Fluoro-alumino-silicate glass, Polyacrylic acid powder, Pigment, Polyacrylic acid, Distilled water, Polybasic carboxylic acid	GC, Tokyo, Japan
GCP Glass Fill	GCP	Glass carbomer	Fluoroaluminosilicate glass > 90% Apatitie < 6% Polyacids < 4%	GCP Dental, Ridderkerk, Netherlands

Table 2. *The dentifrices and their components.*

Dentifrices	Code	Main components	Producer
Sensodyne Pronamel Kids	Brand A	Aqua, Sorbitol, Hydrated Silica, Glycerin, PEG-6, Cocamidopropyl Betaine, Xanthan Gum, Aroma, Sodium Fluoride, Sodium Saccharin, Sucralose, Titanium Dioxide, Sodium Hydroxide, Limonene, Contains: **Sodium Fluoride** 0.315% w/w (1450 ppm Fluoride)	GlaxoSmithKline, Brentford, UK
Elmex Junior	Brand B	Water, sorbitol, hydrated silica, hydroxyethyl cellulose, titanium dioxides, cocamidopropyl betaines, olafluor, flavor, limonene, sodium saccharin, hydrochloride acid (1450 ppm **Amine Fluoride**)	GABA International AG, Therwil, Switzerland
Enamelon	Brand C	Acesulfame K, calcium/sodium maleate methyl vinyl ether copolymer, calcium sulfate, cocamidopropyl betaine, dimethicone, flavors, glycerin, lauroyl-sarcosine, monosodium phosphate, poloxamer 407, polyethylene glycol, silica, sucralose 0.45% **Stannous Fluoride** (1150 ppm F)	Premier Dental Products Company, PA, USA
JackNJill	Brand D	Xylitol, Purified Water, Glycerin (Coconut derived), Silica, Organic Strawberry Flavor (Fragaria Chiloensis), Xanthan Gum, Organic Calendula Officinalis Extract, Potassium Sorbate (Naturally derived) , Citric Acid.	JNJ Operations, Melbourne, Australia

Table 3. *The mean and standard deviations of ΔE_{00} values.[a,b] Values in the same column with different superscripts show the statistical difference ($p<0.05$)[A,B,C] Values in the same row with different superscripts show the statistical difference ($p<0.05$).*

	Restorative Material			p-Value		
	DXP	EF	GCP	Dentifrice	Material	Dentifrice*Material
Dentifrice	Mean±SD	Mean±SD	Mean±SD			
Brand A	1.437±0.56^{a,B}	2.302±0.89^{b,B}	4.98±1.48^{b,A}	<0.001	<0.001	<0.001
Brand B	1.758±0.73 ^{a,B}	2.07±1.01^{b,B}	6.752±0.22^{a,A}			
Brand C	0.981±0.66^{a,C}	2.07±1.16^{b,B}	4.5±0.35^{b,A}			
Brand D	1.793±0.48^{a,B}	3.433±0.77^{a,A}	3.055±0.88 ^{c,A}			

any dentifrice group. ΔE_{00} values of three restorative materials brushed with four dentifrices are presented in Figure 2.

The SEM images (2,000 × magnifications) of the DXP, EF and GCP after simulated tooth brushing with different fluoride content dentifrices were presented in Figure 3. Similar surface features were observed with SEM analysis for the GCP and EF groups. However, the DXP group exhibited smoother surface than the other groups.

Discussion

Main objective of this study was to evaluate the effects of dentifrices with different fluoride content on color change of restorative materials commonly used in pediatric dentistry. As the results of our study, the first null hypothesis was rejected due to the obtaining significant differences between the color changes of restorative materials after brushing with different dentifrices. There were significant differences in ΔE_{00} values of restorative materials after being exposed to dentifrices with different types of fluoride content; therefore, the second null hypothesis was rejected.

Studies generally focused on the color change caused by fluoride gels, mouthwashes and beverages (3, 19-23). While some studies have found that there was no differences of color change between the mouthwashes and distilled water, other studies stated that the low pH of active preventive ingredients such as fluoride in the mouthwashes may affect the color stability (19, 20, 23, 24). It has been reported that fluoride varnish application negatively affects the color stability of restorative materials (25). Fatima *et al.* (26) stated that APF gel application on the GIC and RMGIC materials resulted in significant color change. While there are many studies investigating the color change caused by fluoride containing mouthwashes and local fluoride applications, the number of studies examining the color change caused by dentifrices with different fluoride formulation is very limited (1, 19, 24-27).

