EVALUATION OF THE MICROHARDNESS OF ROOT CANAL DENTIN AFTER DIFFERENT IRRIGATION PROTOCOLS (IN VITRO STUDY)

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ABSTRACT

INTRODUCTION: Different irrigations solutions may cause alteration in the physicochemical properties of dentin structure thereby affecting the microhardness of root canal dentin.
OBJECTIVES: to evaluate the effect of different irrigation protocols on microhardness of human root canal dentin.
MATERIALS AND METHODS: Forty extracted single rooted lower premolars were used. All teeth were instrumented using manual stainless steel files and irrigated by 2ml distilled water between each file, then were sectioned by longitudinal splitting of each tooth. The root halves were randomly assigned into 4 parallel groups (n=10) and immersed for 5 minutes with one of the following irrigants: Group I: 10 ml of 2.5% Sodium Hypochlorite (NaOCL), Group II: 10 ml of 17% ethylene diamine tetra-acetic acid (EDTA) followed by 10 ml of 2.5% NaOCL, Group III: 10 ml of 2.5% NaOCL followed by 10 ml of 2% chlorhexidine digluconate (CHX), Group IV: 10 ml of 2.5% NaOCL followed by 10 ml distilled water then were followed by 10ml of 2% CHX. Ten root halves from each group were prepared to measure dentin microhardness at baseline measurement and after treatment to determine the change in microhardness, using Vickers tester.
RESULTS: Data were analysed using t-test, ANOVA test and Post Hoc test.Group II showed the highest percentage decrease in microhardness values, followed by group III, then group IV and the lowest was group I. All groups showed a significant difference between each other ($P < 0.05$), except group III and IV. The coronal third showed the highest percentage decrease with significant difference between apical and middle thirds ($P < 0.05$), in which there was no significant difference between them.
CONCLUSIONS: CHX is the best final irrigant if there is excellent intermediate flush for prevention of its precipitation with NaOCL.The coronal third needs conservative approach as it is the most affected third.
KEYWORDS: Endodontics, Microhardness, Chlorhexidine, NaOCL, EDTA, Precipitate.

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INTRODUCTION

Success in endodontic therapy depends on chemomechanical debridement of the root canal system through the use of instruments and effective irrigants solutions (1). No irrigant can completely eliminate all organic and inorganic matter and at the same time impart a substantive residual antimicrobial property to the canal wall dentin. It should be also effective against the enterococcus faecalis, thus the combination of auxiliary solutions is necessary to achieve the desired effects (2, 3).

Sodium hypochlorite (NaOCl), because of its broad-spectrum antimicrobial action and tissue-dissolving properties is considered as the gold standard irrigant used in root canal treatment. Despite its germicidal abilities, NaOCl in high concentration is cytotoxic to periapical tissues (2, 3) and can affect dentin structure regarding its physico-chemical and adhesive properties (4,5).

Ethylene diamine tetra-acetic acid (EDTA, PH=7.7) and sodium hypochlorite (NaOCI) solutions have been advocated as an effective irrigation regimen to remove the inorganic and organic remnants of smear layer and has gained wide acceptance (6). Chelating agents decalcify the dentin by combining with the calcium ions of the tooth structure (7) and the organic dissolving properties of NaOCl on the collagen component of dentin explain how the alternated irrigation of these solutions affects the hardness of dentin (5).

Unlike NaOCl, Chlorhexidine gluconate (CHX) has low grade toxicity and has a unique feature that acquires antimicrobial substantivity and improves the adhesive properties of dentin structure (2, 3). Furthermore, it has been stated that CHX failed to significantly alter the microhardness of root canal dentin (8).

However, the drawback of chlorhexidine gluconate is its inability to dissolve the organic matter. Therefore, chlorhexidine gluconate (PH 5.5-6.0) cannot be a replacement irrigant for NaOCl but it is used as a supplemental final irrigation step after NaOCl. This combination between NaOCl and CHX resulted in a dense, orange-brown precipitate (2, 3) that may affect the microhardeness of root canal dentin.

Microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues as it is sensitive to composition and surface changes of the tooth structure (9, 10). Therefore, the objective of this study was to evaluate the effect of interaction of irrigant solutions on the microhardness of root canal dentin. The null hypothesis of this study is that the precipitate resulting from the use of a combination of NaOCl and chlorhexidine gluconate will not affect the microhardness of the root canal dentin when compared to other irrigating regimens

MATERIALS AND METHODS

Preparation of tooth specimens

Forty straight single-rooted lower premolars with relatively similar dimension and morphology, and closed apices were extracted for orthodontic reasons and collected from adult patients. Teeth with previous root caries, cracks, curved canals, endodontic treatment, internal resorption or calcification were excluded.

