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Ayrancı LB
Department of Endodontics,
Faculty of Dentistry, Ordu
University, 52200 Ordu, Turkey

Onder Peker M
Department of Endodontics,
Faculty of Dentistry, Ordu
University, 52200 Ordu, Turkey

Energy dispersive x-ray analysis of apical extruded material using different irrigants and reciprocating systems

Ayrancı LB and Onder Peker M

Abstract

Purpose: To evaluate mineral content of the apical extruded material during irrigation with distile water, Sodium hypochlorite (NaOCl), Ethylenediaminetetraacetic acid (EDTA), QMix 2-in-1 solution, and preparation with Reciproc systems.

Methodology: Single rooted eighty eight teeth were randomly assigned to eight groups (n=11). Irrigation process was performed with distile water, NaOCl, EDTA, QMix 2-in-1 solutions during instrumentation with Reciproc and Reciproc blue systems. Apical extruded material was collected into the Eppendorf tubes, then maintained to Vortex for homojenization. The tubes were stored in an incubator for evaporation. The amount of apical extruded material was calculated by subtracting pre-instrumentation and post-instrumentation weight of the tubes. An amount of homojenized debris was spreaded to the analysis platform and analysed for determining the relative amounts of elements. The Friedman test was used for the repeated measures of multiple groups (mean, standard deviation), Kruskal Wallis test for subgroup comparison

Results: In distile water group Reciproc Blue instrumentation associated with significantly high extruded debris than Reciproc instrumentation ($p = 0.001$), where as no significant difference was found between the Reciproc and Reciproc Blue instrumentation in the NaOCl, EDTA, Qmix groups. In the Reciproc and Reciproc Blue subgroup, the percentage weight of Ca and P, in the Distilled water group was statistically significantly higher than those of the NaOCl, EDTA and QMIX groups ($p = 0.0001$)

Conclusions: Reciproc systems and the irrigating solutions caused extrusion of material. EDTA irrigation produced higher extruded material than the other irrigants however it extruded lower Ca and P levels.

Keywords: extusion, irrigant, mineral content

1. Introduction

A successful endodontic treatment consist in; mechanical preperation without any complications (broken instrument, perforation), copious irrigation, tight obturation and leak proof restoration^[1]. Dentine debris, irrigating solutions, pulp fragmants, necrotic remains and microorganisms could be extruded from the apical foramen during mechanical preparation and irrigation^[2]. This situation can cause 'flare up' which could be formed during or after the root canal therapy that is responsible for pain and swelling^[3].

Sodium hypochlorite (NaOCl) has antibacterial activity and it is using for dissolving organic tissues^[4]. Chlorhexidine (CHX) can adsorb onto the dentin walls (Substantivity) and researchers were prefering this irrigant for its long-lasting antimicrobial effects^[5,6]. Ethylenediaminetetraacetic acid (EDTA) is another irrigating solution that have suggested during chemomechanical preparation^[7]. This chelating agent applied during the root canal treatment to solve the inorganic tissues of smear layer. However it may adversely effect to the microstructure of dentin, also can alter the orginal ratio of organic and inorganic components^[8, 9]. A bisbiguanide antimicrobial agent (2% CHX) combined with a polyaminocarboxylic acid calcium-chelating agent (17% EDTA) called as QMix 2-in-1 solution^[10]. QMix was suggested for removing smear layer and important antimicrobial features^[11-13].

Apical extruded debris (AED) studies were performed with distile water^[14,15]. However in clinical situations endodontists were using different root canal irrigants that may have extruded to the periapical region^[16]. For simulating the clinical situations using irrigating solution was

Correspondence

Ayrancı LB
Department of Endodontics,
Faculty of Dentistry, Ordu
University, 52200 Ordu, Turkey

as important as the styling and kinematics of the endodontic instrument [17]. Collectible debris could not be produced without an irrigant during instrumentation however for the root canal desinfection it is an obligation to use irrigating solutions [18]. Parirokh *et al.* [19] showed that with the same preparation technique and instruments, different irrigating solutions and concentrations can cause varied amounts of AED.

It is generally accepted that apically extruded debris was produced whatever used various techniques and instruments [20-34]. All preparation techniques, instruments and irrigation techniques can cause apical debris but there are differences amongst them. AED initiated an important factor for causing potentially an acute inflammatory reaction in the periapical region [3].

