Original Article / Özgün Araştırma

The Effect of Different Chelating Agents on the Fracture Resistance of Endodontically Treated Teeth

Farklı Şelasyon Ajanlarının Kök Kanal Tedavili Dişlerin Kırılma Dayanımına Etkisinin Belirlenmesi

Hicran Dönmez Özkan[1], Konul Naghıyeva[2], Pınar Açkurt Okutan[1], Tuğrul Aslan[2]

[1]Aydın Adnan Menderes University Faculty of Dentistry, Department of Endodontics, Aydın, Turkey
[2]Erciyes University Faculty of Dentistry, Department of Endodontics, Kayseri, Turkey

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Address for Correspondence/Yazışma Adresi:
Hicran Dönmez Özkan DDS, PhD,
Aydın Adnan Menderes University Faculty of Dentistry, Department of Endodontics, Aydın, Turkey
Phone : +90 535 820 35 55
E-mail : hicrandonmez@hotmail.com

ORCID ID: orcid.org/0000-0002-4495-2746

Abstract

Objective: This study aimed to determine the effect of three different chelating agents on the fracture resistance of endodontically treated teeth with or without passive ultrasonic irrigation (PUI).

Materials and Methods: The crowns of 70 extracted human mandibular premolars were decoronated to obtain a standard root length. Then, root canals were instrumented with ProTaper Next rotary file up to size X4. According to the final irrigation procedures, seven groups (n=10) were formed: Group 1 (control group), 6-mL distilled water with conventional irrigation technique (CIT); group 2, 3-mL 2.5% sodium hypochlorite (NaOCl) +3-mL 17% ethylenediaminetetraacetic acid (EDTA) with CIT; group 3, 3-mL 2.5% NaOCl +3-mL 10% citric acid (CA) with CIT; group 4, 3-mL 2.5% NaOCl +3-mL 1% peracetic acid (PAA) with CIT; group 5, 3-mL 2.5% NaOCl +3-mL 17% EDTA with PUI; group 6, 3-mL 2.5% NaOCl +3-mL 10% CA with PUI; group 7, 3-mL 2.5% NaOCl +3-mL 1% PAA with PUI. Finally, root canals were obturated with X4 gutta-percha cones and epoxy resin-based root canal sealer. Samples were embedded into acrylic resin blocks, and vertical loading was performed on a universal testing machine until the fracture occurred. Values were recorded in Newtons. Data were statistically evaluated using the One-Way ANOVA.

Results: No significant differences were observed among the tested groups ($p>0.05$). Additionally, the activation of the tested chelating agents with PUI does not have any positive or negative effect on the fracture resistance of endodontically treated teeth.

Conclusion: Within the limitations of this study, all tested chelating agents can be safely selected to avoid vertical root fracture.

Öz

Amaç: Üç farklı şelasyon ajanının ve bu ajanların pasif ultrasonik irigasyon (PUI) ile aktive edilmesinin endodontik tedavili dişlerin kırılma dayanımı üzerine etkisinin belirlenmesidir.

Gereç ve Yöntemler: Yetmiş adet çekilmiş insan alt küçük azı dişinin kronları, standart bir kök uzunluğu elde etmek için kesilerek uzaklaştırıldı. Ardından, örnekler ProTaper Next döner eğe sisteminin X4 eğesine kadar şekillendirildi. Sonrasında, örnekler farklı son irigasyon prosedürlerine göre rastgele toplam 7 gruba (n=10) ayrıldı; grup 1 (kontrol grubu): 6 mL distile su + konvansiyonel irigasyon (CIT), grup

2: 3-mL % 2,5 sodyum hipoklorit (NaOCl) +3-mL %17 etilendiamintetraasetik asit (EDTA) + CIT, grup 3: 3-mL %2,5 NaOCl +3-mL %10 sitrik asit (CA) + CIT, grup 4: 3-mL %2,5 NaOCl +3-mL %1 perasetik asit (PAA) + CIT, grup 5: 3-mL %2.5 NaOCl +3-mL %17 EDTA + PUI, grup 6: 3-mL %2,5 NaOCl +3-mL %10 CA + PUI, grup 7: 3-mL %2.5 NaOCl +3-mL %1 PAA + PUI. Son olarak, tüm örnekler X4 güta-perka konları ve epoksi-rezin içerikli kök kanal patı ile dolduruldu. Örnekler, akrilik rezin bloklara gömüldü ve ardından Universal test cihazında kırılma meydana gelene kadar dikey yüklemeye tabi tutuldu ve değerler Newton cinsinden kaydedildi. Verilerin istatistiksel analizi için Tek Yönlü ANOVA kullanıldı.

