Evaluation of the Effect of EDTA, EGTA and Citric Acid on the Microhardness and Roughness of Human Radicular Dentin – An In Vitro Study

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Abstracts: Context: Smear layer is a negative factor which prevents adhesion of the filling material to the dentinal walls. Chelating agents are used during cleaning and shaping of the root canals so as to remove the smear layer. **Aims:** To evaluate the effect of 17%EDTA, 17%EGTA, and 19% CITRIC ACID solutions on microhardness of root canal dentin using Vicker's Hardness Tester and surface roughness using Computerized Roughness Tester. **Settings and Design: Methods and Material:** Sixty non carious specimens were divided into four groups and subjected to varied treatments as follows: 1) Group I – 17%EDTA + 5% NaOCl for 150 sec 2) Group II – 17%EGTA + 5% NaOCl for 150 sec 3) Group III – 19% Citric Acid + 5% NaOCl for 150 sec 4)Group IV – Distilled water. Each group was divided into subgroup 'a' and 'b'. 'a' group were subjected to microhardness testing and 'b' group were subjected to surface roughness. **Statistical Analysis Used:** Results were subjected to Anova and Tukeys test. **Results:** Difference in microhardness values was significant between Ia and IIa ($p<0.05$, 0.029) and groups IIa and IIIa (p-0.001). Citric acid decreased the overall microhardness of the root canal dentin more than other irrigants. EGTA caused minimum reduction in microhardness. Maximum increase in surface roughness was seen in citric acid group and minimum increase in EGTA group. **Conclusions:** EDTA, EGTA and citric acid drastically reduce the microhardness and increase the surface roughness of radicular dentin. EGTA caused minimum reduction in microhardness and citric acid caused maximum increase in surface roughness. [Nayyar A NJIRM 2014; 5(6):24-30]

Key Words: EDTA, EGTA, Citric acid, Microhardness, Surface roughness, Root canal wall

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Introduction: Success of root canal treatment depends on the method and quality of instrumentation, irrigation, disinfection and three dimensional obturation of the root canal[1]. Endodontic instrumentation produces smear layer and plugs off organic and inorganic particles of calcified tissue and organic elements like pulp tissue debris, odontoblastic processes, micro-organisms and blood cells into the dentinal tubules.[2]

Different materials have been used to remove the smear layer, most commonly used is Ethylene-diamine-tetraacetic acid (EDTA). Irrigating the root canals with 10 ml. EDTA followed by 10 ml. of Sodium hypochlorite (NaOCl) is recommended as an effective method to remove the smear layer.[3,4] Citric acid , a weak organic acid, though not effective as EDTA in removing the smear layer, is less cytotoxic to tissues.[5]Ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'–tetraacetic acid (EGTA) is effective in removing smear layer without inducing dentinal erosion commonly caused by EDTA.[6]

Microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues.[7]Microhardness is sensitive to composition and surface. Changes in mineral content can adversely affect the sealing ability and adhesion of dental materials.[8]

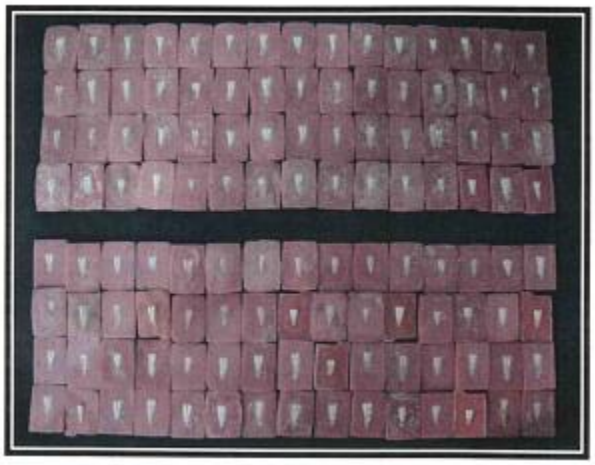
Removal of smear layer by irrigants increases the surface roughness. This is a clinical benefit in case of micromechanical bonding of adhesive materials.[2]

The aim of this study was to evaluate the effect of EDTA, EGTA, and Citric acid on microhardness and surface roughness of radicular dentin after instrumentation with Protaper files.