Reciprocating motion removes the connection between the instrument blades and the root canal walls. This property can reduce the cyclic fatigue of the instrument [35]. In most cases Reciproc and Reciproc Blue single file systems, needs only one file for instrumentation. These files have increased flexibility because of its special Ni-Ti alloy that called M-wire. Reciproc Blue, the new generation Reciproc instrument, is much more flexible and has lower fracture risk than Reciproc file. The Reciproc Blue file system can be used to prepare the curved canals uncomplicatedly. Reciproc Blue offers design features similar to Reciproc that have S-shaped horizontal sections and 2 cutting edges, but to obtain more flexible structure, the file has been improved with a new heat treatment [36, 37]. The blue color was obtained with this new heat treatment process [37]. In this study we compared the apical extruded material (AEM) produced by two types of Reciproc systems in reciproc motion and presence of different root canal irrigants.

The structure of enamel and dentin is defined with the based on the organic and inorganic contents and it consists of high incidence of hydroxyapatites ($\text{Ca}_3(\text{PO}_4)_2\text{OH}$). Calcium (Ca) and phosphor (P) are the main two components of dental hard tissues [38, 39]. Scanning Electron Microscopy coupled with Energy Dispersive X-ray (SEM/EDX) Spectroscopy is the best known and most widely-used for the surface analytical techniques and elemental analysis [40]. Elemental analysis of apically extruded material was important in this present study. To distinguish the teeth structure and the irrigating solutions we preferred to use this technique. This study aimed to compare the elemental analysis and amount of produced AEM from root canals that mechanically prepared by reciproc instruments during irrigation with different irrigants. The null hypotheses tested were that there would be difference in the amount of apical extruded debris between the irrigating solutions but there would be no difference between the reciproc and reciproc blue instruments.

2. Materials and Methods

2.1 Sample selection

In this study single rooted eighty eight extracted human mandibular premolar teeth were used. The teeth were radiographed buccolingual and mesiodistal directions to draw out any abnormal canal morphology and confirm a single root canal. Root canal curvature was $\leq 10^\circ$ measured according to method of Schneider [41] and had a single apical foramen shown by inspection of the root apex under stereomicroscope (Nikon SMZ25, Tokyo, Japan) under X20 magnifications to verify a single apical foramen. To obtain a standard reference point, the occlusal surface of each sample was removed using a high-speed bur to the standardized length of 19 mm and

verified with a digital millimeter ruler (VINCA, Annandale, USA) were included in the study. Access cavities were prepared with round diamond burs with a high speed handpiece and air water spray cooling. A size 10 K-file (Dentsply Maillefer) was positioned into the canal until it was visible at the apical foramen, and the working length was fixed 1 mm short of this length. A size 20 file was inserted until the WL was reached and to make the standardization of apical foramen diameter, the root canals that the file was loose in or can not easily reach the working length were excluded. The totally 88 teeth that had met the inclusion criteria were randomly divided into four main and two subgroups totally 8 groups by tossing coins. The samples were waited in 10% formalin solution until use. Before the root canal preparation the tissue remnants were cleaned with toothbrush that have washed by diluted nitric acid and then washed with distilled water.

2.2 Instrumentation and debris collection

The experimental model described by Myers and Montgomery [23] was used. An Eppendorf tube was numbered for each tooth and a hole was placed on the cap of an Eppendorf tube, and a tooth was inserted up to the cemento-enamel junction. To balance air pressure inside and outside the Eppendorf tubes, a 25-G needle was inserted alongside the cap as a drainage cannula. Each cap with the tooth and the needle was connected to its Eppendorf tube, and the Eppendorf tubes were placed into vials that was covered with aluminium foil to blind the operator to the debris extrusion during the instrumentation. The whole apparatus was handled only by this vial. At any time Eppendorf tubes touched by operator. Before the instrumentation and weighting the recipients, the Eppendorf tubes were weighed with three consecutive measurements on an analytical balance with an accuracy of 10^{-6} (Mettler Toledo XP2U Greifensee, Switzerland), and the mean value was recorded. Each tooth was coded then randomly assigned to 1 of the 8 groups, each of which had 11 specimens. Coronal flaring was performed in the canals using size 2, 3, 4 Gates Glidden Drills (Dentsply Maillefer) and between all Gates Glidden Drills 1 ml distilled water was used (Total 2 ml irrigation for Gates Glidden Drills).