SnF_2 dentifrices have been implicated in surface staining because of incomplete SnF_2 stabilization ion or lack of robust cleaning ingredients (28). Li*et al.* (29) reported that SnF_2 stabilized with zinc phosphate did not induce staining and these dentifrices were very effective in stain removal of surfaces. Ger-

lach *et al.* (30) stated that the stabilized SnF_2/sodium hexametaphosphate dentifrices reduce the development of stain. In the present study, stabilized SnF_2 dentifrice did not cause more staining than the other dentifrices which is in agreement with the previous studies (29, 30). This may be explained with using stabilized SnF_2 dentifrice which provides some benefits without historical stannous objectionable staining.

Conforti *et al.* (27) found that a new dentifrice containing 5.0% potassium nitrate and 0.454% SnF_2 in a silica base did not cause more extrinsic dental staining than the commercially available dentifrices containing NaF. However, Artopoulou *et al.* (31) reported that the porcelain specimens that were exposed NaF showed less surface deterioration and discoloration than those were exposed SnF_2. In our study, we observed that SnF_2 dentifrice caused less color change than NaF dentifrice; however, this difference was not statistically significant.

Clinical researches have investigated the effects of dentifrices and mouth rinses containing AmF and SnF_2 on the dental plaque reduction and compared the effects of the experimental AmF/SnF_2 fluoride mouth rinses on staining (8, 32-34). West *et al.* (34) reported that all experimental AmF/SnF_2rinses caused more tooth staining than placebo with an overall pattern. In this study, AmF containing dentifrice caused significantly high discoloration on GCP restorative material than the other dentifrices. Since there is no study evaluating the color change caused by AmF containing dentifrice, it is not possible to make a comparison with our results.

It has been reported that tooth brushing associated with the use of dentifrices influences the optical features and surface roughness of restorative materials (15). Pires De Souza*et al*. (35) stated that since the staining susceptibility of resins is material-dependent, stains removal ability of dentifrice was not affected by the abrasives in the dentifrice. However, the results of a previous study indicated that only the dentifrices containing SnF_2 and cetylpyridinium chloride caused significant color changes for both composite materials and natural teeth (36). We found that the dentifrices used in this study caused different color changes in DXP, EF and GCP restorative materials which may be explained with the possible staining effects of the ingredients in these dentifrices.

The most common color difference system in dentistry is CIELAB, but a new color formula as CIEDE2000 (ΔE_{00}), that utilizes the concepts of chroma and hue, reinforcing the importance of the original concepts proposed by Munsell (37), has been recommended since 2001. This formula was accepted as the standard to detect color differences in 2013. Since the number of parameters used in this formula was increased, calculations became more complicated than the CIELAB formula. Color perception varies with different brightness levels according to backgrounds; this change in color perception was incorporated into the formula. Although CIELAB formula measured the distance between two points in the space basically, the addition of SL to the formula of CIE2000 had the effect of including brightness in the calculation and offers advantages by implying better clinical relevance (38). In the view of above; ΔE_{00} was chosen to investigate the color change of dental restorative materials for this study.

Detection of color change is based on the noticeable changes in the color values of an object and the amount of color change affecting the aesthetic appearance (39). The extent of differences are defined as Perceptibility threshold (PT) and acceptability threshold (AT) as a control to evaluate the success rate of restorative materials and to interpret visual and instrumental data (18). A color change value that can be visually perceived by 50% of the observers is described as 50:50% PT and the clinically acceptable color change value for 50% of observers is described as 50:50% AT (18, 39). Therefore, color difference at or below the AT is an acceptable match in dentistry. CIEDE2000 reported 50:50% AT as 1.8 ΔE_{00} which means that ΔE_{00} >1.8 values are considered clinically unacceptable (18). Our study found that ΔE_{00} values were lower than 1.8 for only DXP among all restorative materials. This may be explained with that the more regular surface and the small particle size of DXP than the other restorative materials.

Compomer showed less color changes as compare to other groups. Greater color stability of DXP may be explained with the material's composition, as it includes hydrophilic resins, such asurethanedimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA) and carboxyl groups. The filler and resin particles amount affect the color resistance of restorative materials (23). Khokhar *et al*. (40) stated that urethane dimethacrylate (UDMA) content in resin matrix of materials showed lower color change than the materials with other types of dimethacrylate. The high color stability of the DXP in this study may be explained by UDMA content of the resin matrix.

In the literature, some studies have been reported that GICs are more resistant to staining because of their hydrophilic content (3, 41, 42). However, it has been stated that GICs lack color stability and stain resistance due to the degradation of metal polyacrylate salts (2, 21, 23). Ulusoy *et al.* (23) reported that RMGIs showed more aesthetically divergent results following the use of mouthwashes. Similarly, GCP and EF restorative materials with more glass ionomer content showed higher color change with the different pediatric dentifrices in this study. This may be explained with the large particle size of materials containing glass ionomer.