Subjects and Methods: Materials and methods: Tooth Preparation: Sixty non-carious, non-hypoplastic, freshly extracted human permanent

maxillary anterior teeth, between age group 35-45 years were collected for the study. The teeth were stored in distilled water and used within two months after extraction. The teeth were disinfected and handled as per the guidelines laid down by OSHA and CDC. Soft tissue debris and calculus on the root was removed and the crowns of the teeth sectioned at cemento-enamel junction. Cleaning and shaping was done with rotary Pro-taper files. Each root was sectioned longitudinally into labial and lingual segments and examined under optical microscope to eliminate teeth with cracks. Each specimen was mounted horizontally on acrylic resin so that the dentin surfaces were exposed. (Figure 1)

Figure 1: Samples Mounted In Acrylic Resin



A total of 120 specimens were randomly divided into four groups. Baseline values for microhardness and surface roughness for all specimens were recorded before the application of the irrigants. The specimens were treated with irrigant solutions as follows:

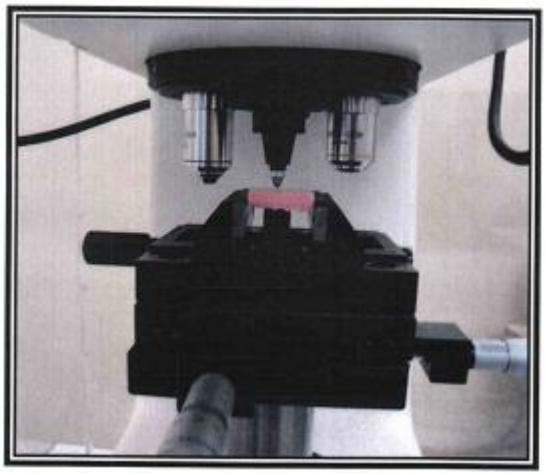
- Group I: 17% EDTA (pH 7.2) for 150 seconds followed by 5% NaOCL for 150 seconds.
- Group II: 17% EGTA (pH 7.2) for 150 seconds followed by 5% NaOCL for 150 seconds.
- Group III: 19% Citric acid (pH 1.3) for 150 seconds followed by 5% NaOCL for 150 seconds.
- Group IV: Control group treated with distilled water.

At the end of active treatment period, the specimens were rinsed with copious amounts of distilled water and dried on a soft absorbent paper. Each group was then divided into two subgroups, 'a' and 'b' of 15 specimens each.

Subgroups Ia, IIa, IIIa, and IVa were used to determine microhardness of root dentin with Vicker's Hardness Tester and subgroups Ib, IIb, IIIb, and IVb were used to determine the surface roughness of tooth dentin with Computerized Roughness Tester.

Micro-Hardness Testing: The specimens were mounted on Vicker's Microhardness Tester (FutureTech, FM300E,Japan). (Figure 2)

Figure 2: Specimen Mounted On Vicker's



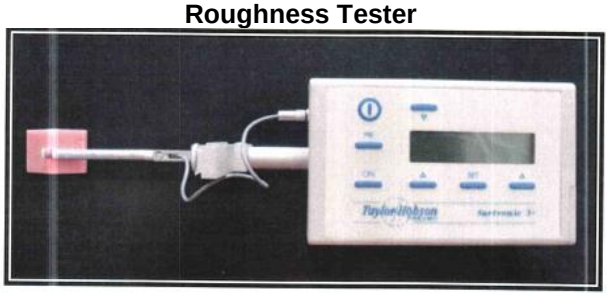
The indentations were made with Vicker's diamond indenter at three locations 0.5mm from the root canal wall in apical, middle and cervical region of the roots. The indentations are made using 300gms. Load and dwell time of 20 seconds. The diamond shaped indentations were observed under an optical microscope. (Olympus Corporation, Japan)

Surface Roughness Testing: The specimens are placed on a flat table surface and the needle of The Computerized Roughness Tester (Taylor Habson, Surtronic 3^{+}) (Figure 3) placed on the tooth surface. The locations are in the apical, middle and cervical regions of the root canal wall. The tested surface roughness values were displayed digitally on the screen of the roughness tester. The roughness values are expressed as Ra,µm.

The results were analyzed statistically using one way Analysis of variance (ANOVA) and the

comparison of means was conducted using Tukey HSD Multiple comparison test.

