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ORIGINAL RESEARCH

The comparative evaluation of the effects of hand and rotary instruments using various irrigation agents on smear layer removal in root canals of primary teeth: a scanning electron microscope study

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Abstract

Background: To assess the efficiency of various irrigation techniques employed for eliminating smear layer removal (SLR) in primary tooth root canals following the use of both rotary and hand instrumentation systems using scanning electron microscopy (SEM). **Methods**: 80 extracted maxillary primary incisors were randomly divided into two main groups: the Easy in Smile X Baby Rotary File Group (ESXF Group) (n = 40) and the K-type Hand File Group (K-File Group) (n = 40). Subsequently, the main groups were divided into four subgroups randomly: one treated with a 5% Ethylene Diamine Tetra Acetic Acid (EDTA) solution, another with 6% (Citric Acid) CA, a third with 1% (Sodium Hypochlorite) NaOCl, and a control group that received 0.9% (Physiological Saline) PS. Following the irrigation procedures, an SEM analysis was performed on three specific areas of the root canal: the coronal, middle, and apical. The data collected underwent statistical analysis using different tests like Friedman's, Mann Whitney U, Kruskal Wallis, and Dunn's, with a significance level of 5%. **Results**: In both instrumentation groups, EDTA and CA showed superior efficacy compared to the others on all root canal thirds ($p < 0.05$) and no difference was observed between EDTA and CA ($p > 0.05$). However, erosive defects were seen in both groups for EDTA, especially in coronal third, were not seen in CA subgroups. Also, all irrigants were less effective in apical third. There was also no statistical difference between ESXF and K-File groups for each irrigant ($p > 0.05$). **Conclusions**: Due to its comparable efficiency to 5% EDTA in terms of SLR and lack of erosive properties, 6% CA is a suitable choice for pulpectomy procedures in primary teeth when using rotary systems.

Keywords

Rotary instrument systems; Irrigation solutions; Smear layer

1. Introduction

Root canal treatment *involves* two essential phases: mechanical preparation and chemical irrigation [1]. Historically, K and H-type stainless steel hand files have been conventionally used for mechanical preparation in primary tooth roots [2]. However, modern practice widely adopts Nickel-Titanium (Ni-Ti) rotary instrument systems due to their elasticity and memory of shape [3]. The variation in root and canal anatomy, especially in primary molars, is defined by a more slender and tighter root canal structure, alongside an increased prevalence of accessory canals [4], along with the changes in apical opening due to the physiological root resorption, clinical challenges in canal and working length determination during primary root canal preparation [5, 6]. The use of pediatric files with pediatric rotary systems is standard in children's root canal treatments, providing advantages like quicker shaping, reduced dentist fatigue, and safer preparation for developing permanent teeth due to the files' shorter length [2, 3]. As a result, the clearance of debris from the canal becomes easier, quicker, and safer, thereby enhancing the effectiveness of the treatment [3, 7, 8].

During root canal treatment, the movement of canal instruments on canal walls generates a smear layer including organic and inorganic components that cover the inner dentinal surfaces. The smear layer, formed from the destruction of dental hard tissues, is an amorphous mass of coagulated proteins, odontoblast debris, blood cells, necrotic pulp remnants, saliva, various microbes and dentin fragments [1]. Since this layer blocks the dentinal tubules and creates a barrier between the canal system and the dentin surface, it negatively affects the adaptation of canal sealer and the effectiveness of irrigation solutions. In primary teeth, the interaction between narrower, more confined root canals and canal instruments exacerbates smear layer formation, highlighting the critical role of chemical irrigation in its removal for treatment success [9, 10].

Although there has been significant research on the levels, mixtures, and ordering of chemical irrigation solutions used in endodontic therapy for permanent teeth [11–14], limited research pertains to primary teeth [1, 15–17]. The effectiveness of irrigation solutions can differ in their abilities to dissolve tissues, their antimicrobial properties and their effectiveness in removing the smear layer [18]. Studies have commonly assessed various irrigation agents such as sodium hypochlorite (NaOCl), ethylene diamine tetra acetic acid (EDTA) and citric acid (CA) at different concentrations to eradicate the smear layer. NaOCl is favored in endodontics for its antimicrobial and tissue-dissolving properties. Commonly recommended at 1% concentration, this dilution minimizes the risk of periapical tissue damage, especially in primary teeth [19, 20]. Nonetheless, its effectiveness in removing the inorganic part of the smear layer is limited [1]. EDTA is a powerful chelating agent that can effectively dissolve the inorganic constituents of the smear layer [21–23]. Nevertheless, because of its limited antibacterial efficacy and insufficient capability to dissolve organic tissues, it is frequently recommended to be used in conjunction with NaOCl, which exhibits superior antibacterial properties and organic tissue-dissolving capabilities [24]. While 17% EDTA are preferred for permanent teeth [20], in primary teeth, this concentration may cause severe erosive effects due to lower inorganic content compared to permanent teeth [16, 25]. As a result, studies have indicated that 5% EDTA solutions, which demonstrate comparable effectiveness in removing smears, are suitable for application in primary teeth [26]. Mild acid CA facilitates the removal of the inorganic component of the smear layer by dentin decalcification [17]. Although concentrations ranging from 1% to 50% have been studied [27], it has been suggested that the use of 6% CA with hand files on primary teeth can effectively remove the smear layer without causing erosive effects on root dentin [1]. The experiments used manual hand files for root canal treatment. It's important to recognize that different endodontic tools, including hand and rotary systems, have varying cutting efficiencies and abilities to create smear layers. The effectiveness of endodontic instruments is affected by their material, cross-sectional shape, flute design, cutting edge, helix and rake angles and radial clearance [28].

Although there is a chemical irrigation protocol for hand instruments in primary teeth, there is no chemical irrigation protocol for rotary instrument systems in primary teeth that has been validated by evidence-based studies. Considering this gap, the aim of our study was to evaluate, under *in vitro* conditions, the efficacy of different irrigation protocols used in rotary instrument systems on the removal of the smear layer formed in the root canal walls of primary teeth using SEM.