Bulgular: Bütün gruplar arasında istatiksel olarak anlamlı fark bulunamamıştır (p>0,05). Ayrıca, test edilen şelasyon ajanlarının PUI ile aktivasyonunun olumlu ya da olumsuz herhangi bir katkı sağlamadığı tespit edildi.

Sonuç: Bu çalışmanın sınırları dahilinde, test edilen tüm şelasyon ajanlarının vertikal kök kırığından kaçınmak amacıyla güvenle tercih edilebilir olduğu görülmüştür.

Introduction

Sodium hypochlorite (NaOCl) is the most widely used irrigation solution in endodontic therapy due to its strong antibacterial activities and organic tissue dissolving capability (1). However, it is ineffective in the removal of inorganic tissue and debris. Therefore, it is often used in combination with decalcifying agents (1). In the common clinical concept, the root canals are irrigated with NaOCl during instrumentation so that the root canal dentin walls are cleaned from organic residues. But still, dentin walls are covered with inorganic residues of the smear layer (2,3).

Ethylenediaminetetraacetic acid (EDTA) is the most commonly preferred chelating agent among practitioners for the removal of inorganic structures of the smear layer (1). Despite its several advantages, the combined use of EDTA with NaOCl erodes the dentin and reduces the microhardness of teeth (4); moreover this combination has limited biocidal ability (1).

In recent years, organic acids such as maleic acid (5), citric acid (CA) (6), etidronic acid (7) and peracetic acid (PAA) (7,8) have been utilized as alternative decalcifying agents to EDTA. CA has been found to be as effective as EDTA in removing the smear layer. Besides, not only is CA solution more effective than EDTA in a short-term application (30 sec) (9), it has been reported to be less cytotoxic and more suitable for clinical use compared to EDTA (10). However, both EDTA and CA do not have strong antimicrobial properties (11). Recently, PAA was been suggested as an alternative to these decalcifying agents in order to disinfect the root canal system and remove the smear layer (7,8). PAA has strong antibacterial, antifungal, antiviral and sporicidal properties (12). Studies have investigated the smear layer dissolution ability (8) and antimicrobial efficacy (13) of PAA, but, to the best of our knowledge, there is no information on the effect of PAA on the fracture resistance (FR) of the endodontically treated teeth. This is the first study in the literature to investigates the effect of PAA on the FR of endodontically treated teeth.

Conventional needle irrigation is regarded as the basic technique for irrigation. However, the mechanical flushing action created by this technique is relatively weak and complete debridement of the root canal system cannot be achieved this way (14). The flushing action of the irrigants may be enhanced using passive ultrasonic irrigation technique (PUI), as suggested several times before (14-16). The final rinse of EDTA with PUI has been shown to significantly increase the removal of the smear layer (16). To the best of our knowledge, no previous research has investigated the effects of agitation of PAA and CA with PUI on FR of endodontically treated teeth.

Therefore, this study aims to determine the effects of EDTA, PAA and CA solutions on the FR of endodontically treated teeth with or without PUI. The first null hypothesis of present study was that there were no differences among tested chelating agents on the FR of endodontically treated teeth. The second null hypothesis was that there was no supplementary or adverse effect of PUI on the tested chelating agents on the FR of root canals.

Materials and Methods

According to the data of a previous research (17), G*Power v.3.1.9.4 software (Heinrich Heine, University of Düsseldorf, Düsseldorf, Germany) was utilized to calculate the required minimum sample size. An alpha-type error of 0.05 and a beta power of 0.95 were determined. Based on these parameters, the minimal estimated sample size per group was found to be 2, however we decided to use 10 samples for each group. Single rooted mature human mandibular premolars were collected for this study under a protocol approved by the Erciyes University Human Research Ethics Committee (protocol no: 2019/760). Before the tooth extractions, consent forms stating that the extracted teeth can be used in scientific research were signed by the patients. Collected teeth were stored in 0.1% thymol solution at 4°C until use. External root surfaces were cleaned using periodontal scaler to remove soft tissue residues and calculus. Buccolingual and mesiodistal radiographs were acquired to include teeth only with one straight root canal. Teeth with root defects, cracks, two or more root canals, internal or external resorption were excluded. Additionally, teeth with curvatures greater than 10° determined by the Schneider criteria (18) were excluded. Next, the crowns of the teeth were decoronated from the cementoenamel junction level using a diamond disc (KG-Sorensen, Barueri, SP, Brazil) with copious water irrigation to obtain a standardized root length of 14-mm. Then, the mesiodistal and buccolingual dimensions of each root were measured with an electronic digital caliper at the 14-mm coronal to the apex to standardize the dimensions of the specimens. The mean of the mesiodistal and the buccolingual root dimensions were 4.7±0.63 mm and 5.52±0.54 mm, respectively. The specimens with a difference in diameter of 20% or more from the mean were excluded from the study (6,17). Based on these criteria, seventy mandibular premolar teeth were utilized for present study.