Teeth were thoroughly cleaned from any soft tissue or calculus deposition then they were stored in isotonic saline solution at room temperature till time of use and then radiographed in proximal view to confirm presence of a patent single canal. The crowns of all specimens were decoronated transversally at the cemento-enamel junction (CEJ) with a double-faced diamond disc (Microdont LDA.Brazil) at low speed with water coolant to ensure a uniform sample length of 14 mm (± 1mm root length).

Canal preparation (11)

All teeth were instrumented as follows: Working lengths were established by inserting a size10 K-file (Mani, Inc, Japan) to the root canal terminus until it became visible through the apical foramen and subtracting 1 mm, the coronal and the middle portions were flared using Gates-Glidden drills in the following sequence (size 3, 2, 1). The apical portion of the canal was instrumented from initial file size 15 k-file to the master apical file (MAF) size 30 K-file then step back flaring was done until reaching the size 45 and recapitulation was done with MAF. Canals in all groups were irrigated
with a standardized volume of 2ml of distilled water using a universal 27-gauge needle between each file.

Specimen preparation for microhardness evaluation (12, 13)

Specimens were longitudinally sectioned in a bucco-lingual direction by using a double faced diamond disk at low speed, without passing through the canal space. This was followed by using a chisel & mallet to split the root. The root segments were then horizontally embedded in autopolymerizing acrylic resin (Acrostone, Dent Product. Egypt) leaving their dentin surface exposed.The dentin surface of the mounted specimens was ground flat and smooth with a series of ascending grades of carbide abrasive papers 500, 800, 1,000, and 1,200 grit (Bigo, Dent Product .Germany) under distilled water to remove any surface scratches and finally polished with 0.1-Mm alumina suspension on a rotary felt disc (Microdont LDA. Brazil) to obtain a smooth glossy mirror-like surface.

Measurement of dentin microhardness

Microhardness was measured for each sample at baseline and after application of different irrigating solution protocols.

Baseline microhardness value was measured using Vickers Microhardness Tester (Model LM-100, FM 1159 LECO Corporation Michigan, and U.S.A) at magnification of x100 using a 25gm load for 10 seconds. The microhardness measurements were taken either on the buccal or lingual side and were determined at three different points for each sectioned root: on the coronal, middle and apical thirds. Each sectioned root was equally divided into three thirds representing coronal, middle and apical third. In each third (corono-apically), two centrally located points were determined, the first point of measurement represents the baseline value while the second point of measurement represents the post application value after immersion in the tested irrigants such that the distance between the two points was at least 1mm. The indentation was made on the dentin surface approximately at 200µm from the canal-dentin interface for standardization (14, 15) as shown in figure (1a, 1b,). The Vickers hardness is obtained by dividing the test force by the area of the sloping faces of the indentation. The resulting impression of the two diagonals was observed with an optical microscope and the average length of the two diagonals was measured by the built-in scaled micrometer and converted into Vickers hardness number (VHN) as shown in figure (1c, 1d) with the following equation (5,16):

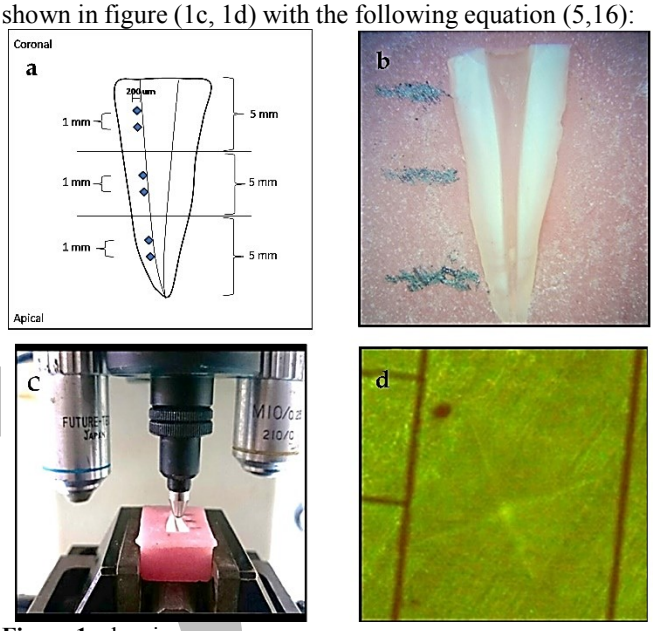

Figure 1: showing:
a) Diagram showing the first and second point measurement location.
b) The analogue image of the diagram
c) Mounting the specimen on Vicker's hardness tester for measurement
d) Analogue image (photomicrograph) of the square based diamond pyramid of the indenter impression on the root canal surface.