2.3 Root canal preparation

Four main groups prepared by two Ni-Ti instrumentation (Reciproc and Reciproc Blue VDW) separately. After used size 4 Gates Glidden the pulp chamber and canals were initially flooded with 1,25 ml irrigant for 30 seconds that belonging to each group. The apical preparation was started with R25 (25/06) and again pulp chamber and canals were initially flooded with 1,25 ml irrigant that belonging to each group and finalized with size R40(40/06). Reciproc and Reciproc Blue Ni-Ti instruments using VDW Gold motor in the Reciproc programming. The torque and speed of the motor were based on the manufacturer's instructions. The files were used in back and forth movements and the instruments were cleaned after every 2 pecks. 4 pecks were completed for R25 and 2 pecks for R40 for reaching the working length. After R40 instrumentation the root canals were irrigated with 1ml distilled water. After the instrumentation was completed, tooth were separated from the Eppendorf tube, and the debris adhered to the root surface was collected by washing the root with 1,5 ml distilled water in the tube. Total irrigation volume was 7ml. All irrigation procedures were performed with 30 gauge side port needle (medical brokers, Poland) that were

inserted 2mm short of the predetermined working length. The Eppendorf tubes were maintained to Vortex mixer (MX-F, Dragon Lab instruments, China) for 5 seconds homogenisation before the SEM-EDX (Hitachi, SU 1510, Japan) analysis.

The tubes were then stored in an incubator at 37 °C for 15 days to evaporate the irrigating solutions before weighing the dry debris [42]. Three consecutive measurements obtained for each tube, and the mean value was reported. The net weight of the dry debris was calculated by subtracting the original weight of the empty Eppendorf tube from the gross weight. All preparations performed by the same operator and on the same day at the same temperature to avoid temperature changes that alter the demineralization process [39].

2.4 SEM EDX analysis

This method allowed determining the relative amounts of calcium (Ca), phosphorus (P), oxygen (O), carbon (C), and sodium (Na), chlor(Cl), by weight percent. After weighting the extruded debris, the tubes that could be analysed were separated. Eight tubes were selected from each group. An amount of homogenized debris was spreaded to the analysis platform. SEM images were taken under X300 magnification. Two samples of EDX analysis were shown in Figure 2 and Figure 3

After the coronal flaring the specimens were divided as shown

Distile water group

1a- Reciproc

1,25 ml distile water irrigation, Reciproc R25 instrumentation, 1,25 ml distile water irrigation, Reciproc R40 instrumentation, 1ml distile water irrigation, 1,5 ml distile water (apical irrigation)

1b- Reciproc blue

1,25 ml distile water irrigation, Reciproc Blue R25 instrumentation, 1,25 ml distile water irrigation, Reciproc Blue R40 instrumentation, 1ml distile water irrigation, 1,5 ml distile water (apical irrigation)

NaOCl group

2a- Reciproc

1, 25 ml 5% NaOCl irrigation, Reciproc R25 instrumentation, 1,25ml 5% NaOCl irrigation, Reciproc R40 instrumentation, 1ml distile water irrigation, 1,5 ml distile water (apical irrigation).

2b- Reciproc blue

1, 25 ml 5% NaOCl irrigation, Reciproc Blue R25 instrumentation, 1,25ml 5% NaOCl irrigation, Reciproc Blue R40 instrumentation, 1ml distile water irrigation, 1,5 ml distile water (apical irrigation).

EDTA group

3a- Reciproc

1, 25 ml 17% EDTA irrigation, Reciproc R25 instrumentation, 1,25ml 17% EDTA irrigation, Reciproc R40 instrumentation, 1ml distile water irrigation, 1,5 ml distile water (apical irrigation).

3b- Reciproc blue

1, 25 ml 17% EDTA irrigation, Reciproc Blue R25 instrumentation, 1,25ml 17% EDTA irrigation, Reciproc Blue R40 instrumentation, 1ml distile water irrigation, 1,5 ml distile water (apical irrigation).

QMix group

4a- Reciproc

1, 25 ml QMix irrigation, Reciproc R25 instrumentation, 1,25ml QMix irrigation, Reciproc R40 instrumentation, 1ml distile water irrigation, 1,5 ml distile water (apical irrigation).

4b- Reciproc blue

1, 25ml QMix irrigation, Reciproc Blue R25 instrumentation, 1,25ml QMix irrigation, Reciproc Blue R40 instrumentation, 1ml distile water irrigation, 1,5 ml distile water (apical irrigation).

2.5 Statistical Analysis

The amount of extruded debris was analysed statistically using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. In the evaluation of the data, the Friedman test was used for the repeated measures of multiple groups (mean, standard deviation), Kruskal Wallis test for subgroup comparison, Dunn's multiple comparison test for subgroup comparison, Mann-Whitney-U test for comparison of two groups, and Fisher's reality test. The results were evaluated with a significance level of $p < 0.05$ and a confidence interval of 95%. SEM EDX datas of elements were analysed statistically using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. In the evaluation of the data, the Kruskal Wallis was used for the repeated measures of multiple groups (mean, standard deviation), Dunn's multiple comparison test for subgroup comparison, Mann-Whitney-U test for comparison of two groups. The results were evaluated with a significance level of $p < 0.05$.