The root length of the specimens was confirmed by inserting a #10 stainless steel K-files (Dentsply-Maillefer, Ballaigues, Switzerland) into the root canal until its tip was observed beyond the apical foramen. Working lengths were set at 1-mm short of this length. Specimens were instrumented using Protaper Next rotary files (Dentsply, Ballaigues, Switzerland) up to size X4 by the same endodontist according to the instructions of manufacturer. Root canals were irrigated with 2-mL 2.5% NaOCl between each instrument with a 30-gauge irrigation needle (NaviTip; Ultradent, USA). After the instrumentation procedures, paper points were used to dry the root canals. Then, the apices of the samples were sealed with sticky wax to create a closed-end channel. Finally, the samples were randomly distributed into 7 groups (six experimental groups and 1 control group) using a computer algorithm program (http://random.org) (n=10, in each group) using the following sequence of irrigation:

Group 1 (control group): Irrigation procedure was performed with 6 mL distilled water for 180 sec with conventional needle irrigation technique (CIT).

Group 2: Irrigation procedure was performed with 3-mL 2.5% NaOCl for 90 sec followed by 3-mL 17% EDTA for 90 sec with CIT.

Group 3: Irrigation procedure was performed with 3-mL 2.5% NaOCl for 90 sec followed by 3-mL 10% CA for 90 sec with CIT.

Group 4: Irrigation procedure was performed with 3-mL 2.5% NaOCl for 90 sec followed by 3-mL 1% PAA for 90 sec with CIT.

Group 5: Initially, irrigation procedure was performed with 3-mL 2.5% NaOCl using a 30-gauge irrigation needle (NaviTip, Ultradent) for 90 sec. Then, a total of 3-mL 17% EDTA was applied to root canal with PUI as follows: 1-mL 17% EDTA was applied to the root canal with an irrigation needle for 10 sec. Next, an ultrasonic tip (ESI, EMS, Nyon, Sweden) was applied into the root canal 2 mm short of the working length. The tip was activated at a frequency cycle of 28-32 kHz for 20 sec. This irrigation protocol with 17% EDTA was triplicated after 2.5% NaOCl use was finalized.

Group 6: Initially, irrigation procedure was performed with 3-ml 2.5% NaOCl using a 30-gauge irrigation needle (NaviTip, Ultradent) for 90 sec. Then, a total of 3-mL 10% CA was applied to the root canal performed with PUI as the same procedures as group 5.

Group 7: Initially, irrigation procedure was performed with 3-mL 2.5% NaOCl using a 30-gauge irrigation needle (NaviTip, Ultradent) for 90 sec. Then, a total of 3-mL 1% PAA was applied to the root canal performed with PUI as the same procedures as group 5-6.

All the irrigation procedures described above were performed with a 30-gauge irrigation needle (NaviTip, Ultradent, USA). The needle was applied into the root canal 2 mm shorter than the working length. After the final irrigation procedures, the root canals were rinsed with 2 mL distilled water and dried with paper points. Then, all root canals were sealed with a Protaper Next X4 (Dentsply Maillefer) gutta-percha point and epoxy resin-based root canal sealer (AH Plus, Dentsply De Trey, Konstanz, Germany) utilizing the single-cone technique.

All specimens were kept in an incubator at 37 °C and 100% humidity for one week to ensure the proper setting of the sealer. The root samples were prepared according to the methods of Turk et al. (17). As follows, in all groups, the root surfaces were covered with one layer of parafilm. Then, only the apical 9-mm of the specimens were embedded into the auto polymerizing acrylic resin (Heraeus Kulzer, Hanau, Germany). After the polymerization of the acrylic resin, specimens were removed from the resin blocks and then, parafilm layer was removed. A light body silicone-based impression material (Zhermack, Badia Polesine, Italy) was injected into the resin blocks and the teeth were carefully repositioned in the resin blocks. This silicone layer was created to mimic the periodontal ligament.