The color stability of restorative materials is also depended on its surface features. Increased surface roughness of restorative material causes water absorption through the polymer chains, influences the bonds between the matrix and filler particles resulting more staining (2, 22). In this study, significantly higher color change in GCP may be explained with its irregular surface. The irregular surface features of GCP were seen in the SEM images, as well. Besides, some cracks were observed in the SEM image of the GCP group (Figure 3). The discoloration process may be affected by these cracks.

In this study, all dentifrices were diluted in distilled water to mimic the oral environment during simulated tooth brushing. Normally, this dilution occurs in saliva which includes the enzymes, specific proteins, and ions that may decrease the effect of toothbrush abrasiveness on the specimens that may change the color stability of dental materials. Certain kind of food and beverages consumption may also cause discoloration of restorative materials in the oral environment. Limitations of this study include the possible effects of different diet and oral hygiene habits on color change of restorative materials. Considering that our results are valid for in vitro conditions, we believe that in vivo studies will reveal more comprehensive information.

Conclusion

Glass carbomers caused significant color change when brushed with all types of dentifrices. Compomers seem to be more resistant to the color change for all dentifrices used in this study. The fluoride content of dentifrices is important for the color change of restorative materials. Stannous fluoride containing dentifrice provided better color stability for all restorative materials when compared to sodium fluoride and amine fluoride containing dentifrices.

Türkçe Özet: *Farklı florür içeriğine sahip çocuk diş macunlarının restoratif materyallerin renk değişimi üzerine etkisi. Amaç: Bu çalışmada, farklı flor içerikli diş macunlarının pediatrik diş hekimliğinde yaygın olarak kullanılan restoratif materyallerin renk değişimi üzerindeki etkilerinin değerlendirilmesi amaçlandı. Gereç ve yöntem: Disk şeklinde toplam 120 adet örnek hazırlamak için üç restoratif materyal (cam hibrit [Equia Forte (EF)], cam karbomer [GCP Glass Fill (GCP)] ve kompomer [Dyract XP (DXP)]) kullanıldı. Sodyum Florür (NaF), Amin Florür (AmF), Kalay Florür (SnF_2) ve florürsüz (n = 40) olmak üzere dört diş macunu grubu oluşturuldu. Her örnek için toplam 6720 fırça darbesi uygulanarak diş fırçalama simülasyonu gerçekleştirildi. Renk değişim değerleri CIEDE2000 (ΔE_{00}) renk sistemine göre hesaplandı ve veriler çift yönlü varyans analizine tabi tutuldu. Bulgular: NaF ve AmF diş macunu grupları için en yüksek renk değişim değeri GCP restoratif materyalinde gözlendi ($p < 0.05$). SnF2 diş macunu grubu ile test edilen restoratif materyallerin renk değişim değerleri istatistiksel olarak birbirinden farklıydı ($p < 0.05$) ve ΔE_{00} değerleri GCP> EF> DXP olarak gözlendi. SnF_2 diş macunu, NaF ve AmF diş macunlarına kıyasla tüm restoratif materyallerde daha az renk değişimine neden oldu; ancak bu istatistiksel olarak anlamlı değildi. Tüm diş macunu gruplarında GCP en yüksek renk değişim değerleri gösterdi. Sonuç: Tüm diş macunu gruplarında, cam karbomerler önemli renk değişimine neden olurken, kompomerlerin renk değişimine karşı daha dirençli görüldü. Diş macunlarının florid içeriği restoratif materyallerin renk değişimi için önemlidir. Anahtar Kelimeler: Çocuk diş macunları, florürler, renk değişimi, renklenme, restoratif materyaller.*

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: EK participated in designing the study. SY participated in generating the data for the study. EK participated in gathering the data for the study. SY participated in the analysis of the data. EK wrote the majority of the original draft of the paper. SY participated in writing the paper. SY has had access to all of the raw data of the study. EK has reviewed the pertinent raw data on which the results and conclusions of this study are based. EK, SY have approved the final version of this paper. EK, SY guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2022; 56(1): 49-54

Original research

Micro-computed tomography assessment of triple antibiotic paste removal using different irrigation methods

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Purpose
The study aimed to compare four irrigation methods for triple antibiotic paste (TAP) removal using micro-computed tomography (micro-CT) analysis.