3. Results

The mean and standart deviation of extuded material of main groups were as follows: 0,001000+0,004000 for distile water group, 0,008000+0,004000 for NaOCl group, 0,026000+0,004000 for EDTA group and 0,008000+0,004000 for QMIX group. Distile water group caused significantly less extruded debris than EDTA, NaOCl and QMIX groups ($p=0,0001$). EDTA irrigation produced more extruded material than the NaOCl and QMIX groups ($p=0,005$, $p=0,002$). The mean values and the standart deviation of each experimental main groups are shown in Table 1.

In distile water group Reciproc Blue instrumentation associated with significantly high extruded debris than reciproc instrumentation ($p = 0.001$), where as no significant difference was found between the Reciproc and Reciproc Blue instrumentation in the NaOCl, EDTA, Qmix groups.

3.1 Reciproc Group

In Reciproc instrumentation distile water group was associated with less apical extruded debris than the other irrigating solutions ($p = 0.0001$). There was statistically significant difference was found between the EDTA and Qmix groups. In the EDTA group, instrumentation produced significantly more extruded debris than Qmix group ($p = 0.039$) and no statistically significant difference was observed between the other groups ($p > 0,05$).

3.2 Reciproc blue group

In the Reciproc Blue instrumentation, there was a statistically significant difference between Distilled water, Sodium Hypochlorite, EDTA and QMIX groups ($p = 0.0001$). In the EDTA group, apically extruded debris was statistically significantly higher than the Sodium Hypochlorite and QMIX groups. ($p=0,023$, $p=0,02$). In the distilled water group, apical

extruded debris was statistically significantly lower than Sodium Hypochlorite, EDTA and QMIX groups ($p=0,009$, $p=0,0001$). No statistically significant difference was observed between the other groups ($p>0,05$).

3.3 EDX analysis

In this study some elements from apically extruded debris were analysed such as; Na, Ca, P, Cl and rate of Ca/P. C and O elements were common in the tooth structure and the formula of irrigating solutions. Therefore these elements were not included in the statistical analysis.

3.4 Na analysis

In the EDTA group, the percentage weight of Na in the Reciproc Blue subgroup, was statistically significantly lower than the Reciproc group ($p = 0,036$). No statistically significant difference was observed between the other groups ($p> 0,05$)

In the Reciproc and Reciproc Blue subgroups, the percentage weight of Na in the Distilled water group were statistically significantly lower than NaOCl, EDTA and QMIX groups ($p = 0,0001$), but no statistically significant difference was observed between the other groups ($p> 0,05$).

3.5 Ca analysis

In the QMIX group, the percentage weight of Ca in the Reciproc Blue subgroup was statistically significantly higher than the Reciproc subgroup. No statistically significant difference was observed between the other groups ($p> 0,05$). The mean percentage weight of extruded Ca in the Reciproc and Reciproc Blue subgroups according to the irrigants showed in Figure 1

In the Reciproc subgroup, the percentage weight of Ca in the Distilled water group was significantly higher than those of NaOCl, EDTA and QMIX groups ($p = 0,0001$) and no statistically significant difference was observed between the other groups ($p> 0,05$).

In the Reciproc Blue subgroup, the percentage weight of Ca in the Distilled water group was statistically significantly higher than those of the NaOCl, EDTA and QMIX groups ($p = 0,0001$), and the percentage weight of Ca in the EDTA group was statistically lower than NaOCl and QMIX groups ($p= 0,0001$, $p = 0,023$), no statistically significant difference was observed between the other groups ($p> 0,05$).

3.6 P analysis

In the QMIX group, the percentage weight of P in the Reciproc Blue subgroup was statistically significantly higher than the Reciproc subgroup ($p = 0,0001$). No statistically significant difference was observed between the other groups ($p> 0,05$)

In the Reciproc subgroup, the percentage weight of P in the Distilled water group was statistically significantly higher than NaOCl, EDTA and QMIX groups ($p = 0,0001$), but no statistically significant difference was observed between the other groups ($p> 0,05$).

In the Reciproc Blue subgroup, the percentage weight of P in the Distilled water group was statistically significantly higher than NaOCl, EDTA and QMIX groups ($p = 0,0001$) and the percentage weight of P in the EDTA group was statistically lower than Sodium Hypochlorite and QMIX groups ($p=0,0001$, $p = 0,03$), no statistically significant difference was observed between the other groups ($p> 0,05$).