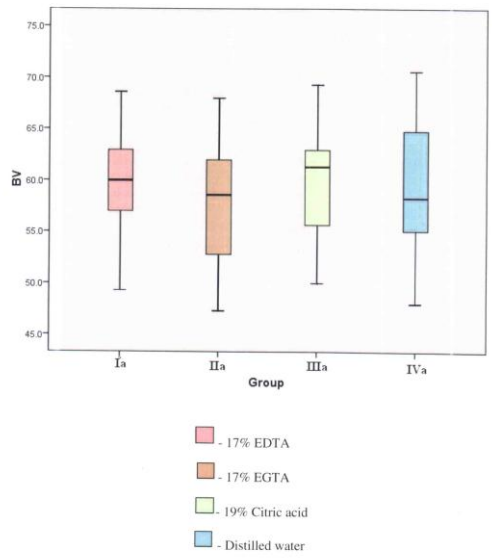
Figure 3: Specimen Mounted On Surface Roughness Tester



Results: The results showed that the lowest mean for microhardness was observed in Group IIIa – Citric acid group (35.43), followed by Group Ia – EDTA group (40.32), Group IIa-EGTA group (46.3) and highest in Group IVa – Control group (59.1). The differences in mean values of microhardness after irrigation are statistically significant (p=0.01).Tukey's test showed that there was a statistically significant difference between Groups Ia and IIa (p value 0.029), Groups IIa and IIIa (p value 0.00) , but there was no statistically significant difference between Groups Ia and IIIa (p value 0.101). (Graph 1; Graph 2)

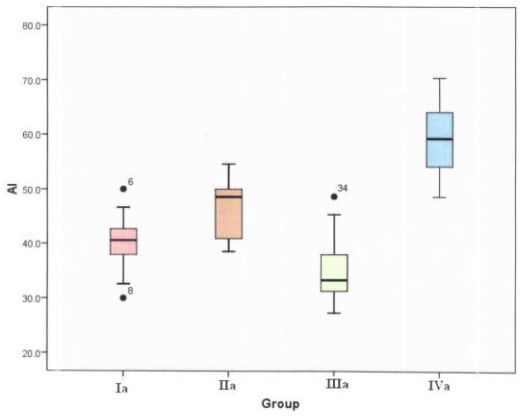
Graph 1:

Graph 1: BOX PLOTS OF BASE-LINE MICROHARDNESS VALUES OF THE FOUR STUDY GROUPS (MEDIAN, 25TH, 75TH PERCENTILES



Graph 2:

Graph 2: BOX PLOTS OF MICROHARDNESS VALUES OF THE FOUR STUDY GROUPS AFTER IRRIGATION (MEDIAN, 25TH, 75TH PERCENTILES, MINIMUM & MAXIMUM VALUES, OUTLIERS & EXTREME VALUES)

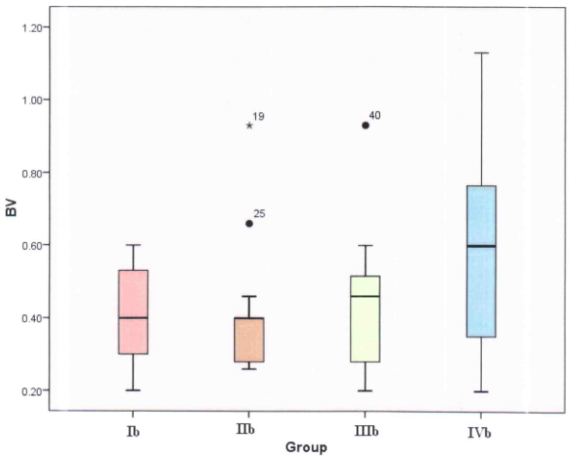


- Group III (Citric acid group) showing lowest median for the microhardness followed by Group I (EDTA group) and Group II (EGTA group).

The highest mean for roughness was observed in Group IIIb – Citric acid group (0.88), followed by Group Ib – EDTA group (0.67), Group IIb – EGTA group (0.62) and lowest in Group IVb – Control group (0.57). Tukey's test showed that there was no statistically significant difference between Groups Ib and IIb (p value 0.92), Groups Ib and IIIb (p value 0.06) but there was statistically significant difference between groups IIb and IIIb (p value 0.01).(Graph 3)

Graph 3:

Graph 3: BOX PLOTS OF BASE-LINE SURFACE ROUGHNESS VALUES OF THE FOUR STUDY GROUPS (MEDIAN, 25TH, 75TH PERCENTILES)



Discussion: The success of root canal therapy depends on the method and quality of instrumentation, irrigation, disinfection and three dimensional obturation of the root canal.[1] Removal of vital and necrotic remnants of pulp tissues, micro-organisms and microbial toxins from the root canal system is essential for endodontic success. Although this might be achieved through chemo-mechanical debridement, it is impossible to shape and clean the root canal completely because of the intricate nature of root canal anatomy. Even with the use of rotary instrumentation, the Ni-Ti instruments currently available only act on the central body of the canal, leaving the canal fins, isthmi and cul-de-sacs untouched after completion of the preparation. These areas might harbor tissue debris, microbes and their by-products, which might prevent close adaptation of the obturation material and result in persistent peri-radicular inflammation. Therefore, irrigation is an essential part of root canal debridement because it allows for cleaning beyond what might be achieved by root canal instrumentation alone.[9]