Materials and Methods
Forty bovine central incisor teeth were selected, and the root canals were prepared up to #6 Peeso reamer drills. Equal portions of metronidazole, ciprofloxacin, and minocycline were used for the TAP preparation. The TAP was prepared by mixing the powder with distilled water (with a powder to liquid ratio of 1 mg/1 mL). The TAP was introduced to the canals with a lentulo spiral; then, the access cavities were temporarily sealed. After 21 days of storage, the teeth were randomly divided into four equal groups according to irrigation techniques: open-ended, side-vented, double side-vented needle irrigations and EndoActivator irrigation device. The TAP was removed using 17% EDTA (20 mL) and distilled water (5 mL) for all of the groups. The volume of the intracanal medicament before and after the irrigation procedure was recorded by scanning the samples with micro-CT, and the TAP percentage was calculated. The percentages obtained from each group were compared using ANOVA. The significance level was set at $p<0.05$.

Results
The results showed that there was no statistically significant difference among the TAP percentage volumes removed by the different irrigation techniques.

Conclusion
The irrigation techniques used in this study showed similar TAP removal efficiency, however, they could not completely remove the TAP from the root canal systems.

Keywords: *Endoactivator, irrigation, regenerative endodontic treatment, side-vented irrigation needle, triple antibiotic paste*

Introduction

The immature teeth are at risk for pulp necrosis due to trauma, dental anomalies or caries; which leads the cessation of the root formation (1, 2). Regenerative endodontic treatment is an essentially recommended treatment approach for the reconstruction of a functional pulp-dentin complex in necrotic immature permanent teeth (1). This treatment approach consists of several stages including the disinfection of the root canal system, bleeding and the formation of an intracanal blood clot, and coronal sealing (3, 4). This treatment is basically conducted by disinfecting the root canal system, promoting the stem cells of the apical papilla to generate in further root development, and eliminating the signs and symptoms of infection (5). These outcomes are affected by the adequate elimination of microorganisms from the root canal space (6). The disinfecting procedure is accomplished after chemical debridement with minimal or no mechanical preparation, and the root canals are disinfected by using a medicament (7).

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Received: 8 February 2021
Revised: 24 June 2021
Accepted: 16 October 2021

DOI: 10.26650/eor.2022200002

How to cite: *Saricam E, Yusufoglu SI, Kucuk M, Geneci F, Ocak M, Celik HH. Micro-computed tomography assessment of triple antibiotic paste removal using different ırrigation methods. Eur Oral Res 2022; 56(1): 49-54.*

Triple antibiotic paste (TAP), which is a 1:1:1 mixture of metronidazole, minocycline, and ciprofloxacin, is a commonly used medicament for regenerative endodontic protocols because of its antimicrobial efficacy (8). However, it has some drawbacks because it is difficult to totally remove the medicament from the root canals and it promotes tooth discoloration (1, 9, 10). Residual TAP may have adverse effects on the adhesion and penetration of the barrier materials to the root canal dentin (11). Moreover, the presence of residual TAP can also negatively impact the survival of the stem cells of the dental apical papilla (7, 11). In order to eliminate these negative properties, TAP should be totally removed from the root canals.

Conventional irrigation with syringes is an irrigation method that is widely accepted by both general practitioners and endodontists, although several irrigants and sonic/ultrasonic activation techniques have been introduced (12, 13). Manufacturers have designed different types of needles to make syringe irrigation more efficient, such as side-vented (SV) and double side-vented needles (DSV) (14-16). A sonic-driven irrigation solution activation system, called the EndoActivator (EA) (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA), was manufactured to produce vigorous fluid agitation in the root canals by using flexible, plastic, and non-cutting tips of various sizes (17). It has been shown that EA has a superior irrigation efficacy when compared to conventional needle irrigation (18).

In order to eliminate the negative properties of residual TAP, as stated above, it should be totally removed from the root canals. Although irrigation techniques and irrigant types are essential for the removal of the medicament, minimal or no instrumentation has been advised for regenerative procedures (3). Therefore, this study was designed to determine the optimal irrigation technique for the removal of TAP from the root canal system by evaluating the remaining TAP using a micro-computed tomography (micro-CT) system. The null hypothesis was that the removal of the TAP was not related to the irrigation technique, including open-ended (OE) needles, SV needles, DSV needles, and the EA.