3.7 Cl analysis

In the EDTA group, the percentage weight of Cl in the Reciproc Blue subgroup was statistically significantly higher than the Reciproc subgroup ($p = 0,004$). No statistically significant difference was observed between the other groups ($p> 0,05$).

In the Reciproc subgroup, the percentage weight of Cl in the NaOCl group was statistically significantly higher than Distilled water, EDTA and QMIX groups ($p = 0,0001$) and no statistically significant difference was observed between the other groups ($p> 0,05$).

In the Reciproc Blue subgroup, the percentage weight of Cl in the Sodium Hypochlorite group were found to be statistically significantly higher than Distilled water, EDTA and QMIX groups ($p = 0,005$, $p = 0,01$) and no statistically significant difference was observed between the other groups ($p> 0,05$)

3.8 Ca/P analysis

For Ca / P ratio, there was no statistically significant difference was found between Distilled water, Sodium Hypochlorite, EDTA and QMIX groups in Reciproc subgroup ($p = 0,190$).

There was no statistically significant difference between the Ca / P averages of Distilled water, Sodium Hypochlorite, EDTA and QMIX groups in Reciproc Blue subgroup ($p = 0,274$).

4. Discussion

The present study showed that different types of instruments extruded similar amounts of debris, however different irrigating solutions extruded different amounts of material, so the null hypothesis of this study was accepted.

In this study, to eliminate possible complications, straight and single rooted teeth were selected and these teeth were decoronated for obtaining flat reference points. The generally accepted method of Myers and Montgomery was used to collect the apically extruded material^[23]. According to previous studies, it has been shown that the root canal curvature can effect the amount of apical extrusion of material^[28, 43]. Therefore in the present study only straight canals with similar lengths were used. One of the limitation of this study model is the absence of an apical barrier that can affect the results. Some researchers have suggested to use flower foams for simulating the resistance of periapical tissues that may promote irrigation and debris absorption^[44-46]. Therefore this situation may affect the results. In the current study we did not mimicked the periapical tissues. Another limitation that the different microhardness values of specimens may affect the results^[17].

The coronal flaring was performed with Gates-Glidden drills before canal preparation in the present study. Peters *et al.*^[47] reported that less debris might extrude into periapical region if the preparation of the apical third is completed in a short time, and it allows instruments to prepare the apical part of the canal with less wall contact. Topcuoğlu *et al.*^[48] stated that performing the coronal flaring can produce a reservoir in the coronal part of the root and this wide area can supply removal without cumulant of debris in the apical and middle third during root canal preparation.

The depth and placement of the needle tip is an important factor in root canal irrigation and apical extrusion. The needle tip was recommended to be placed 2 mm below the working length or to be placed to when resistance was met before reaching the desired length^[49, 50]. Yeter *et al.*^[51] have reported that a significant difference was observed between

needles. Open-ended needles were produced significantly more debris than side-vented needles. Therefore in the present study the needle penetration depth was 2 mm shorter from the working length and side vented needling was used.

Reciproc systems have superior cutting effectiveness. As a result of this situation more debris and dentinal chips were produced [52]. However in a recent study, Arslan *et al.* [53] stated that reciprocating motion caused less extrusion than continuous rotation. Uslu *et al.* [54] compared Reciproc Blue, Hyflex EDM and XP Endoshaper ni-ti instrument for apical extrusion of debris and they reported that reciproc blue caused more debris from the XP Endoshaper however there was no statistically significant difference between the reciproc blue and hyflex EDM. To our knowledge no previous study has compared the apical debris extrusion of the Reciproc and Reciproc Blue instruments. The present study showed that there was no statistically significant difference between the Reciproc and Reciproc Blue systems except distilled water irrigation. Reciproc Blue subgroup produced higher extruded material than the Reciproc group in the distilled water group.