Irrigation is presently the best method for the removal of tissue remnants and dentin debris during instrumentation. The simple act of irrigation in itself flushes away loose, necrotic, contaminated materials before they are inadvertently pushed deeper into the canal and the apical tissues.[2] During irrigation, radicular and coronal dentin is exposed to solutions deposited in the pulp chamber. This may cause alterations on the surface of dentin. NaOCl, EDTA, Chlorhexidine and Citric acid are widely used irrigants. The introduction of EGTA as an endodontic irrigant in various in-vitro studies has been encouraging due to its lower erosive potential on root canal dentin.

Iris Slutzky et al studied the effect of instrumentation with stainless steel and rotary Ni-Ti files and using irrigation with 2.5% NaOCl and reported a reduction in microhardness of root dentin with less reduction using rotary files.[10]

The base-line microhardness and surface roughness values for each specimen were measured after instrumentation and before the application of the irrigants to have a reference value for every specimen.

David Pashley et al. reported that the microhardness of dentin declined when tested from superficial to deep regions[11]. The increased number of widely opened dentinal tubules free of peri-tubular dentin near the pulp offered little resistance to the microhardness testing in dentin. Thus, he proposed an inverse correlation between dentin microhardness and tubular density. This histologic pattern probably contributes to the hardness reduction at the cervical region of the root.

As microhardness of dentin may vary considerably within the same tooth, the Vicker's Hardness values were measured from different regions of the root, cervical, middle and apical regions. The average microhardness was calculated by taking the means of the three values for each specimen.[2]
The indentations were made at 0.5mm level from the root canal wall for standardization.[2] Clinically, heavy bacterial invasion inside root dentinal tubules was found at a depth of 200-400 µm. Therefore, this would be the maximal desired extent of dentinal penetration by the irrigating solutions. Further penetration of this irrigation solution may harm dentin and cause unnecessary reduction in microhardness.[10] There is no difference in the hardness of dentin in the crown or root if the root dentin farther than 0.5mm from the root canal is compared with crown dentin farther than 0.5mm from dentino-enamel junction.[12]

The measurement of the hardness of a material is one of the simplest non destructive mechanical characterization methods. Hardness is measured as the resistance to the penetration of an indenter that is necessarily harder than the sample to be analyzed. The microhardness values obtained depend on several factors such as: the Young's Modulus of the material, the Yield stress in compression, anisotropy, amongst the others. Hardness value cannot be considered as a basic property of the material, but rather an indication of its behaviour given the specific conditions of the penetration test.[13] Dentin microhardness depends on the amount of calcified matrix per mm^2

(Pashley, 1985)[11] and its determination provides an indirect evidence of mineral loss or gain in the dental hard tissues.

Investigations have shown the suitability and practicality of Vicker's microhardness test for evaluating surface changes of dental hard tissues treated with chemical agents.[2] The microhardness test, being a simple and effective method to evaluate and compare the effect of different substances, can contribute to the comparison of their demineralization power. Thus, this method was adopted in this study.[13] However, the information provided by microhardness testing alone may then be complimentary and thus the use of another method is necessary for comprehensive understanding of the surface changes. Thus, surface roughness measurement has also been included in this study to determine the surface changes of dental hard tissues.[2, 14]

Instrumentation of the root canal produces smear layer that consists of two confluent components, a l-2µm thick layer on the surface of the canal wall and a layer packed into the dentinal tubules upto 40µm.[15] Irrigating solutions have been used during and after instrumentation to increase the cutting efficiency of root canal instruments and to flush away debris.

The purpose of irrigation is two-fold:

- To remove gross debris originating from pulp tissue and possible bacteria – organic component.
- To remove the smear layer - mostly inorganic component.