Lastly, the resin blocks were mounted to the lower platform of UTM (Universal Testing Machine, Instron Corp, Canton, USA). A stainless-steel ball tip with a diameter of 4.88 mm was placed on the upper jig of UTM. The tip was centered on the root canal orifice and a compressive loading was applied vertically at a crosshead speed of 0.5 mm/minute until fracture occurred. A sudden drop during continuous compression with the maximum fracture load was recorded in Newtons (N). Finally, crack line of the fracture was confirmed using a dental operating loupe at x4 magnification.

Statistical Analysis

The data were evaluated statistically with SPSS-PASW 18.00 software. They were normally distributed according to the Kolmogorov-Smirnov normality test. Therefore, the data were analyzed by One-Way ANOVA (α=0.05).

Results

The mean FR and the standard deviations of the experimental and the control groups were presented in Table 1. There were no statistically significant differences among the tested groups ($p>0.05$).

Discussion

Effects of three different chelating agents (17% EDTA, 10% CA, 1% PAA) on the FR of the endodontically treated teeth were evaluated in present study. According to the findings, there was no significant difference between the tested groups or the control group. Therefore, the first null hypothesis was accepted. We also observed that the ultrasonic activation of these agents did not have any supplementary or adverse effect on the FR of endodontically treated teeth compared to conventional needle irrigation. Therefore, the second null hypothesis also was not rejected.

The use of endodontic instruments results in the production of a smear layer consisting of both

Table 1. The mean, minimum and maximum fracture resistance values (Newton) of the tested groups are shown

Groups	Mean	Min-max
Group 1 (Control)	1086,84±284,91[a]	844,06-1705,11
Group 2 (EDTA+CIT)	1014,49±270,48[a]	621,45-1502,91
Group 3 (CA+CIT)	1052,74±381,48[a]	532,63-1546,28
Group 4 (PAA+CIT)	1032,63±340,86[a]	558,53-1614,10
Group 5 (EDTA+PUI)	1006,66±258,73[a]	709,32-1404,88
Group 6 (CA+PUI)	1064,03±374,32[a]	571,02-1655,55
Group 7 (PAA+PUI)	1081,71±303,89[a]	492,96-1362,89

EDTA: Ethylenediaminetetraacetic acid, CIT: Conventional irrigation technique, CA: Citric acid, PAA: Peracetic acid, PUI: Passive ultrasonic irrigation, min: Minimum, max: Maksimum inorganic and organic materials that covers the root canal walls. Sodium hypochlorite cannot dissolve particles of inorganic materials and thus cannot fully remove the smear layer alone (1).

Therefore, the use of chelating agents such as EDTA combined with the NaOCl was recommended for the removal of the smear layer (19,20). Removal of the smear layer is associated with dentin demineralization induced by chelating agents. However, the effect of chelating agents is generally not limited to the smear layer, they also affect the dentin by changing the Ca:P ratio (7) and reduce dentin hardness by exposing collagen fibers (21). Baumgartner and Mader (22) have shown that the combined use of EDTA and NaOCl causes dissolution of the peritubular and intertubular dentin. Another study showed that using 17% EDTA after 2.5% NaOCl causes a decrease in dentin microhardness (23). This result was attributed to the EDTA's ability to dissolve the mineral content of dentin (24,25). It is known that the microhardness of dentin is an important factor for FR. One study demonstrated that long-term administration (10 minutes) of 17% EDTA adversely affected the FR of endodontically treated teeth. The same study reported that a short-term administration (1 minute) of 17% EDTA increased FR compared to the positive control group which had only distilled water for the final rinse (26). This result was explained by the removal of the smear layer that contributes to the penetration and bonding of the epoxy resin-based root canal sealer (AH-Plus) (26). Similarly, Meryon et al. (27) declared that the smear layer was completely removed *in vivo* with 10% EDTA for 60 seconds. All these findings indicate the importance of determining the ideal application time and ideal volume for chelating agents. As per the aforementioned studies, we applied all chelating agents 3-mL for 90 seconds to accurately reflect the clinical conditions as well as the refrain from long term detrimental effects of these agents.

Several studies have shown that the presence of the smear layer negatively affects bonding, penetration, and adaptation of root canal sealers. In addition, the removal of the smear layer was been proved to result in better FR outcomes (28-30). Previous studies have also reported that resin-based root canal sealers adhere better to root canal dentin. Subsequently, the root canal wall and gutta-percha create a monoblock structure that helps to prevent tooth fracture (31,32). Considering all these factors, AH-Plus, an epoxy-resin based root canal sealer, was utilized in this study.