Postoperative pain and flare-ups were could be significant results of extrusion of materials during root canal instrumentation. [55]. The results of this study revealed that extrusion of debris occurred in different irrigant groups independent of type of instruments that were used. da Silva *et al.* [56] compared the postoperative pain after the foraminal instrumentation using 5.25% NaOCl or 2% CHX gel irrigation. They reported there was no statistically significant difference was found at any observation time between the used irrigants. They stated that the irrigants that used in their study, had little or no response on postoperative pain during instrumentation. However these short term follow up postoperative pain studies were lack of long term observation and prognosis. Not only postoperative pain and also cytotoxicity was important during using the irrigating solutions. Al Kahtani *et al.* [57] evaluated the cell viability and cytotoxicity of QMix and NaOCl solutions on human bone marrow mesenchymal stem cells. They reported that both QMix and NaOCl irrigants were toxic to human bone marrow mesenchymal stem cells. If we handled the present study apical extruded material included irrigating solutions' elements. Therefore apical extruded material is an important factor for tooth prognosis. Mollashahi *et al.* [58] cytotoxic effects of various endodontic irrigation solutions on the survival of stem cell of human apical papilla. They noted that Chlorhexidine had the lowest cytotoxicity compared to EDTA, MTAD, QMix and NaOCl and its cytotoxicity did not change by the time compared to other irrigants.

The present study showed that different root canal irrigants can produce different weights of AEM with the same instruments and preparation technique. In the present study, to be meaningful, distilled water, NaOCl, Qmix and EDTA was preferred as an irrigation solution to mimic clinical practices. After the evaporation of the irrigant sodium crystals cannot be separated from the extruded material [17]. To solve this problem we used SEM EDX analysis for separating elements. In this section Ca and P elements are important to identify the tooth structure [39]. In this study we preferred to use Energy dispersive X-ray analysis (SEM-EDX) for allowing researcher to perform elemental mapping at the desired depth and area of interest [59].

Cobankara *et al.* [39] evaluated the effects of chelating agents on the mineral content of root canal dentin. They showed that Ca levels significantly decreased in paracetic acid, citric acid and EDTA. However in the present study Ca levels were

significantly higher for evaluating the extruded material in distilled water group than the other groups. Therefore it means that irrigating solutions tend to extrude from the apical foramen than the tooth structure.

In the present study we predicted that EDTA irrigation produced more extruded Ca ions because of its chelating ability. However EDTA irrigation caused least apical extruded Ca ions. Therefore we decided that calcium chelates could not extrude from the apical foramen because of its complex molecule structure.

In the Reciproc and Reciproc Blue subgroups, the percentage weight of Na in the Distilled water group were statistically significantly lower than NaOCl, EDTA and QMix groups. Due to the presence of sodium salt in all used irrigants in the present study, apical extruded material included Na elements. In the Reciproc and reciproc blue subgroups, the percentage weight of Cl in the NaOCl group was statistically significantly higher than Distilled water, EDTA and QMix groups. There is no study to compare these results.

The main inorganic components of the dentin are Ca and P, which are present in the hydroxyapatite crystals [7]. Due to its chelating action, EDTA can cause changes in dentin by modifying the Ca/P ratio [38, 60], including changes in hardness, permeability and solubility properties [39, 60, 61]. Lima Nogueira *et al.* [62] evaluated the effects of different irrigating solutions on mineral content of root canal dentin and they reported that intermediate irrigation with EDTA had lower Ca and P values in all root thirds and changes in the Ca/P ratio in the apical third. In their study percentage weight of Ca and P in saline solution group was higher than we reported in distilled water group. This may have varied because of different mineral content for all teeth we randomly selected. Randomization was made by tossing coins.

When NaOCl used alone, according to the concentration, the Ca / P ratio in the dentin may reduce or increase. [63-65]. In the present study Ca / P ratio in apical extruded material was higher in all groups than the other studies that were evaluated the mineral content of root canal dentin [39, 62].

This could be a limitation of this study that the root canals could not be analysed with EDX after preparation and irrigation of the specimens. Therefore we could not conclude the effect of irrigating solutions on the mineral content of root canal dentin.

In Reciproc and Reciproc Blue subgroups, distilled water irrigation produced statistically lower apical extruded material than other solutions. The EDTA irrigation caused statistically higher apical extruded material than other irrigants in Both Reciproc and Reciproc Blue subgroups. However percentage weight of Ca and P elements in EDTA group was statistically less than distilled water group in both during reciproc and reciproc blue instrumentation and also when using EDTA as an irrigant in reciproc blue subgroup, percentage weight of Ca and P elements were less than NaOCl and QMix groups. Percentage weight of Ca and P elements in distilled water group, was statistically higher than the other groups. There is no investigation comparing these four irrigating solutions that based on apically extruded material or postoperative pain. In addition another randomized clinical study compared 5.25% NaOCl, 2% CHX irrigating solutions on postoperative pain [66]. They stated that NaOCl showed statistically significant more pain at the 6-h time point. If we handled Reciproc subgroups for assessing the percentage weight of Ca and P elements, Reciproc blue instrumentation produced higher values than Reciproc instrumentation but statistically difference was found in QMix group ($p=0,001$).