The first H0 hypothesis is that there will be no difference in the effectiveness of different irrigation solutions used in rotary instrument systems; the second H0 hypothesis is that there will be no difference in the effectiveness of different irrigation solutions used in hand instruments; and the third H0 hypothesis is that there will be no difference in the effectiveness of rotary instrument systems and hand instruments in cleaning the smear layer in the root canals of primary teeth.

2. Materials and methods

2.1 Sample selection

As the study was planned to be conducted with two main groups and four subgroups, a total of 80 samples, 10 in each group, were found to be sufficient at 80% power and 5% type I error levels in the power analysis (effect size (f) = 0.4).

This study utilized single-rooted maxillary primary incisors and primary canines scheduled for extraction due to various reasons (*e.g.*, dentoalveolar traumatic injuries, periapical abscess, fistula or advanced bone loss-related untreatable dental caries). The selected teeth for the study had intact crowns amenable to restoration, normal roots, and visible apical 1/3 root resorption, measured from the enamel-cement junction to the deepest resorption point [29], and had apical openings not wider than the diameter of a #50 K-File. Samples displaying pathologies like resorption lacunes or canal obliteration, identified through stereomicroscope examination, were excluded from the study [15].

A total of 80 extracted and intact (caries free and not coronal damaged) single-rooted primary teeth (53 maxillary primary incisors and 27 primary canines) meeting the specified inclusion criteria were included in the study protocol.

2.2 Study design and sample preparation

2.2.1 Storage media and sample pre-preparation

Teeth were preserved in 0.9% physiological saline (PS) until the study procedures. Prior to the study, any residual tissue on the tooth surface was brushed off under running water after soaking in a 2.5% NaOCl solution for 48 hours to control infection and remove organic debris.

2.2.2 Description of the main study groups and sample randomization

All randomisation procedures in this study protocol were performed based on simple randomisation procedures and using the website of www.randomizer.org. Accordingly, all the samples were randomly allocated into two main groups: the Easy in Smile X Baby Rotary File Group (ESXF Group) (n = 40) and the K-type Hand File Group (K-File Group) (n = 40).

2.2.3 Endodontics access cavity preparation and working length determination

Endodontic access cavities for each sample were prepared based on root canal anatomy of the prepared teeth by using high-speed rotating diamond fissure burs (Meisinger, 840, Hager & Meisinger GmbH, Neuss, NRW, Germany). The coronal pulp tissue and debris residues were then removed using a sharp excavator. Subsequently, the pulp chamber was irrigated with 0.9% PS followed by removal of tissue debris from the root canal with a tirnerf (#25, Medin Barbed Broach, Vlachovice, Czech Republic) followed by washing of the root canals with 0.9% PS. Afterwards, the tooth samples were ready for the chemo-mechanical preparation of the root canals for the main study procedures. However, in order to standardise the chemo-mechanical preparation procedures of each root canals, a working length was determined. In this context, a re-accessible and fixed reference point was determined for method standardisation in the coronal structure. Therefore, decoronation procedure was not included in this study methodology. For each sample, a reference point was created by notching a certain point on the crown with a rotating bur. Accordingly, the root-canal working length was determined by measurement the length between the coronal reference notch and the apical foramen. For the determination of the location of apical foramen, a #15 size K-file (Denco Shenzhen Denco Medical Co., LTD, China) was placed to the root canal and the length at the time that K-file was visible at the apex was recorded as the root canal length for each specimen. Subsequently, 1 mm shorter than this length was recorded as root canal working length [12]. A wax block (Polywax, Bilkim, Izmir, Turkey) was placed at the root apex to prevent irrigation solution overflow from the apical foramen and to simulate apical irrigation pressure.

2.2.4 Chemo-mechanical preparation steps

In the ESXF Group (n = 40), root canal preparation *involved* a reaming motion using ESXF pediatric Ni-Ti rotary instrument systems (Easy in Smile USA International Corp., Changsha, Hunan, China) of sizes 20/04, 25/04, and 30/04. The rotation speed was set at 300–350 revolutions per minute (RPM) with a torque of 2 newton-metre (N.m). The K-File Group (n = 40) underwent root canal preparation using K-type hand files (Denco Shenzhen Denco Medical Co., LTD, China) of sizes #15, #20, #25, #30, #35 and #40 respectively. In both groups, each set of canal files was dedicated to a single tooth throughout the procedure. Since there were four different irrigation protocols in the study methodology, the subgroup distributions of irrigation agents and related irrigation steps in chemo-mechanical preparation were given below.

2.2.5 Description of the study subgroups and sample randomization

In both experimental groups (ESXF and K-File), 10 mL of 1% NaOCl was utilized for irrigation during root canal preparation prior to moving on to the next file size. Paper points were employed to dry the canals before the final irrigation solution was administered. Subsequently, four subgroups were formed by selecting 10 samples from each group, and four distinct chemo-mechanical preparation protocols were implemented on these subgroups. As in the randomization of the main groups, simple randomization was performed by using www.randomizer.org to assign the samples to the subgroups. Above-mentioned four different irrigation subgroups are described below:

1- 5% EDTA Group (n = 10): In this group, 10 mL of 5% EDTA solution was used as final irrigation.

2- 6% CA Group (n = 10): In this group, 10 mL of 6% CA solution was used as final irrigation.

3- 1% NaOCl Group (n = 10): In the final irrigation of this group, 10 mL of 1% NaOCl solution was used.

4- 0.9% PS Group (Control Group) (n = 10): Final irrigation of this group was performed using 10 mL of 0.9% PS solution (Fig. 1).