$HV=1854(F/D^2)$.

The constant value of the equation was calculated from the specific geometry of the indenter, F being the applied load in gram force and D being the diagonals of the indentation in (µm).

Evaluation of microhardness for the tested irrigants

The samples were randomly divided into four parallel groups according to the irrigant used (n=10). Then each sample was immersed in each tested irrigant solutions for 5 minutes.

Grouping

Group I: 10ml of 2.5%NaOCl (Clorox for Chemical Industries, A.R.E.).
Group II: 10ml 17% EDTA (Prevest Denpro limited, India.); followed by 10ml of 2.5% NaOCl.
Group III: 10ml of 2.5% NaOCl; followed by 10ml of 2% CHX (Kempetro for Chemical Industries, A.R.E).
Group IV: 10ml of 2.5%NaOCl then 10ml distilled water (intermediate immersion) followed by 10ml of 2% CHX.

This mean that for each individual irrigant solution the total immersion time per sample was 5 minutes; for group I samples were subjected to a total of 5minutes, while group II samples were subjected to a total of 10 minutes, then group III sample were subjected to a total of 10 minutes, lastly group IV sample were subjected to a total of 15 minutes.

STATISTICAL ANALYSIS

The collected data was fed to statistical software IBM SPSS version 20. All statistical analysis was done using two tailed tests and alpha error of 0.05 P value less than or equal to 0.05 was considered to be statistically significant. Quantitative normally distributed data were described using mean and standard deviation as well as, minimum and maximum. Comparison between two independent populations was done using the independent student t-test while comparisons between more than two populations were analyzed using the F-test (ANOVA) and Post Hoc tests (Scheffe).

RESULTS

There were no statistically significant differences between the mean of pre-treatment

Vickers's hardness number (VHN) values for all tested groups, while after immersion of the specimens in the irrigating solution for 5 minutes, all irrigating solutions significantly decreased the microhardness of the canal dentin surface compared to the pre-treatment values.

Group II (EDTA+NaOCl) showed the highest percentage decrease in microhardness values and was equal to 30.97±5.90 VHN, followed by group III (NaOCl + Distilled water + CHX) where the percentage decrease was equal to 18.00±2.62 VHN, then group IV (NaOCl+CHX) where the percentage decrease was equal to 14.07±1.09VHN. The lowest was group I (NaOCl) and the percentage decrease was equal to 9.10±1.11VHN. All groups showed a significant difference between each other ($P < 0.05$), except group III and IV, as shown in table (1).

The coronal third showed the highest percentage decrease in microhardness values with significant difference with apical and middle thirds ($P < 0.05$), while there was no significant difference between apical and middle thirds as shown in table (2) and figure (2).

Table 1: showing comparison between overall percentages decrease value of the mean of the three zones different studied groups.

Coronal, middle And apical thirds	Group I	Group II	Group III	Group IV
Range Mean±S.D	8.19 - 11.78 9.10±1.11	18.97-39.08 30.97±5.90	14.92 - 22.95 18.00±2.62	12.29 - 15.63 14.07±1.09
F P	22.1 0.0001*			
P1		0.0001*	0.002*	0.031*
P2			0.001*	0.001*
P3				0.077

P1 comparison between group I and other groups.
P2 comparison between group II and group III, IV
P3 comparison between group III and IV
F-test (ANOVA) * $P < 0.05$ (significant)
Post Hoc test (Scheffe)

DISCUSSION

The use of irrigation solutions is mandated in order to improve chemo-mechanical debridement of the root canal system and its anatomic complexity lying in the form of fins and accessory canals (1). However, none of the available irrigating solutions can be regarded as optimal, thus the combination of auxiliary solutions with the correct irrigation sequence is necessary to achieve a successful treatment outcome (3, 17).

The interaction of irrigants individually or in combination with root dentin is capable of causing alteration of the physicochemical properties of the dentin structure such as the microhardness, permeability and solubility of root canal dentin due to change in the original proportion of the inorganic and organic components of root canal dentin (9,14,18).

Table 2: showing comparison between different studied groups regarding the overall percentage decrease in the all different root thirds.