Parirokh *et al.* [19] compared the apically extruded debris when using different irrigants. They reported that 5,25% NaOCl caused significantly more debris than 2% chlorhexidine and 2,5%NaOCl. In an another study Zhang L *et al.* [67] have reported that 2,5% sodium hypochlorite can produce more apically extruded debris than%1 sodium hypochlorite and%17 EDTA. According to these studies with the increased consantration of NaOCl, apical extruded material was rising. Contrary to the previous study in the current study, EDTA

irrigation caused significantly higher extruded material than NaOCl and QMiX irrigation. Further studies are needed in this regard.

Previous studies related to apical extruded debris used different materials and methods and no research has been done in this method. Therefore a direct comparison could not be made in this topic. We tried to simulate the clinical conditions to give similar results to the real teeth preparations.

Table 1: Mean and standart deviations of apical extruded material of groups. The p value written in bold showed significant difference.

		Reciproc	Reciproc Blue	p
DistileWater	Mean+SD	0,000263±0,000275	0,000829±0,000387	0,001
NaOCl	Mean+SD	0,009950±0,007794	0,005206±0,006852	0,224
EDTA	Mean+SD	0,031657±0,036424	0,020353±0,023891	0,324
QMIX	Mean+SD	0,010104±0,011764	0,006756±0,008552	0,922

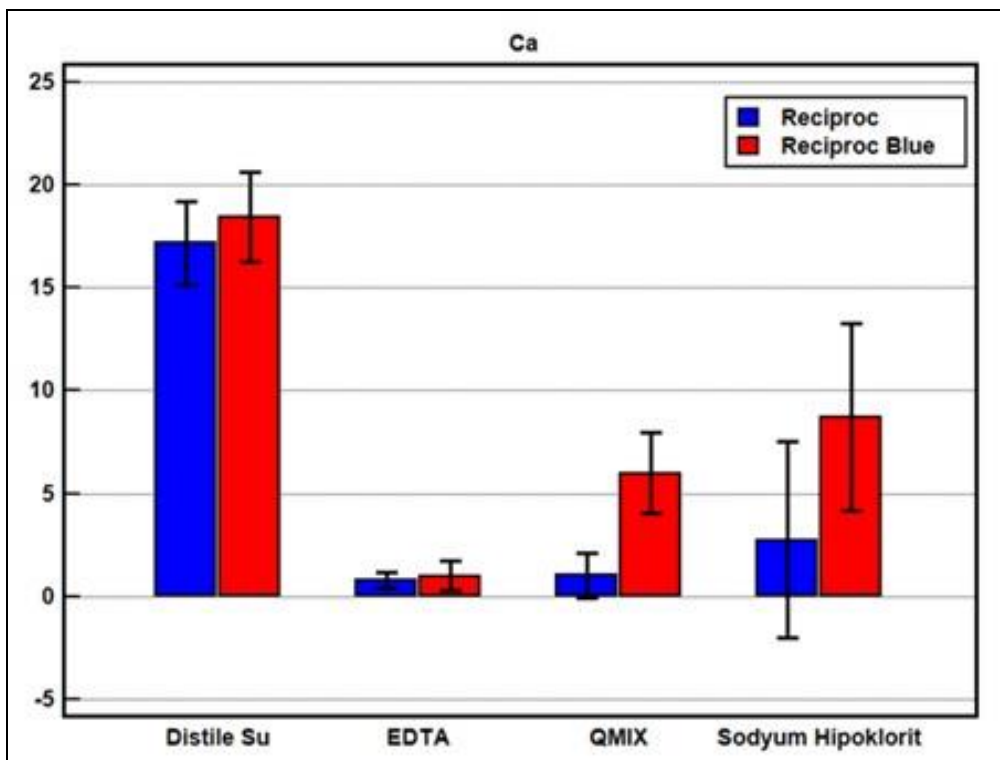


Fig 1: The mean percentage weight of extruded Ca in the reciproc and reciproc blue subgroups according to the irrigants