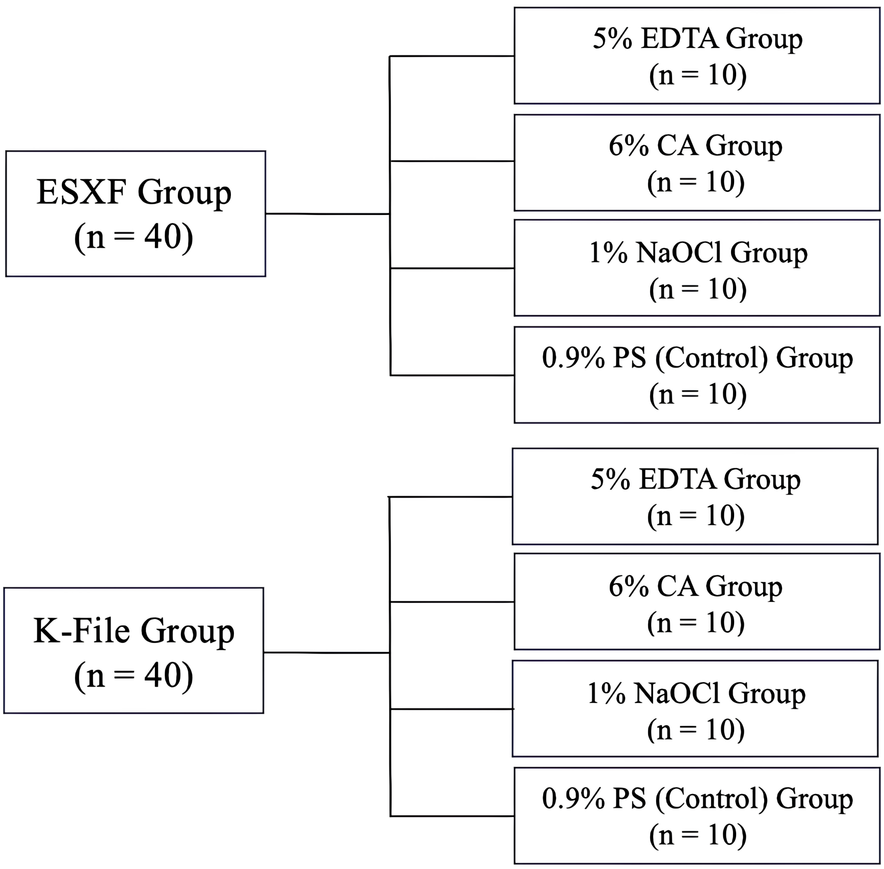

FIGURE 1. Schematic view of the main and subgroups of the study. ESXF: Easy in Smile X Baby Rotary File, EDTA: Ethylene Diamine Tetra Acetic Acid, CA: Citric Acid, NaOCl: Sodium Hypochlorite, PS: Physiological Saline.

In all samples, single side-vented irrigation needle (Endo-Top, Pph Cerkamed, Poland) with 30 gauge closed tip diameter was used for canal irrigations, inserted 2 mm short of the root apex into a syringe with 10 mL solution (Genject, Zhejlang Kangkang Medical-Devices Co. Ltd., China).

2.3 SEM evaluation

Following the removal of wax from the apices, the canals were dried using air spray. The tooth samples, embedded in acrylic blocks, were mesiodistally bisected with a Micracut 201 device (Micracut 201, Metkon, Bursa, Turkey) under water cooling, employing sharp diamond disks. The root halves were then detached from the acrylic blocks using an enamel chisel. Subsequently, all tooth samples were forwarded to Kayseri Erciyes University Technology Research and Application Center for SEM examination. The vacuum-dried samples underwent coating with Au/Pd (80%/20%) to a thickness of approximately 135 Angstroms using a Sputter-Coater (Polaron SC7620 Sputter Coater, Quorum Tech., Lewes, UK) and were affixed onto the sample holder with double-sided tape.

SEM examination was conducted using a SEM device (Zeiss GeminiSEM 500-71-08, Carl Zeiss, Jena, TH, Germany) under vacuum conditions. Microphotographs were captured at ×1500 and ×3500 magnifications from the approximate center of the coronal, middle and apical thirds of the roots. Following the analysis of the images, the effectiveness of removing smears in the three distinct regions of each root was assessed according to the triple scoring criteria detailed in Table 1 [10, 22]. The SEM photographs were blindly assessed by the same researcher (NSÖ) at one-week intervals. Intra-examiner reliability was assessed using the Kappa test, indicating a reliability value of 0.9, demonstrating good reliability.

2.4 Statistical analysis

Data were analyzed using SPSS version 23 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). Mean ± standard deviation and median value (minimum–maximum) for quantitative variables and number of teeth (percentage) for qualitative variables were given as descriptive data. The difference between coronal-middle-apical measurements was analyzed by Friedman's test. The difference between ESXF and K-File groups was evaluated with Mann Whitney U test, and the difference between subgroups was evaluated with Kruskal Wallis test. Pairwise comparisons were made with Dunn's test. $p < 0.05$ indicates a significant difference and $p > 0.05$ indicates no significant difference.

3. Results

3.1 Efficacy of irrigation solutions in smear layer removal within the ESXF group

Evaluation of the scores presented in Table 2, which assess the efficacy of the irrigation solution in removing debris from different areas of the root surface in the ESXF group, revealed notable findings. In the 5% EDTA group, a mean score of 1 was observed across all root surfaces, with a statistically significant superiority in smear removal in the middle third compared to the apical third ($p = 0.043$). On the other hand, the 6% CA group demonstrated a mean score of 0 for the coronal and middle thirds, while scoring an average of 1 in the apical third. This suggests that smear removal was more effective in the coronal third compared to the apical third when using this irrigation solution ($p = 0.022$).