	Group I	Group II	Group III	Group IV
APICAL			14.55-	11.11-
Range	7.61-11.38	9.62-38.41	22.10	14.45
Mean±S.D	8.36±1.16	29.56±8.01	17.18±2.35	13.23±1.01
MIDDLE		10.34-	14.67-	12.08-
Range	7.67-11.38	38.69	22.34	15.55
Mean±S.D	8.92±1.08	30.37±8.02	17.68±2.52	13.82±1.10
CORONAL		20.17-	15.53-	13.19-
Range	8.70-12.59	40.15	24.42	16.88
Mean±S.D	10.02±1.15	32.98±6.06	19.15±3.09	15.16±1.25
P1	0.312	0.087	0.452	0.236
P2	0.011*	0.041*	0.036*	0.047*
P3	0.021*	0.032*	0.041*	0.035*

P1 comparison between apical and middle
P2 comparison between apical and coronal
P3 comparison between middle and coronal
F-test (ANOVA) * $P < 0.05$ (significant)
Post Hoc test (Scheffe)

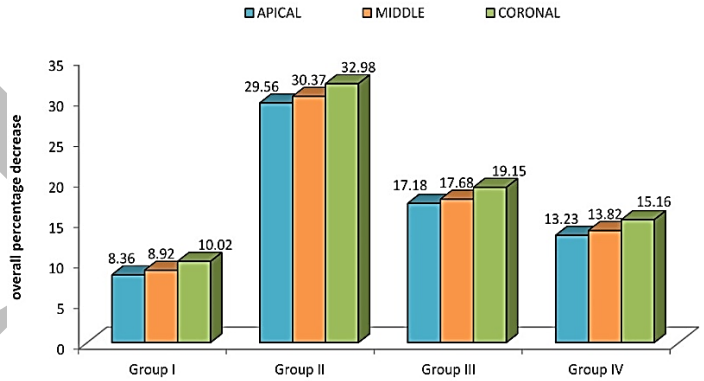

Figure 2: showing comparison between different studied groups regarding the overall percentage decrease in the all different root thirds.

Hardness measurements can be correlated with other mechanical and adhesive properties such as fracture resistance, modulus of elasticity, yield strength and the respective bond strength. Therefore, microhardness provides a first step toward predicting the behavior of dentin and restoration interfaces (9, 19, 20).

Although a reduction in microhardness facilitates the instrumentation throughout the root canal, it may also weaken the root structure, consequently root canal-treated teeth are more prone to fracture (19, 20). In addition, it may increase the permeability, solubility of the root canal dentin and adversely affects the sealing ability and adhesion of dental materials to dentin which in turn inhibits resistance to bacterial ingress and permits coronal leakage (9, 21).

In the present study, the canals were prepared by using the hybrid technique of instrumentation which is said to

maintain the integrity of the dentin as it avoids excessive removal of radicular dentin (11). Furthermore, this allowed providing a plane of measurement as close as possible to the canal lumen. Pashley et al (22) reported that the microhardness of dentin decreased near the pulp due to increase of the tubular density. As the tubule density increases the amount of calcified matrix between tubules decreases. This was also associated with a decrease in the amount of intertubular dentin (22).

The standardization of the prepared specimens was proved by the absence of a statistical significant difference between the pretreatment vicker hardness values of all the tested groups.

In the current study, the longitudinal sectioning of the roots was preferred instead of cutting transversally into discs as Cruz-Filho et al (13) observed that it can show accurate representations of clinical situations. Additionally, the irrigants first contact the most superficial layer of dentin in the root canal lumen and so, the present study measured the microhardness of the most superficial layer of root canal dentin.

On the contrary, previous studies (23,) used the transverse sectioning of the root into discs to evaluate the hardness value in the region between the main canal and the cementum layer.

The microhardness of the radicular dentin varied at different locations within the same tooth. Thus, in the present study, to measure the Vickers hardness values for the dentin, indentations were made in the cervical, middle, and apical thirds of the radicular dentin and were done at the 0.2 -mm level from the root canal walls for standardization (14, 18, 21, 24). Furthermore, less than 0.2 mm may lead to fracture of the specimen and more than 0. 2 mm may lead to error in the reading of microhardness values (16, 18).

There is a lack of consensus on application time, concentration and irrigation sequence for obtaining optimum result in root canal therapy.