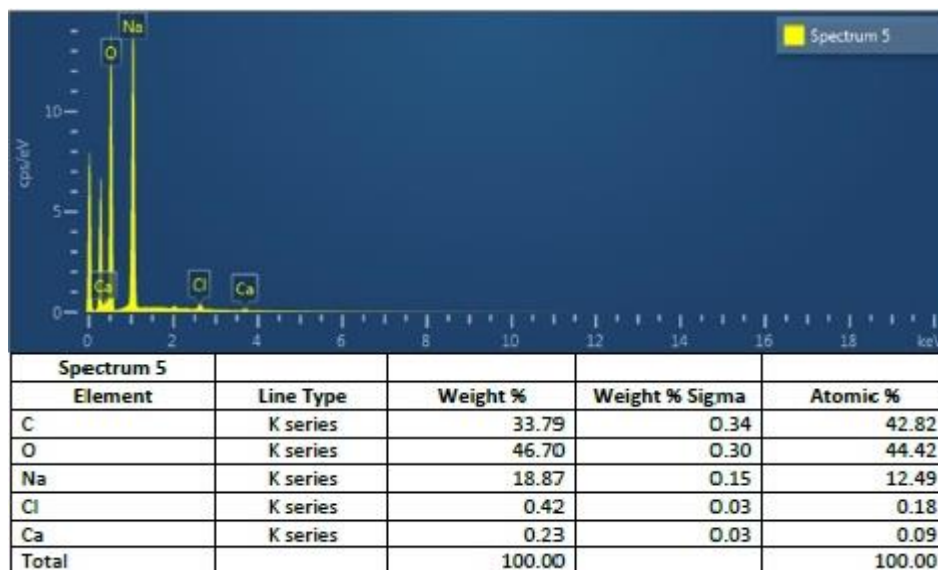


Fig 2: EDX analysis of a specimen from EDTA irrigation and Reciproc blue subgroup

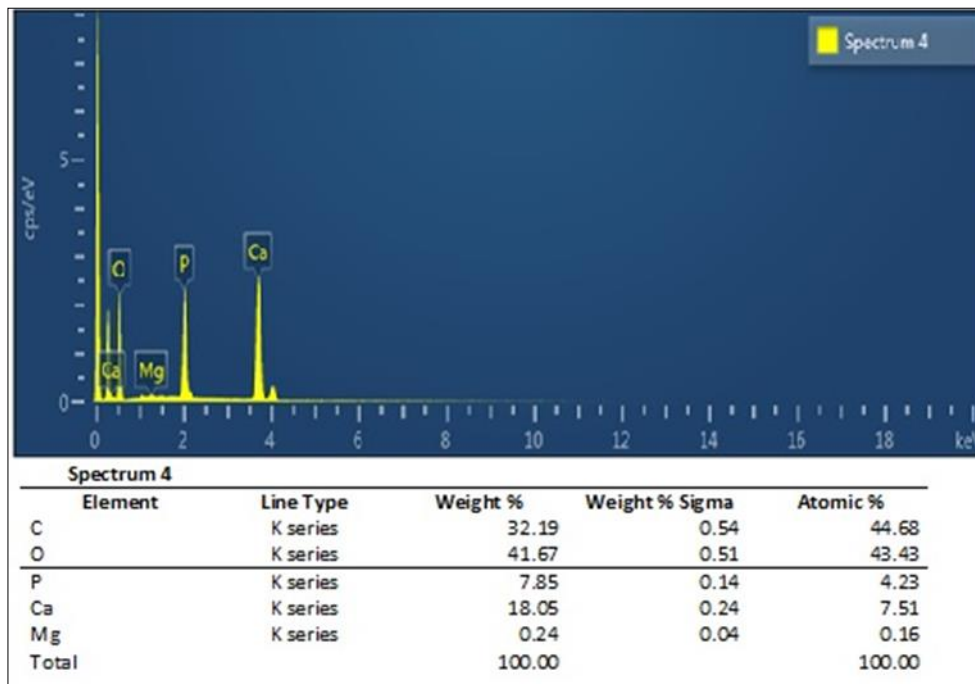


Fig 3: EDX analysis of a specimen from distile water irrigation Reciproc subgroup

5. Conclusion

Within the limitations of the present study, all the file systems and irrigation solutions led to apical extrusion during root canal preparation. There was no significantly difference among Ni-Ti files except distilled water irrigation group. In the Reciproc and Reciproc Blue subgroups, distilled water irrigation statistically produced lower extruded material than Sodium Hypochlorite, EDTA and QMix irrigation ($p=0,0001$). Percentage weight of Ca and P elements in distile water group, was statistically higher than the other groups. EDTA irrigation produced higher extruded material than the other irrigants however it extruded lower Ca and P levels. In conclusion, the results obtained under the experimental conditions showed that distile water could be used during instrumentation for obtaining lower extruded material, lower cytotoxicity and lower postoperative pain. Irrigating solutions could be used as final irrigation.

6. Acknowledgement

The authors deny any financial affiliations related to this study or its sponsors.

7. Conflict of Interest

The authors have declared that no COI exists

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