Materials and Methods

Sample selection and root canal preparation

Forty bovine central incisor teeth were selected and sectioned horizontally to standardize the root lengths at 17 mm. The working length was established at 16 mm. The root canals were instrumented with using hand instrumentation technique up to the #80 K- file, and they were prepared with #1-6 Peeso reamer drills. At each instrument change, 2 mL of 1.5% sodium hypochlorite (NaOCl) was used for irrigation. After finishing the instrumentation protocol, 20 mL of 1.5% NaOCl (5 min), 5 mL of distilled water, and 20 mL of 17% ethylenediaminetetraacetic (EDTA) (5 min) were applied to the root canals. Then, the root canals were dried using paper points (Dentsply Maillefer, Ballaigues, Switzerland). Equal portions of metronidazole (Eczacibasi, Istanbul, Turkey), ciprofloxacin (Biofarma, Istanbul, Turkey), and minocycline (Ratiopharm, Ulm, Germany) were used for the TAP preparation. The medicament was prepared by mixing the powder with distilled water (with a powder to liquid ratio of 1 mg/1 mL) and it was introduced into the root canals with a Lentulo spiral at 900 rpm until the medicament extrusion was visible at the apical foramen. The apical portions of the canals were sealed with a flowable composite resin, and the access cavities were temporarily sealed (Cavit G; 3M ESPE, Seefeld, Germany). The teeth were stored at 37 °C and 100% humidity.

Medicament removal

The teeth were randomly assigned to one of four groups. After 21 days of storage, the intracanal medicaments were removed via irrigation with 17% EDTA (20 mL, 4 min) and distilled water (5 mL, 1 min) as follows: OE needle irrigation: A 27 G beveled OE dental irrigation needle (Ayset, Adana, Turkey) was used for the irrigation procedure. SV needle irrigation: A 30 G SV irrigation needle (Max-i-Probe; Dentsply Maillefer, OK, USA) was used for irrigation procedure. DSV needle irrigation: A 30 G DSV needle (i-Tips; i dental, Siauliai, Lithuania) was used for the irrigation procedure. EA irrigation: A 2.5 mL of the irrigation solution was flushed into the canal by using a 27 G needle and it was activated with the red tip of the EA (25/.04) at 10,000 cycles/min. After every 2.5 mL of irrigation, the irrigant was activated for 30 seconds.

The tips/needles were moved with an up and down motion in all of the groups. The maximum depth of the tips/needles was positioned at 2 mm short of the apical foramen. During the irrigation procedures, each canal was flushed with a 2.5 mL of irrigant for a total of 30 seconds. Therefore, 20 mL of EDTA solution was applied in 4 min, and 5 mL of distilled water was applied in 1 min. The same amount of irrigant and the same irrigation time were applied to every root.

Micro-computed tomography evaluation

For the volumetric analysis of the filling materials, the teeth were scanned using micro-CT (SkyScan 1174; Bruker micro-CT, Kontich, Belgium) before and after the irrigation protocols were performed with the following scanning conditions: 50 kVp, 800 µA, a pixel size of 33 um, a beam hardening correction of 30%, a smoothing of 2 and a ring artifact correction of 6. The scanning was performed with a 180° rotation around the vertical axis, a camera exposure time of 2.700 ms, a rotation step of 0.4°, and a frame averaging of 3. Flat field corrections and geometric corrections for random movement were performed in all of the scans. The scanning procedure took approximately 1 h per sample.

The three-dimensional reconstruction data was obtained by using NRecon reconstruction software (version 1.6.9.4; Bruker micro-CT). Serial section images obtained with NRecon software were opened in CTAn program (version 1.17.7.2; Bruker micro-CT) for the calculation of TAP volume. The region of interest (ROI) area was determined to measure the TAP volume. Care was taken to position the ROI between the root canal wall and the tooth surface. This situation has been checked for all sections. Dental tissue and TAP were clarified by adjusting the upper and lower values from the histogram section in the CTAn software. The area included for the measurement of the TAP volume (TAP volume = volume of interest-VOI) was shown with the red colour. The green and black coloured areas in the figure are not included in the measurement. These settings