In the present study, the study groups revealed similar FR values compared to the control group in which chelating agents were not included. This outcome might have resulted from the negative effect of the smear layer on the bonding capacity of AH-Plus in the control group. The smear layer which was preserved in this group probably prevented the formation of a monoblock structure, which was previously shown by Uzunoglu et al. (26).

Previous studies have demonstrated that EDTA, PAA, and CA show similar effects in the removal of the smear layer (7). This similar effect of PAA and CA has been linked to their similar mechanism of action (33). It is thought that the similarities in their mechanism of action and their smear removal capacities may be one of the factors that explain the similar FR outcomes obtained in our study. Ayad et al. (34) investigated the effect of 10% and 20% lactic acid and 15% EDTA solutions for irrigation on the removal of the smear layer and the FR of endodontically treated teeth, however, they did not find a statistically significant difference between the groups. In another study, Arslan et al. (6) applied different concentrations of CA (10% and 50%) to root canals for different durations (1 minute and 10 minute) and compared the FR outcomes; and they did not find a statistical difference between the groups. Similar to the above-mentioned studies, the tested chelating agents in this study had similar FR outcomes in endodontically treated teeth.

Keine et al. (35) also examined the effects of peracetic acid on the smear layer and the penetration of the epoxy resin-based root canal sealer AH-Plus into the dentin tubules. They did not find a statistically significant difference between the group treated with NaOCl, EDTA and NaOCl, respectively and the group where peracetic acid was applied as the single agent. In both groups, the bonding of the sealer and root canal dentin was found to be higher than the groups where the smear layer was not removed. Another relevant study by De Deus et al. (8), showed that even at low concentrations (0.5%), PAA can remove the smear layer at a rate similar to 17% EDTA. For this reason, in this study, we utilized the commonly preferred 1% concentration for PAA to limit dentin erosion while still sufficiently removing the smear layer. Also, since PAA only removes the inorganic portion of the smear layer, Hartmann et al. (13) recommend using it in combination with NaOCl. As per this recommendation, we preferred to use all chelating agents in this study by combining them with NaOCl.

To the best of our knowledge, there are only a few studies in the literature on the effects of different chelating agents on the FR of the endodontically treated teeth (6,23,25,26). Also, this is the first study in the literature to investigate the effects of PUI on the CA and PAA solutions regarding the FR of the endodontically treated teeth. A recent study applied sodium hypochlorite as the main root canal irrigant with the conventional irrigation procedure in one group, and with the PUI activation method in another group (36). Similar to our study, they did not find that FR was significantly affected by the irrigation method (36). We also found that the PUI activation of different chelating agents did not positively or negatively affect the FR. In addition to this, a recently published study reported that PUI activation of EDTA and PAA solutions which were used for final rinse did not result in increased antibacterial activity on *Enterococcus faecalis* compared to manual agitation (13).

It has been reported that the FR of the endodontically treated teeth is affected by many factors such as occlusion type, remaining crown structure, coronal restoration quality, and parafunctional habits (17,37). Therefore, one of the limitations of this study is that the crown was cut from the cementoenamel junction to provide standard test conditions. Another limitation of this study is that the experimental methods determined for the laboratory environment do not fully reflect the clinical practice.

Conclusion

In the light of the findings of this *in vitro* study, we observed that organic chelating agents such as CA and PAA have a similar effect on FR of the endodontically treated teeth, compared to its inorganic compartment EDTA. This result confirms the reliability of the short-term application (90 sec) of 17% EDTA solution, which is frequently preferred in routine clinical practice. It has also been shown that the activation of the tested chelating agents with PUI does not positively or negatively affect the FR of endodontically treated teeth. There are currently only a few studies on this subject, and our results need to be supported by further studies.

Ethics

Ethics Committee Approval: Single rooted mature human mandibular premolars were collected for this study under a protocol approved by the Erciyes University Human Research Ethics Committee (protocol no: 2019/760).

Informed Consent: Before the tooth extractions, consent forms stating that the extracted teeth can be used in scientific research were signed by the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: T.A., H.D.Ö., Design: H.D.Ö., K.N., P.A.O., T.A., Supervision: T.A., H.D.Ö., Fundings: H.D.Ö., K.N., P.A.O., T.A., Materials: H.D.Ö., K.N., P.A.O., T.A., Data Collection or Processing: H.D.Ö., K.N., P.A.O., T.A., Analysis or Interpretation: K.N., H.D.Ö., T.A., Literature Search: P.A.O., H.D.Ö., Writing: H.D.Ö., P.A.O., Critical Review: T.A.

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