Original article

Journal of International Medicine and Dentistry 2014; 1 (2): 75-85

JOURNAL OF INTERNATIONAL MEDICINE AND DENTISTRY
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JIMD
ISSN.No.2350-045X

Relation between Calcium Loss and Its Effect on Microhardness of Root Canal Dentin Following Treatment With 17% Ethylene Diamine Tetraacetic Acid(EDTA) at Different Time Intervals: An Ex-Vivo Study

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Abstract:

Decalcification and its effect on microhardness of root canal dentin by an aqueous solution of 17% EDTA at different time intervals were studied. Single rooted forty extracted human premolar teeth after adequate processing and embedded with acrylic resin were randomly divided into 8 groups based on different time intervals. Each sample group was assessed for Ca^{2+} release into the test solution by Atomic Absorption Spectrophotometer, respectively and then were subjected for microhardness testing. 17% EDTA and 0.9% Saline, test solutions were used; EDTA was adjusted to 7.5 pH. Data analysis was done by One Way analysis of variance (ANOVA) and the comparison of means was done by using Tukey's multiple comparison tests. The decalcifying activity of 17% EDTA solution was time dependent and increased immersion time showing very high significant influence on the amount of calcium extracted ($F=154.304$, $p<0.0005$). The reduction in microhardness for 17% EDTA solution was time dependent and increased immersion time showing very high significant decrease in the microhardness among the different time intervals tested ($F=249.925$, $p<0.0005$).Effect of 17 % EDTA solution as root canal irrigant is time dependent as evidenced by dentin microhardness reduction.

Key words: Calcium, EDTA, Microhardness, Spectrometry, Time dependent

Introduction:

The endodontic therapy success depends on optimized chemomechanical preparation and disinfection followed by root canal obturation together with coronal restoration to prevent re-infection.[1,2] An amorphous layer which is known as smear layer, is deposited on the root canal walls during chemomechanical preparation. At present irrigation is considered as one of the best methods for the removal of tissue remnants and the smear layer produced during root canal instrumentation[3,4]. It is generally suggested to use endodontic chelating agents followed by tissue solvents for effectual removal of both organic and inorganic components of smear layer, as no single solution is capable of removing organic and inorganic material.[5,6,7]

The microhardness reduction of the most superficial layer of the root canal lumen dentin is desired. The utilization of chelating agents during chemomechanical preparation of root canals reduces dentin microhardness, facilitates the action of endodontic instruments, removes smear layer and increases the access of the irrigant into the dentin tubules to allow adequate disinfection[8]. Various chelating agents like EDTA, Citric acid, Mixture of Tetracycline isomer, an acid and a detergent (MTAD) etc. have been used, but out of which EDTA is generally accepted as the most useful chelating agent with outstanding lubricant properties and is commonly used in endodontic therapy [9,10].

Calcium ions of the hydroxyapatite crystals react with the chelating agents

resulting in dentin micro-structural changes by changing the Ca^{2+}: PO_4^{3-} ratio. Change in the Ca^{2+}: PO_4^{3-} ratio changes the unique proportion of organic and inorganic components of hydroxyapatite resulting in changes of the microhardness, permeability and solubility characteristics of dentin adversely affecting the sealing ability of resin-based cements and root canal sealers to dentin[11,12]. If mineral loss or gain occurs in dental hard tissues it can be determined by Microhardness testing which acts as indirect evidence [3,13], as it is sensitive to composition and surface changes of the tooth structure. Demineralization, dentin surface microhardness changes and cleanliness of root canal walls depends on the time the chelating agents are in contact with dentin[13,14,15].

As there is no definite recommended optimum working time for the use of chelating agents during clinical practice, this study was done to assess decalcification and its effect on root canal dentin microhardness after treatment with 17% EDTA solution at different time intervals.

Materials and Methods:

The test solutions used were 17% EDTA and 0.9% Saline, freshly prepared in laboratory conditions from Yenepoya research centre, Mangalore. EDTA was adjusted to7.5 pH

Experimental procedure: Forty freshly extracted single rooted human premolar teeth were taken for the study.

Inclusion criteria: Non-carious, non-fractured, non-restored, single rooted premolar teeth.

Exclusion criteria: Carious, fractured, restored teeth, multi rooted teeth.