were applied for all sections by selecting the from dataset option. The CTAn software provided the TAP area. No methodological modifications were applied. A 3D model of TAP was created with the Create 3D model option. In order to create a 3D model of the tooth with TAP, the ROI area was determined again with the same sections. The ROI was positioned outside of the tooth surface. All measurements were performed with these settings. Only the tooth tissue was included in the VOI by adjusting the upper and lower values from the histogram section in the CTAn software. This could be made by the difference in radiopacity between the tooth and TAP. The green and black areas in the figure were not included in the measurement, the red area was included in the measurement (VOI = tooth volume). These settings were applied for all sections by selecting the from dataset option. A 3D model of tooth was created with the Create 3D model option. All of the created 3D files were opened using the CTVol software (version 3.3.0; Bruker micro-CT). The three-dimensional visualization and qualitative evaluation of the TAP were performed using CTVol software. TAP was coloured to make it more distinct and the tooth was made more transparent to provide TAP to be seen in the tooth (Figure 1 A-B). TAP volume image was fitted into the tooth model. All these procedures were performed using opacity and colors settings in the objects menu of CTVol. The examination of the images was performed by a blinded observer. The volume of the TAP before and after the irrigation procedure was recorded, and the TAP percentage was calculated (Figure 1 A-B). The horizontal sections of the teeth were obtained using DataViewer software. Black and white images were available as raw data obtained from the scanning (Figure 1 C-E). To ease the TAP detection, the black and white raw images were colourized in the DataViewer program (Figure 1- D-F). DataViewer created this color difference owing to the radiopacity difference.

Statistical analysis

The Shapiro-Wilk test was used to evaluate the assumption of normality. The pre-operative TAP volume for the groups showed a non-normal distribution ($p < 0.05$). The percentage volumes of the remnant TAP for each irrigation technique were normally distributed. The pre-operative TAP volumes of the groups were compared with Kruskal-Wallis test. The percentages obtained from each group were compared using ANOVA. The significance level was set at $P < 0.05$ (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY, USA).

Results

There was no statistically significant difference among the techniques in the TAP residue percentages.The statistical results and the volumes of the intracanal medicament before and after the irrigation procedure are shown in Table 1. The 3D and 2D images taken before and after removal of TAP for

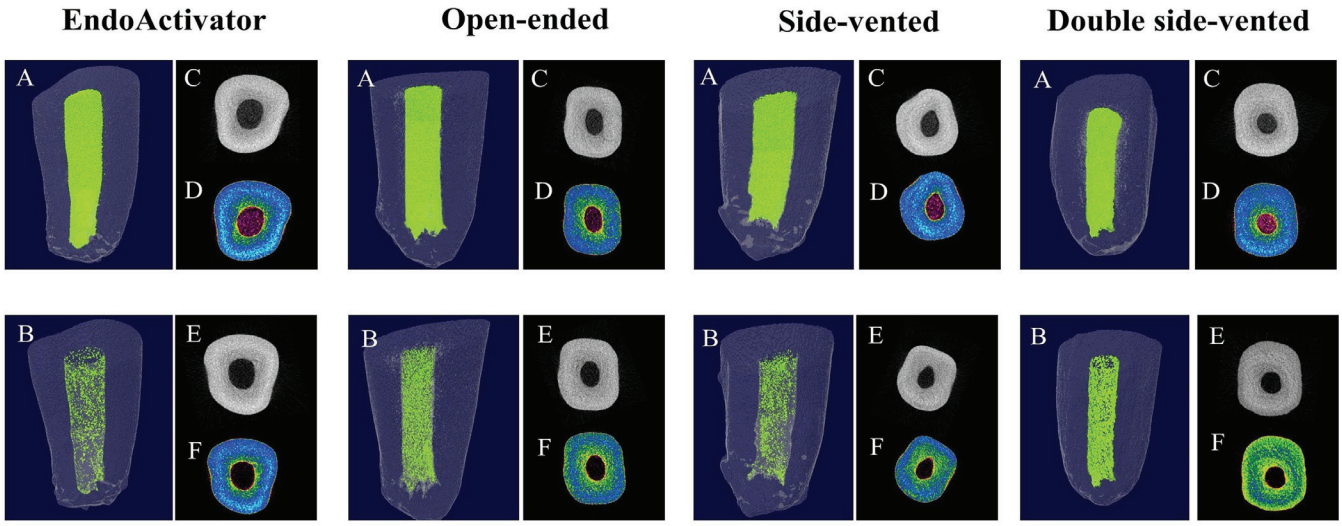

Figure 1. *Images obtained with different observational methods. 3D images observed (A) before, and (B) after TAP removal. (C) Black and white, (D) coloured cross-sectional 2D images taken before TAP removal. (E) Black and white, (F) coloured cross-sectional 2D images taken after TAP removal.*

Table 1. *TAP volumes before and after removing procedures, and comparison of residual TAP percentages for groups (ANOVA)*

Groups	TAP volume before removal (mm³)		TAP volume after removal (mm³)		TAP percentage (%)	F	*P*
	Mean-Median	Min-Max	Mean-Median	Min-Max	Mean±sd		
OE	58.46-53.68	27.34-106.21	9.11-9.01	6.76-12.69	7.05±1.16	0.321	0.810
SV	65.82-62.93	19.02-164.85	7.76-7.22	6.05-10.54	5.82±0.72		
DSV	44.85-30.06	19.99-90.48	7.12-6.54	4.74-11.28	6.97±1.37		
EA	58.35-28.71	24.18-140.84	7.43-7.62	4.35-11.40	7.41±1.46		

sd: standard deviation; Min: minimum; Max: Maximum; TAP: Triple antibiotic paste; OE: Open-ended needle irrigation; SV: Side-vented needle irrigation; DSV: Double side vented needle irrigation; EA: EndoActivator irrigation.

each technique are represented in Figure 1. No statistically significant difference was found among the groups for pre-operative TAP volumes (chi-square = 2.129, P = 0.546).