Comparative assessment of four irrigation solutions for smear layer removal in distinct root regions in the ESXF group (Table 2) showcased compelling results. The 5% EDTA group exhibited significantly higher success than the 1% NaOCl and 0.9% PS groups in the coronal 1/3 region ($p = 0.020$, $p = 0.020$, respectively). Similarly, the 6% CA group outperformed the 1% NaOCl and 0.9% PS groups with notable statistical differences ($p < 0.001$, $p < 0.001$, respectively). In the middle 1/3 region, both the 5% EDTA and 6% CA groups demonstrated statistical superiority over the 1% NaOCl group ($p = 0.007$, $p = 0.004$, respectively). Notably, within the apical 1/3 region, the 6% CA group exhibited significantly enhanced smear layer removal compared to the 1% NaOCl and 0.9% PS groups ($p = 0.008$, $p = 0.008$, respectively). While no disparity was observed in scores across the entire root surface between 5% EDTA and 6% CA, erosion defects, characterized by intertubular and peritubular dentin removal, were apparent in the 5% EDTA group. These defects were primarily observed in the coronal (4 samples) and middle thirds (2 samples) of the root, while absent in the apical third (Fig. 2).

3.2 Efficacy of irrigation solutions in smear layer removal within the K-File group

The results detailing smear removal efficiency of 4 different irrigation solutions across distinct root surface regions within the K-File group are presented in Table 3. In the 5% EDTA group, the mean score for all root regions was 1, with statistically more significant smear removal noted in the coronal third compared to the apical third ($p = 0.022$). Conversely, the 6% CA group exhibited a mean score of 1 in the coronal and middle thirds and 1.5 in the apical third, highlighting more effective smear layer removal solely in the coronal third compared to the apical third ($p = 0.044$).

Comparative analysis of smear removal efficiency among the four irrigation solutions across root segments in the K-File group (Table 3) yielded compelling findings. The 5% EDTA

TABLE 1. Triple scoring scale for the effectiveness of smear layer removal in root canals.

Score 0	Score 1	Score 2
No smear layer on the canal walls, free of debris, dentin tubules are open	Moderate smear layer, the borders of the dentinal tubules are visible or partially covered with debris	Excessive amount of smear layer, indistinguishable boundaries of dentinal tubules

TABLE 2. SEM scores of the smear removal effectiveness of 4 different irrigation solutions along the root surface in the ESXF group.

	SEM scores on root surfaces Median (min–max)			p	Pairwise comparisons	p
	Coronal 1/3	Middle 1/3	Apical 1/3			
Irrigation solutions						
5% EDTA	1 (0–1)	1 (0–1)	1 (0–2)	**0.015**	C *vs.* M C *vs.* A M *vs.* A	p = 0.737 p = 0.180 **p = 0.043**
6% CA	0 (0–1)	0.5 (0–2)	1 (0–2)	**0.002**	C *vs.* M C *vs.* A M *vs.* A	p = 0.539 **p = 0.022** p = 0.539
1% NaOCl	2 (1–2)	2 (0–2)	2 (1–2)	0.607		
0.9% PS (Control group)	2 (1–2)	2 (0–2)	2 (1–2)	0.174		
	<0.001	**0.001**	**0.001**			
	0.020^a	**0.007**^{a}				
p	**<0.001**^{b}	**0.004**^{b}	**0.008**^{b}			
	0.020^c					
	<0.001^d		**0.008**^{d}			

C: Coronal 1/3, M: Middle 1/3, A: Apical 1/3, min: minimum, max: maximum, SEM: scanning electron microscopy, EDTA: Ethylene Diamine Tetra Acetic Acid, CA: Citric Acid, NaOCl: Sodium Hypochlorite, PS: Physiological Saline.
^{a}: 5% EDTA vs. 1% NaOCl solutions, ^{b}: 6% CA vs. 1% NaOCl solutions, ^{c}: 5% EDTA vs. 0.9% PS solutions and ^{d}: 6% CA vs. 0.9% PS solutions. A statistically significant p value is < 0.05 and is highlighted in bold in the table.

group significantly outperformed both the 1% NaOCl and 0.9% PS groups in the coronal 1/3 region ($p < 0.001$, $p = 0.014$, respectively). Similarly, the 6% CA group displayed superior efficacy over the 1% NaOCl and 0.9% PS groups with notable statistical differences ($p = 0.001$, $p = 0.031$, respectively). In the middle 1/3 region, both the 5% EDTA and 6% CA groups were significantly more successful than the 1% NaOCl group ($p = 0.001$, $p < 0.001$, respectively) and the 0.9% PS group ($p = 0.004$, $p = 0.002$, respectively). Within the apical 1/3 region, the 5% EDTA group exhibited statistically superior performance compared to the 1% NaOCl and 0.9% PS groups ($p = 0.018$, $p = 0.018$, respectively). While there were no noticeable differences in scores covering the entire root surface between the 5% EDTA and 6% CA groups, erosion defects *involving* the removal of intertubular and peritubular dentin were only observed in the 5% EDTA group. These erosion defects were primarily concentrated in the coronal third of the root, as evidenced in three samples (Fig. 3).

3.3 Comparison of smear removal efficiency of irrigation solutions in different areas of the root in ESXF and K-File group

Comparative results of the effectiveness of each irrigation solution (5% EDTA, 6% CA, 1% NaOCl and 0.9% PS) in the coronal, middle and apical thirds of the root in the ESXF and K-File groups are presented in Table 4. Accordingly, there was no statistically significant difference between the ESXF and K-File groups in terms of the effectiveness of the irrigation methods used to remove the smear layer in each region of the root ($p > 0.05$).

4. Discussion

The absence of a widely acknowledged evidence-based guideline for rotary instrument systems in primary teeth, coupled with limited research on irrigation procedures during preparation, highlights the necessity for further investigation in this domain. The aim of this study was to evaluate the effectiveness of different irrigation protocols used in rotary instrument systems to remove the smear layer in root canals of primary teeth, using SEM analysis.

In dentistry literature, Fourier Transform Infrared Spectrometer (FT-IR), Transmission Electron Microscope (TEM), and SEM represent common methods for analysing dental hard and soft tissues as well as biomaterials [30]. SEM stands out in various protocols for its capacity to evaluate microcracks, surface topography, subsurface structures, and differentiate dental hard tissues and biomaterial structures [31]. The reason for choosing its utilization in this study lies in its high magnification capabilities and sensitive imaging performance [32, 33].