The advantages and disadvantages of smear layer and whether it should be removed or not from the instrumented root canals is still controversial.[1] However accumulating evidence suggests the importance of removing the smear layer because it can result in a more thorough disinfection of the root canal system and the dentinal tubules, which would ensure a better adaptation between the obturation materials and the root canal walls.[3] Complete removal of the smear layer requires use of chelating agents followed by tissue solvents, because there is no single solution which has the ability to dissolve organic tissues and to demineralize the smear layer.[1]

Irrigating the root canals with 10ml of 17% EDTA followed by 10ml of 5% NaOCl has been recommended as an effective method to remove the smear layer.[4] Chelation is a physicochemical process that prompts the uptake of multivalent positive ions by specific chemical substances. In the case of root dentin, the chelating agent reacts with the calcium ions in the hydroxyapatite crystals. Initially, EDTA solution was proposed by Nygaard Ostby in 1957 to assist with instrumentation of calcified, narrow or blocked canals. The most commonly used chelating solutions are based on different concentrations of EDTA. An in vitro study showed that chelating solutions significantly reduced dentin microhardness.[13] EDTA used in this present study was buffered to a pH of 7.2.

Hulsmann et al reviewed the mode of action of EDTA using gravimetrical analysis.[16] They showed that the properties of EDTA were self limiting. EDTA with neutral pH (7.3) shows chemically, two co existing reactions.

- Complex formation
- Protonation

The exchange of calcium from the dentin by hydrogen results in a subsequent decrease in pH. Because of the release of the acid, the efficacy of EDTA decreases with time. On the other hand, the reaction of the acid with hydroxyapatite affects the solubility of dentin.[16]

Recently, a more specific calcium ion chelator, EGTA was evaluated for smear layer removal by Semra Calt and Ahmet Serper.[7] Antonio Cruz Filho et al evaluated the effect of 15% EDTAC, 1% CDTA and 1% EGTA on radicular dentin microhardness and reported significant reduction in microhardness.[17] Another in vitro study evaluated the effect of different EGTA concentrations (1%, 3% and 5%) and reported a reduction in microhardness of root canal dentin.[18] In the present study, 17% EGTA at pH 7.2 was evaluated to compare the results between EDTA and EGTA.

Citric acid as a weak organic acid has been applied previously on root surfaces altered by periodontal disease.[2] Citric acid was reported to be an effective root canal irrigant when used alternately with sodium hypochlorite. Yamaguchi et al reported that both 10% and 19% citric acid removed calcium ions from dentinal matrix.[19] Di Lenarda et al reported that after three minutes of irrigation, both 19% citric acid and 15% EDTA opened the dentinal tubules.[20] In the present study 19% citric acid at pH 1.3 was evaluated.

In the present study, irrigating solutions were used on root canal dentin surface for 150 seconds (two and half minutes) to obtain optimum results and a more realistic time in terms of clinical practice.

For surface roughness, as a result of the smear layer removal after applying these solutions to the endodontic surfaces, dentinal tubules become patent and the surface roughness increases. This could be of clinical benefit as in the case of micromechanical bonding of the adhesive materials that require the presence of irregularities on the surface of the adherend into which the adhesive can penetrate.[2]

The observations in the present study suggest that canal irrigation with these chemical solutions leads to structural changes, as evidenced by reduction of dentin microhardness and augmentation in surface roughness. This effect may be related to the solutions demineralizing effect on root canal dentin. The softening effect of chemical solutions on the dentinal walls could be beneficial in the clinical practice as it permits rapid preparation and negotiation of narrow and calcified root canals. However, the degree of softening and demineralization action may have an influence on the physical and chemical properties of this heterogenic structure.[21] These chemicals may also affect the adhesion of sealers and cement to the dentin.

The surface microhardness and roughness findings of this study show that EDTA / NaOCl ,Citric acid / NaOCl and EGTA / NaOCl treated surfaces have statistically significant difference in microhardness and roughness values when compared to control group as well as the baseline values of respective groups. Because the thickness of root dentin varies at different levels of the root, the mean values were taken into consideration minimizing the bias in the results.

Ayce Unverdi, Ali Erdemir and Semi Belli compared 17% EDTA and 19% Citric acid on microhardness and surface roughness and reported a stronger difference in dentin microhardness and roughness values for citric acid.[2]The results of the present study are in comparison with their study. The difference between EDTA and citric acid group in this study is not statistically significant. In this study, EGTA has shown minimum reduction of microhardness compared to EDTA and citric acid.

In the present study, the effect of 17% EDTA, 17% EGTA and 19% Citric acid on the microhardness and surface roughness of radicular dentin was evaluated and compared using a Vicker's Hardness Tester and Computerized Roughness Tester.

Conclusion: EGTA caused minimum reduction in microhardness when compared to EDTA and Citric acid. Citric acid caused maximum increase in surface roughness compared to EDTA and EGTA. 17% EGTA seems to be an appropriate irrigating solution, because of its harmless effect on the microhardness and surface roughness of root canal dentin. Although Citric acid caused maximum increase in surface roughness, it should be cautiously used as an endodontic irrigant.

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