Discussion

This study was designed to compare the efficacy of OE, SV, DSV needles, and the EA technique for removing TAP from root canals using a micro-CT imaging system. The tested null hypothesis was accepted because there was no statistically significant difference among the tested irrigation methods.

Dental trauma to immature teeth could lead the cessation of the root formation and thinner root canal walls (19). The root canal spaces of immature teeth are larger than the canal spaces of mature teeth requiring regenerative endodontic treatment. In this study, the root canals were prepared with Peeso reamer drills up #6 in order to provide larger canals to simulate the clinical conditions of immature teeth. In some previous endodontic studies, bovine teeth had been used, since the bovine dentine has a similar structure, chemical composition and number of tubules to human root dentin (20-23). It was found that, the adhesion ability of different sealers to the human and bovine dentin were similar (22). Instead of using human teeth, bovine incisors were used in this study because of the mentioned reasons.

The intracanal application of medicaments is a powerful way to combat pulp necrosis pathogens. More specifically, TAP has been recommended for regenerative procedures because its success with eliminating the endodontic infections has been proven (2, 24). Although TAP provides a significant disinfection, it has some disadvantages, such as notable tooth discoloration and significant stem cell death, especially at dense concentrations (2, 7, 25, 26). Because it has been proven that the use of TAP at 1 mg/mL has no adverse effects on cell survival, the TAP was prepared at that concentration in this study in order to mimic the clinical conditions (6). EDTA has been shown to remove TAP effectively from root canals, and also release growth factors from the dentin, and support the adhesion, migration, and differentiation of the stem cells of the dental pulp (27, 28). For the TAP removal, the irrigation procedure was performed with 17% EDTA and distilled water as suggested (29). The maximum depth of the tips/needles was maintained at 2 mm above the apical foramen during the irrigation procedure based on the recommendations of European Society of Endodontology (ESE) for revitalization procedures (29).

Residual TAP is a challenge for regenerative procedures as it acts as a barrier between filling material and root canal walls. Therefore, the medicament should be efficiently removed. However, current study showed that TAP can not be totally removed, regardless of the irrigation technique. TAP was proved to have higher diffusion and retention capacities toward the dentin tubules, induced by the chelation of calcium ions by minocycline which probably caused the lack of adequate medicament removal (1).

One study compared the needle types via a computational fluid dynamics model, which showed that the flow conditions of OE and close-ended needles were different (30). It was stated that the shear stress observed with SV or DSV needles increases on the root canal walls, which means they achieve a higher debridement efficacy. However, different from this study, that study was created on a simulated 6% tapered root canal model. In this study, the canals were not tapered, and the widths of the root canal spaces were standardized by preparing them with #6 Peeso-reamer drills to mimic the enlarged root canals of immature teeth. Therefore, the similarity of the results obtained from all of the groups could be related to the untapered shape of the root canals, which may have allowed irrigation solution to easily flow back and remove the apical medicament to the coronal portion of the root canal.

Previous studies that compared EA and classic needle irrigation techniques for removal of the antibiotic paste and root canal sealer found EA more effective (31-33). EA has a non-cutting tip, and the tip generates short vertical strokes by vibration and up-and down movements, which ease the elimination of debris from the root canals (18). Activating the irrigation solutions are claimed to ease the irrigation flow and therefore cleaning the paste from the root canal walls. In these studies, the mature teeth with tapered root canals which had been preparing up to #40 apical size were used. It has been claimed that an increase in the taper of the root canals facilitates the irrigant flow, and debridement from the apical to the coronal part of the root canals (34). In this study, the teeth were prepared as non-tapered form, similar to a cylinder. The similarity among the techniques may depend on the shape of the root canals.