Under normal circumstances, Primary roots, like permanent teeth, have a root and canal anatomy that tapers apically. Root canal instruments are similarly designed, tapering towards the apical end [4, 34]. Nonetheless, the process of natural root resorption in primary teeth results in a widening of the apical opening, which affects the consistency of mechanical cleaning using canal instruments along the entire root structure [4]. In addition, chemical canal irrigation does not irrigate the coronal part of the canal, which is wider and more visible, and the apical part, which is further away and invisible to the naked eye, with the same efficiency [22]. To address this, we segmented root canals into coronal, middle, and apical regions [16, 17, 24, 35]. Microphotographs were employed at

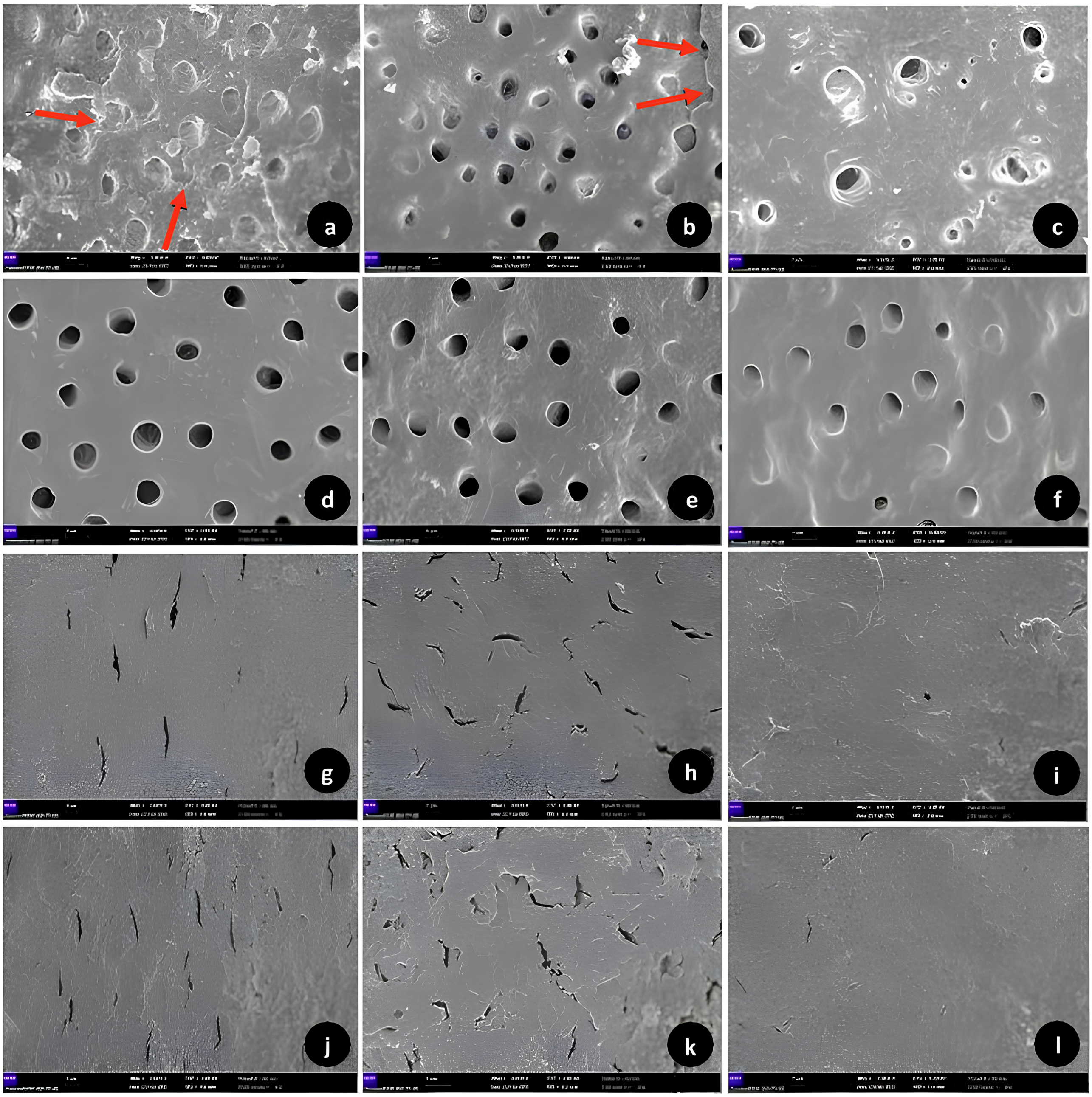

FIGURE 2. Representative SEM images (×3500 mag.) of the root surfaces of tooth samples from the ESXF group. Representative SEM images of root samples applied with 5% EDTA solution (a–c). Coronal third (a, score 1), middle third (b, score 1) and apical third (c, score 1). Erosive defects in inter- and peritubular dentin in coronal and middle thirds (a,b) are shown marked with red arrows. Representative SEM images of root samples applied with 6% CA solution (d–f). Coronal third (d, score 0), middle third (e, score 0) and apical third (f, score 1). Representative SEM images of root samples applied with 1% NaOCl solution (g–i). Coronal third (g, score 2), middle third (h, score 2) and apical third (i, score 2). Representative SEM images of root samples applied with 0.9% PS solution (Control group) (j–l). Coronal third (j, score 2), middle third (k, score 2) and apical third (l, score 2).

a magnification of ×3500 to allow for a detailed examination of the surfaces of root canals and dentinal tubules to ensure accurate scoring [11, 14, 17, 26]. Utilizing the triple scoring scale in our research provided a more distinct differentiation, as it represents various degrees of smear layer elimination, assisting in more accurate evaluations compared to numerous scoring systems [11, 20].