All teeth were stored in normal saline containing 0.1% thymol until the further period of the study. The teeth were decoronated at the cemento enamel junction (CEJ) by using diamond disc and pulp extirpation was done and root canals were enlarged using K files up to ISO # 25 to remove the pulpal debris.

Each tooth was sectioned longitudinally into two halves using a low-speed diamond disc making a total of 80 samples, to expose the root dentin surfaces for evaluation. The sectioning was done under water cooling in order to prevent desiccation of teeth. Each half was weighed on a precision balance and standardized to 0.24 gm before use. Then each tooth sample was embedded in horizontal direction using two L-shaped metallic moulds with auto polymerizing resin so that their dentin surface was exposed. 400, 600 and 1200-grit silicon carbide abrasive papers were used to flatten and polish the samples through wet grinding. The samples were then divided into 7 groups randomly according to time intervals of 1 min, 3 min, 5 min, 7 min, 10 min, 12 min and 15 min respectively. Each group had 10 samples (n=10).

- Group 1: 17% EDTA Solution for 1 min.
- Group 2: 17% EDTA Solution for 3 min.
- Group 3: 17% EDTA Solution for 5 min.
- Group 4: 17% EDTA Solution for 7 min.
- Group 5: 17% EDTA Solution for 10 min.
- Group 6: 17% EDTA Solution for 12 min.
- Group 7: 17% EDTA Solution for 15 min.
- Group 8: 0.9% Saline (control)

Initially, 20 ml of 17% EDTA solution was prepared as a blank to determine the level of calcium in absence of the specimen. Then each sample was immersed in a beaker containing 20 ml of test solution according to the different time intervals and maintained under constant agitation using magnetic stirrer to homogenize the test solution. After respective time interval the sample was taken out of beaker and 1ml of test

solution was extracted from the beaker using calibrated micropipette and transferred to another beaker, which was then diluted with 500 ml deionized water. Five ml of diluted solution was then extracted with automated micropipette into labeled glass tube and then hermetically sealed. This procedure was followed for each sample at respective time intervals and ethical clearance for this study was obtained from the institutional Ethical committee.

Then these samples were evaluated for the amount of Ca^{2+} release into the solution by means of Atomic Absorption Spectrophotometer using flame technique as reported by Machado-Silveiro et al[16]. The readings expressed were in parts per million (ppm), which were then multiplied by dilution factor. The obtained readings were then converted into mg of Ca^{2+} lost per gram by using the formula:

mg Ca^{2+} (ppmCa^{2+}) x 10^{-3}L/ml x V/P

Where, ppm Ca^{2+} = ppm of Ca^{2+} in each time period,

V = volume of solution in ml

P = the weight of the sample in mg.

Then the microhardness of the samples used for determining the Ca^{2+} loss was evaluated at five different locations of root canal dentin with a Vickers microhardness tester. The locations were chosen from coronal to apical region of root canal dentin. Pyramid shaped diamond indentations were made at 0.5 mm from the root canal lumen with 100g load for 15s dwell time. Care was taken to avoid overlap between each indentation. Optical microscope with a digital camera and image analysis software was used for accurate digital measurements of the diamond shaped indentations. The microhardness value was calculated by measuring the average length of the two diagonals. The average of the five indentations values was taken as the representative hardness value for each sample.

The data were recorded and the statistical comparison between the different test groups and control group for calcium loss and microhardness evaluation were carried out using One Way analysis of variance (ANOVA) and the comparison of means was done by using Tukey's multiple comparison tests. The testing was performed at the 95% level of confidence ($p<0.05$) and $p<0.05$ is considered to be significant.

Results:

1. Determination of calcium loss:

The mean and SD values of the root canal dentin calcium loss for 17% EDTA solution and 0.9% saline at different time intervals are listed in **Table I** and graphically represented in **Graph I**. The mean of calcium loss is expressed in ppm and ANOVA test showed that decalcifying activity of 17% EDTA solution was time dependent and increased immersion time showing very high significant influence on the amount of calcium extracted (**$F=154.304$, $p<0.0005$**). There was highly significant difference between 17% EDTA and 0.9% saline. In the control group, there was no significant difference in the Ca^{2+} loss at different intervals. Even though there was minimal amount of calcium loss in the control group, it may be due to the presence of trace amount of calcium in the saline solution itself.