In some previous studies that evaluated several irrigation methods or solutions for antibiotic paste removal, a stereomicroscope was used for the determination of the remnant medicament (11, 27, 31, 32, 35-39). In another study, radio-labeled TAP was introduced into the root canals, and the residual material was evaluated radiographically (1). In stereomicroscopic evaluation, the roots are longitudinally sectioned and residual material is scored, or the areas of remnant material and root canal surface are measured on the images. The sectioning procedure may cause the remnant material removal and thus causing misleading results. The stereomicroscopic and radiographic evaluations are both made on 2D images. The micro-CT imaging lets volumetric calculations on 3D imaging, and previously used to evaluate the effects of irrigation techniques on removal of filling material and calcium hydroxide from the root canals (40, 41). Previous studies using micro-CT imaging to evaluate volumetric solubility of TAP used artificial fabricated resin acrylic roots (42, 43). In this study, bovine teeth were used to simulate clinical conditions such as the bonding between TAP and dentin, and the effect of irrigation techniques to remove paste from dentinal walls. However, the radiopacity level of TAP may not be enough for micro-CT imaging, and this could be a limitation for the current study.

It has been claimed that irrigation tips lead to apical extrusion, which may cause some complications, such as flare-ups, periapical inflammation, and the delayed healing of apical lesions (14). Causing less debris extrusion may be another important factor for immature teeth in order to prevent the TAP from going beyond the apical foramen. Several studies have compared the techniques used in this study with regard to apical extrusion at mature teeth (14, 44-46). Because this study proved that the irrigation techniques had similar effects with regard to TAP removal, another important factor, apical extrusion, could affect the selection of the irrigation method. Further studies evaluating debris extrusion using

these methods with immature teeth may help to specify the optimum irrigation technique for immature teeth.

Conclusion

Although the SV needle irrigation method provided a lower remnant material percentage; the OE, SV, DSV and EA irrigation methods left statistically similar amounts of TAP in the root canals. None of the methods investigated were able to totally remove the TAP from the root canal system.

Türkçe Özet: *Farklı irrigasyon yöntemlerinin üçlü antibiyotik patı uzaklaştırma etkinliğinin mikro-bilgisayarlı tomografi ile değerlendirmesi. Amaç: Bu çalışmanın amacı, dört farklı irrigasyon tekniğini, üçlü antibiyotik patını (ÜAP) uzaklaştırma etkinliği açısından mikro bilgisayarlı tomografi (mikro-BT) yöntemi ile karşılaştırmaktır. Gereç ve Yöntemler: Kırk adet sığır keser dişi seçildi ve kök kanalları # 6 Peeso reamer kalınlığına kadar genişletildi. ÜAP tozu için eşit miktarda metronidazol, siprofloksasin ve minosiklin kullanıldı. ÜAP tozunun distile suyla (1 mg / 1 mL toz-likit oranında) karıştırılmasıyla pat elde edildi. ÜAP, lentülo spiral ile kanallara iletildi, ardından giriş kaviteleri geçici olarak kapatıldı. Yirmi bir gün sonra, dişler irrigasyon tekniğine göre rastgele dört eşit gruba ayrıldı: açık uçlu, tek yandan delikli, ve çift yandan delikli irrigasyon iğneleri ve EndoActivator irrigasyon cihazı. ÜAP, tüm gruplarda % 17 EDTA (20 mL) ve distile su (5 mL) irrigasyonu ile uzaklaştırıldı. Kanal içi medikaman hacmi, irrigasyon işleminden önce ve sonra mikro-BT cihazı ile taranarak kaydedildi ve artık ÜAP yüzdesi hesaplandı. Bulgular: Farklı irrigasyon teknikleriyle uzaklaştırılan ÜAP yüzdesi hacimleri arasında istatistiksel olarak anlamlı bir fark olmadığı görüldü. Sonuç: Bu çalışmada kullanılan irrigasyon teknikleri ÜAP uzaklaştırma etkinliği açısından benzerlik gösterdi, ancak hiçbir sistem kök kanal sisteminden patı tamamen uzaklaştıramadı. Anahtar kelimeler: endoactivator; irrigasyon, rejeneratif endodontik tedavi; yandan delikli irrigasyon iğnesi, üçlü antibiyotik patı.*

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: ES, SİY, MK participated in designing the study. ES, SİY, MK participated in generating the data for the study. ES, SİY, MK, FG MO, HHC participated in gathering the data for the study. ES, SİY, MK, FG participated in the analysis of the data. ES wrote the majority of the original draft of the paper. ES participated in writing the paper. ES, SİY, MK, FG MO, HHC have had access to all of the raw data of the study. ES, SİY, MK, FG MO, HHC have reviewed the pertinent raw data on which the results and conclusions of this study are based. ES, SİY, MK, FG MO, HHC have approved the final version of this paper. ES guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

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