The immersion time in the present study was five minutes for each irrigating solution as it may simulate the clinical application time of the irrigant solution. In accordance with the current study were those of Sayin et al (24), Cruz-Filho et al (13), Ulusoy et al (14) and Aslantas et al (8) who used the root canal irrigants for five minutes in their microhardness tests, stating that this duration is more realistic in terms of clinical practice. In addition De-Deus et al (25) evaluated the effect 17% EDTA for one,three and five minutes on the microhardness of radicular dentin .They found that EDTA produced the greatest decrease in microhardness from reference state to 3 min and then microhardness did not change after 5 min. Goldberg et al (5) evaluated the effect of 2.5% and 6% sodium hypochlorite solutions on root dentin microhardness for various irrigation periods and they found that irrigation for 5 minutes did not lead to a significant change in dentin microhardness.

Meanwhile, Calt et al (28) showed that irrigation with 17% EDTA was time dependant; increased treatment time led to increased calcium loss and microhardness reduction. In addition, they found that 1 min EDTA irrigation is effective in removing the smear layer, however a 10-min application of EDTA caused excessive peritubular and intertubular dentinal erosion especially when using NAOCL after EDTA.

In addition to contact time, the concentration of the irrigation solution needs to be considered as another determinant in the post-treatment microhardness.

In the present study, 2.5% concentration of NaOCl was used as it has greater effectiveness than 0.5% and 1% concentrations and has lower cytotoxicty than the 5.25% concentration (5, 14, 24). Furthermore, the 2.5% NaOCl is capable of inhibiting 100% of the Enterococcus faecalis in 5 minutes (27)

However, some reasearchers (28) concluded that NaOCl caused a concentration-dependent reduction of microhardness values and 0.5%, NaOCl, is recommended as the predominant concentration for routine use during root canal therapy to minimize any NaOCl-induced dentin deproteination. However, both volume and time must be increased to enhance irrigation efficiency with low concentration of NaOCL (28).

In the present study, 17% concentration of EDTA solution was used as it is the most commonly concentration in clinical practice (3, 7, 12, 14). However, some studies have indicated that EDTA with lower concentration (e.g: 15, 10%, 5%, and even 1%) removes the smear layer equally well after NaOCl irrigation and they are recommended to use this lower concentration of EDTA in clinical practice to avoid excessive erosion of root canal dentin (3, 29).

Regarding CHX, concentration of 2% was used as it is the most desired concentration in clinical practice for root canal irrigation (3,8,30,31). Furthermore, CHX at low concentration will result in bacteriostatic effect but at higher concentrations, it is bactericidal (2,3).

The present study revealed that all irrigation solutions decreased significantly dentin microhardness in the following sequence; group II, group III then group IV and finally group I. In group I, we found that 2, 5% NaOCl reduced significantly dentin microhardness when compared to base line values. This result is consistent with previous studies (5, 8, 30).

Kinney et al (32) suggested that the decrease in hardness is caused by a decrease in stiffness of intertubular dentin matrix caused by heterogeneous distribution of the mineral phase within the collagen matrix.In addition NaOCL is an efficient organic tissue solvent that causes dissolution of collagen by the breakdown of the bonds between carbon atoms and disorganization of the protein's primary structure and change in magnesium and phosphate ions.

On the other hand Hue et al (28) investigated the effect of NaOCl on the surface chemical changes to human dentin (dentin deproteinization). The attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy technique was used to analyze the amide:phosphate ratio and carbonate:phosphate ratio. They found that the amide phosphate ratio decreased significantly after NaOCl treatment but did not affect the carbonate phosphate ratio.

GroupII showed the highest decrease in microhardeness due to the fact that the effect was mainly from EDTA not from NaOCL (7,8,15,19,23), as the chelating action of EDTA solution induces an adverse softening potential on the calcified components of dentin and consequently a reduction in the microhardness was expected (6-8). Also, the dissolving action of NaOCl on the organic collagen components of dentin explains how the alternated irrigation with these solutions affects the hardness of dentin (5, 8, 30).

The high inorganic content of the dentin which is approximately 70% compared to 20% the organic content; this explains the more reduction in dentin microhardness caused by EDTA (15, 20).

Furthermore, the effect of the combination of EDTA with NAOCL in this sequence causes this decrease in microhardness values (3, 6, 17, 20, 24). Other researchers (33) reported that the final irrigation with NaOCl will accelerate dentinal erosion following treatment with EDTA and causes a marked erosion of the root canal wall dentin, with dentin microhardness reduction. Indeed; this was demonstrated in the current study.