In the present study, when the results of the removal efficiency of the smear layer in the coronal, middle, and apical thirds of the root surfaces of 4 different irrigation solutions in the ESXF and K-File groups were evaluated, it was found that final irrigation with 5% EDTA and 6% CA was superior to 1% NaOCl and 0.9% PS irrigation agents. According to the results of our study; The first and second H0 hypotheses, which stated that "There is no difference between various irrigation solutions used in rotary instrument systems and hand instruments in terms of the effectiveness of removing the smear layer formed on the root canal walls of primary teeth", were

TABLE 3. SEM scores of the smear removal effectiveness of 4 different irrigation solutions along the root surface in the K-File group.

	SEM scores on root surfaces Median (min–max)			*p*	Pairwise comparisons	*p*
	Coronal 1/3	Middle 1/3	Apical 1/3			
Irrigation solutions						
5% EDTA	1 (0–1)	1 (0–1)	1 (1–2)	**0.002**	C *vs.* M C *vs.* A M *vs.* A	*p* = 0.943 ***p* = 0.022** *p* = 0.281
6% CA	1 (0–1)	1 (0–1)	1.5 (1–2)	**0.015**	C *vs.* M C *vs.* A M *vs.* A	*p* = 0.737 ***p* = 0.044** *p* = 0.094
1% NaOCl	2 (1–2)	2 (1–2)	2 (2–2)	0.368		
0.9% PS (Control group)	2 (0–2)	2 (1–2)	2 (2–2)	0.174		
p	**<0.001** **<0.001**[a] **0.001**[b] **0.014**[c] **0.031**[d]	**<0.001** **0.001**[a] **<0.001**[b] **0.004**[c] **0.002**[d]	**0.002** **0.018**[a] **0.018**[b]			

C: Coronal 1/3, M: Middle 1/3, A: Apical 1/3, min: minimum, max: maximum, SEM: scanning electron microscopy, EDTA: Ethylene Diamine Tetra Acetic Acid, CA: Citric Acid, NaOCl: Sodium Hypochlorite, PS: Physiological Saline.
[a]: 5% EDTA vs. 1% NaOCl solutions, [b]: 6% CA vs. 1% NaOCl solutions, [c]: 5% EDTA vs. 0.9% PS solutions and [d]: 6% CA vs. 0.9% PS solutions. A statistically significant p value is < 0.05 and is highlighted in bold in the table.

rejected. Smear removal efficacy was similar between the 5% EDTA and 6% CA groups across root sections. The superior performance of EDTA and CA over other agents is due to their strong acidity, effectively dissolving the inorganic dentin layer. 1% NaOCl and 0.9% PS were not successful in removing the smear layer on their own because they could only dissolve organic tissues and neither organic nor inorganic tissues, respectively. In line with our research findings, Arruda *et al*. [11] demonstrated that the efficacy of smear removal was higher when using a combination of 17% EDTA + 1% NaOCl with rotary instrument systems compared to using 1% NaOCl alone.

In studies using hand files, Hariharan *et al*. [35] found that the combination of 10% EDTA + 5.25% NaOCl removed the smear layer more effectively than 5.25% NaOCl alone. Demirel found that the EDTA groups with concentrations of 5%, 10%, and 17% exhibited higher effectiveness compared to the group using 1% NaOCl [26]. Vallabhaneni *et al*. [17] found that 6% CA was more effective on smear layer removal than 5.25% NaOCl. Vallabhaneni *et al*. [17] also reported that 5.25% NaOCl was ineffective in removing the smear layer when used alone. K-File was employed by Demirel *et al*. [1] to demonstrate that 10% EDTA + 1% NaOCl and 6% CA + 1% NaOCl solutions exhibited superior smear layer removal efficacy compared to 1% NaOCl and 0.9% PS in the coronal and middle thirds of the root. In a separate investigation *involving* rotary files, Darrag demonstrated that the use of only 2.5% NaOCl was ineffective in eliminating the smear layer, mirroring the findings of our study [12]. In the studies conducted by Hariharan *et al*. [35] and Toyota *et al*. [16], NaOCl and PS used alone were not successful in removing the smear layer in parallel with our study, too.

The ESXF and K-File groups had their root surfaces treated with different irrigation solutions. The 5% EDTA and 6% CA solutions removed the smear layer in the coronal and middle thirds, which were higher than the apical third. It is a well-known fact that the apical portions of the root canal system in teeth are characterized by their relatively narrower and thinner dimensions compared to other areas. It is also known that the histological properties of dentin along the root surface differ between regions. Therefore, it is possible that the canal instruments, which had more contact with the root surface, produced more smear layers in the apical third. Conversely, because of the similar anatomical structure, it is widely agreed that irrigation solutions are unable to penetrate the apical region effectively to ensure proper rinsing. In addition, in our study, the working length of the canal was determined to be 2 mm above the apical orifice, and preparation and irrigation were performed at this level. Nonetheless, due to the utilization of a closed-tip needle with a solitary side vent for irrigation, and positioning the side vent to be around 1 mm above the needle's tip, the irrigation solutions initiated the irrigation process higher than the working length. As the needle's tip was sealed, the solutions were unable to be effectively delivered apically, resulting in insufficient rinsing effectiveness. Therefore, the apical region becomes a disadvantageous region in the evaluation of the SEM examination regions formed by dividing the total root length into three. With root lengths averaging around 10–12 mm for the maxillary primary incisors and canines [36]. Even in an unresorbed root, the apical region corresponds to an apical root length of approximately 3–4 mm. Although a smear was created while filing at the 3 mm mark

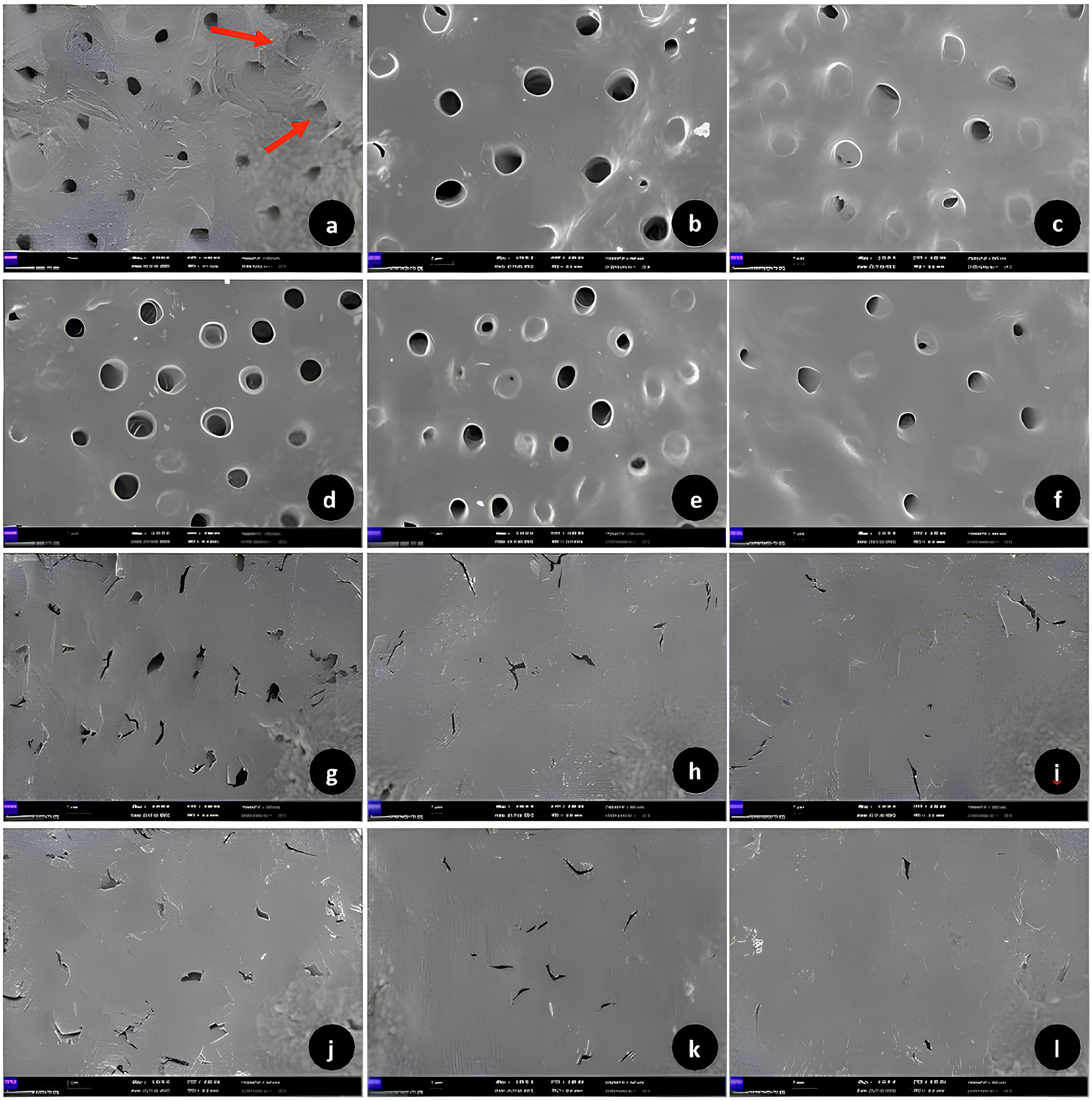

FIGURE 3. Representative SEM images (×3500 mag.) of the root surfaces of the tooth samples in the K-File group. Representative SEM images of root samples applied with 5% EDTA solution (a–c). Coronal third (a, score 1), middle third (b, score 1) and apical third (c, score 1). Erosive defects in the inter- and peritubular dentin in coronal third (a) are shown marked with a red arrow. Representative SEM images of root samples applied with 6% CA solution (d–f). Coronal third (d, score 1), middle third (e, score 1) and apical third (f, score 1). Representative SEM images of root samples applied with 1% NaOCl solution (g–i). Coronal third (g, score 2), middle third (h, score 2) and apical third (i, score 2). Representative SEM images of root samples applied with 0.9% PS solution (Control group) (j–l). Coronal third (j, score 2), middle third (k, score 2) and apical third (l, score 2).

from the root tip, the working length was found to be 2 mm shorter. The closure of the needle tip suggests that effective irrigation may not have been achieved in this instance. Based on this information, it may be possible to explain that both acidic washing solutions failed to remove enough smears in the apical 1/3.

Darrag also reported a comparable outcome, indicating that the efficacy of 17% EDTA and 10% CA groups in eliminating the smear layer in the upper and middle portions was higher when contrasted with the lower portion [12]. Vallabhaneni *et al.* [17], Demirel *et al.* [1] and Demirel [26] have also shown superior removal of the smear layer in the coronal and middle thirds compared to the apical third.

Another finding of the study was that erosion defects were observed much more severely in the coronal third and less in the middle third in the 5% EDTA subgroup of the ESXF and K-File groups. The EDTA solution, a strong inorganic tissue solvent, penetrates the coronal third more effectively

TABLE 4. SEM evaluation scores in the coronal, middle and apical thirds of the root according to the irrigation solutions used in the ESXF and K-File groups.