The dentin microhardness of group III was less than that of group I and group VI. This may be due to the orange-brown precipitate that forms in response to the combination of NaOCL with CHX without intermediate flush in between .However, when distilled water was added in group IV as an intermediate flush between NaOCL and CHX, the microhardness reduction was less than group III, although there was no significant difference in between them but still there was a decrease in microhardness more than group I due to the fact that the precipitate formed was reduced by distilled water (2, 3).

Chlorhexidine agent had no effect on the dentin structure when compared to EDTA or NaOCL (8,34,35), because CHX has neither tissue dissolving properties proteolytic properties nor chelating properties (2,3). The result of this study is contradicting with the study (30) which reported that 2.0% CHX showed a statistically significant decrease in the microhardness of root dentin which could not be explained by the authors and could not be confirmed by any other study. This might be attributed to the difference in the methods used in the study such as difference in exposure time (prolonged exposure time, 15minutes).

Regarding the effect of the variation in immersion time between groups on the results, comparing group I and other tested groups; the shortest immersion time used in group I (5 minutes) in comparison with other tested groups might affect its result as it showed the lowest decrease in microhardness value (5). Meanwhile, comparing groups II, III and IV, the immersion time has no effect on their results. As group II showed the highest decrease in microhardeness value followed by group III then group IV. Although, groups II and III have 10 minutes' immersion time which is less than the time used in group IV (15 minutes). This might be attributed due to the fact that the effect was mainly from type of irrigating solutions and the sequence applied but no noticeable effect of the variation in immersion time (3, 15, 24, 33, 35).

In the present study, the coronal segments had significantly lower dentin microhardness in comparison with the middle and the apical segments in all groups. This finding agrees with several studies (14, 24, 36). This may be attributed to the histological pattern of the root canal dentin and relative nature of dentin in the apical region as Carrigan et al (21) showed that tubule density decreased from cervical to apical dentin and Pashley et al (22) reported that there was an inverse correlation between dentine microhardness and tubular density.

Previous researchers (37) reported that the apical portion of human teeth showed marked variations in structure, including accessory root canals, varied amounts of irregular secondary dentine, cementum-like tissue, low content of non-collagenous proteins (NCPs) and even dentin sclerosis. Hence, EDTA may not have such a pronounced action in apical third because it acts on smear layer and noncollagenous proteins (NCPs).

Sayin et al (24) reported that NaOCl and or EDTA was not as effective in the apical region as it was in the coronal and middle thirds, probably because it has been shown to be less effective in reducing the surface tension at the apical region than in the middle and coronal thirds and or may be because of the less penetration of irrigating solution in the apical third of the canal with little amount of irrigating solution in contact with root canal walls at apical third.

On contrary the results of this study disagree with the work of Ballal et al (12) who reported that there was an increase in microhardness from apical to coronal third. Meanwhile, Singh et al (38) reported that there was no significant difference in microhardness reduction in the coronal, middle, and apical thirds of root dentin when treated with the tested solutions. The controversy of these results with other studies might be attributed to differences in methods of applications of these irrigants, different evaluation techniques for evaluation of smear layer hardness and different types of root canal preparation.

A possible limitation of the present study is the immersion treatment as the volume of the irrigant in a root canal clinically is small compared with the immersing root dentin in irrigating solutions and the experiments were performed at room temperature and not body temperature. In addition, the use of hand instruments creates a significant amount of smear layer. However, standardized circumstances for all study groups allowed for comparable results.

Finally, it is recommended that chelators as EDTA can be used in low concentrations (3, 29) or shorter chelating time (3, 26) and NaOCl solutions can be used in low concentrations (3, 28). A final flush of NaOCL after EDTA must be considered (3, 17, 33) which could be replaced by CHX (2, 3) or other new irrigant solutions (3, 31, 36, 38, 39). As well as remineralizing agent may be used before bonding procedures for promoting remineralization and improving the microhardness of eroded root dentin (19, 40).

CONCLUSIONS

Within the limitations of this study, it could be concluded that:

1) All irrigating solution used this study significantly decreased dentin microhardness.
2) 17% EDTA followed by 2.5% NaOCl showed the highest percentage decrease in microhardness values.
3) The precipitate formed as a result of the interaction between 2.5% NaOCl and 2% CHX has significant effect on dentin microhardness values.
4) The coronal third of the root canal is the most affected third by irrigation solution.
5) Chlohexidine is the best final irrigant if there is excellent intermediate flush for prevention of its precipitation with NaOCL.
6) The coronal third needs conservative approach as it is the most affected third.

CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest.

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