	SEM scores on root surfaces Median (min–max)											
	Coronal 1/3				Middle 1/3				Apical 1/3			
	Irrigation solutions				Irrigation solutions				Irrigation solutions			
	5% EDTA	6% CA	1% NaOCl	0.9% PS (Control group)	5% EDTA	6% CA	1% NaOCl	0.9% PS (Control group)	5% EDTA	6% CA	1% NaOCl	0.9% PS (Control group)
Groups												
ESXF group	1 (0–1)	0 (0–1)	2 (1–2)	2 (1–2)	1 (0–1)	0.5 (0–2)	2 (0–2)	2 (0–2)	1 (0–2)	1 (0–2)	2 (1–2)	2 (1–2)
K-File group	1 (0–1)	1 (0–1)	2 (1–2)	2 (0–2)	1 (0–1)	1 (0–1)	2 (1–2)	2 (1–2)	1 (1–2)	1.5 (1–2)	2 (2–2)	2 (2–2)
p	0.481	0.063	0.739	0.684	0.481	0.436	0.971	0.436	0.579	0.143	0.739	0.739

SEM: scanning electron microscopy, min: minimum, max: maximum, EDTA: Ethylene Diamine Tetra Acetic Acid, CA: Citric Acid, NaOCl: Sodium Hypochlorite, PS: Physiological Saline, ESXF: Easy in Smile X Baby Rotary File.

and in higher concentrations, aiding smear layer removal but risking erosion defects due to its acidity. Erosion is less common in the middle and apical thirds due to reduced solution concentration. Niu *et al*. [36] noted significant erosion defects with a 15% EDTA and 6% NaOCl combination using rotary instruments. According to the findings of Hariharan and colleagues, while EDTA is successful in eliminating smears, it results in unwanted erosive damage on the dentin surfaces of the root, particularly in the coronal third [35]. Along with our study, Toyota *et al*. [16] and Demirel *et al*. [1] also found that EDTA solution damaged the root walls and eroded the dentin surfaces of the roots. These defects were most noticeable in the coronal third. Demirel observed erosive lesions in the coronal and middle thirds with 10% EDTA. However, 5% EDTA showed similar smear layer removal efficacy with less erosion compared to higher EDTA concentrations [26]. Erosion defects in the samples from the 5% EDTA group were not discussed in the studies conducted by Arruda *et al*. [11] and Darrag [12]. This difference may be explained by differences in the rotary instrument systems used in the studies and variability in the injectors used for irrigation.

It was found that the 6% CA group was similar to the 5% EDTA group in terms of smear removal. There were no erosion defects in any part of the root in the groups. This finding has been attributed to the fact that CA is a moderate acid. Indeed, Demirel and colleagues also found in their study using K-File that 6% CA achieved the same level of smear removal efficacy as EDTA. However, they did not observe any signs of erosion [1].

In the present study, we compared how well 4 different irrigation solutions worked on different parts of the root between the ESXF and K-File groups. On all surfaces of the root, there was no difference in how well rotary instrument systems and hand files removed the smear layer. According to the results of our study; The third H0 hypothesis, "There is no difference in the effectiveness of removing the smear layer in the root canals of primary teeth between rotary instrument systems and hand instruments with the use of different irrigation solutions" was accepted. Moreover, the reduction in the efficacy of removing the smear layer from the coronal third to the apical third closely aligns with findings reported in existing literature [13, 14, 37].

Similar to our study, Prati *et al*. [14] found no difference in efficacy between rotary and hand files on all surfaces of the root. When the literature is examined from this point of view, unlike our study, there are also studies showing a difference between rotary and hand files in terms of smear layer removal efficiency. Manjunatha *et al*. [13] found hand files superior to rotary systems for removing the smear layer in the apical third, with no significant difference in the coronal and middle thirds. Reddy *et al*. [37] showed that rotary instrument systems were more effective in the group using rotary instrument systems than hand files. The efficacy of root canal files in cutting dentin during root canal shaping varies based on their design, which is determined by the cross-section, tip design, and bevel angle of the file. Therefore, their ability to form a smear layer will also vary depending on the file used. Irrigation solutions are known to remove the resulting smear layer. The fact that there was no difference in smear layer removal efficiency between the rotary instrument systems and hand files used in our study and some similar studies in the literature may be due to the similar smear layer forming capacity of the files used in the studies. The difference in smear layer removal effectiveness between our study and some literature may stem from variations in the files' smear layer creation capabilities. Differences in outcomes might also result from the range of irrigation solutions and their concentrations used in the studies.

Current study had some limitations. The initial one research design was *in-vitro* nature, unable to replicate the oral environment with teeth and surrounding tissues. Wax blocks at root tips simulated oral conditions, controlling irrigation solution flow to prevent leakage. Future clinical trials will adjust irrigation solution flow dynamics and periapical pressure, which may cause statistical variations in results. Secondly, by using extracted teeth from different individuals, the dentin characteristics of all the teeth collected may not be identical and this may alter the results. However, to overcome this limitation, randomization procedures were included in the study protocol. Finally, irrigation with a working length set at 2 mm above the open-ended and single-vented or double side-vented needles may also lead to different results, especially in the apical third. Randomised controlled trials in various lab and clinical settings are needed to evaluate the efficacy and safety of irrigation protocols for initial rotary file canal preparation.

5. Conclusions

Based on this study's results, the following conclusions can be made:

1. The 6% CA irrigation protocol was found to be more effective and reliable for the use of both rotary instrument systems and K-type hand files.
2. Especially in the rotary instrument systems group, the high score of 0 was obtained with 6% CA solution.
3. In the 5% EDTA group, erosion was observed as a disadvantage.

As a result, the use of CA solution, which was found effective in the current study, can be recommended because it does not cause erosion damage and is found to be reliable.

AVAILABILITY OF DATA AND MATERIALS

Data generated and/or analyzed during this study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

NSÖ and ŞS—formulated the idea and designed the study protocol; analyzed the data. NSÖ—performed the *in-vitro* experiments; written the first draft of the article. ŞS—analyzed the manuscript in a critical way. All authors contributed to design of study. All authors have read and approved the submission of the final versions of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study received ethical approval (dated 20 January 2021, numbered 02/09) from the Ankara University Faculty of Dentistry Ethics Committee. Informed consent was obtained from all patients and their parents. The study was conducted in accordance with the principles of the Declaration of Helsinki.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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