In **Table II**, when Tukey's multiple comparison tests were performed, the mean calcium loss of control group with group1 showed no statistically significant difference ($p=0.098$), but showed a significant difference with group2, group3, group4, group5, group6 and group7. The amount of calcium extraction is increasing from group 1 to group 7 respectively i.e. from 1minute to 15 minutes.

2. Microhardness determination:

The mean and SD values of microhardness of the root canal dentin for 17% EDTA solution and 0.9% saline at different time intervals are listed in **Table III** and graphically represented in **Graph II**. The mean of microhardness values were expressed as Vickers microhardness

number (VHN) and ANOVA test showed significant difference between each group. The reduction in microhardness for 17% EDTA solution was time dependent and increased immersion time showing very high significant decrease in the microhardness among the different time intervals tested (**F=249.925, p<0.0005**). Reduction in microhardness was statistically highly significant for 17% EDTA solution when compared with control group.

Table IV: ANOVA test showed significant difference between each group and when Tukey's multiple comparison tests were done, the mean microhardness value of control group with group 1 showed no statistically significant difference (p=0.090), but showed a significant difference with group 2, group 3, group 4, group 5, group 6 and group 7. The Microhardness value (MHV) is decreasing from group 1 to group 7 respectively i.e. from 1minute to 15 minutes.

Comparison of the mean MHV between all groups from group1 to group7 showed statistically significant difference (p<0.0005) except with group3 when compared with group4 showed no significant difference (p=0.135).

Table I: Mean and SD Comparison of amount of Calcium extracted by control and 17%EDTA group at different time intervals

Group	N	Mean(ppm)	Std. Deviation	Minimum	Maximum
Control	10	0.03000	0.018480	0.008	0.068
17% EDTA@1min	10	2.64350	0.806578	1.571	3.971
17%EDTA @3min	10	4.27840	0.886721	3.580	6.219
17% EDTA @5min	10	6.18140	1.184749	4.742	8.342
17% EDTA @7min	10	8.71480	1.323255	7.076	11.085
17% EDTA @10min	10	10.82340	1.893690	7.923	13.066
17% EDTA @12min	10	14.71560	1.476664	13.228	17.114
17% EDTA @15min	10	21.42330	3.223602	17.171	27.742
Total	80	9.17272	6.573259	0.008	27.742

Table II: Tukey's HSD Multiple Comparisons between the groups

Group		Mean Difference	P
Control	17% EDTA@1min	-2.613500	0.098ns
	17%EDTA @3min	-4.248400	<.0005
	17% EDTA @5min	-6.151400	<.0005
	17% EDTA @7min	-8.684800	<.0005
	17% EDTA @10min	-10.793400	<.0005
	17% EDTA @12min	-14.685600	<.0005
	17% EDTA @15min	-21.393300	<.0005
17% EDTA @1min	17%EDTA @3min	-1.634900	0.371ns
	17% EDTA @5min	-3.537900	<.0005
	17% EDTA @7min	-6.071300	<.0005
	17% EDTA @10min	-8.179900	<.0005
	17% EDTA @12min	-12.072100	<.0005
	17% EDTA @15min	-18.779800	<.0005
17%EDTA @3min	17% EDTA @5min	-1.903000	0.194ns
	17% EDTA @7min	-4.436400	<.0005
	17% EDTA @10min	-6.545000	<.0005
	17% EDTA @12min	-10.437200	<.0005
	17% EDTA @15min	-17.144900	<.0005
17% EDTA @5min	17% EDTA @7min	-2.533400	0.024
	17% EDTA @10min	-4.642000	<.0005
	17% EDTA @12min	-8.534200	<.0005
	17% EDTA @15min	-15.241900	<.0005
17% EDTA @7min	17% EDTA @10min	-2.108600	0.106ns
	17% EDTA @12min	-6.000800	<.0005
	17% EDTA @15min	-12.708500	<.0005
17% EDTA @10min	17% EDTA @12min	-3.892200	<.0005
	17% EDTA @15min	-10.599900	<.0005
17% EDTA @12min	17% EDTA @15min	-6.707700	<.0005

Table III: Mean and SD Comparison of Microhardness Reduction by control & 17%EDTA group at different time intervals

Group	N	Mean(VHN)	Std. Deviation	Minimum	Maximum
Control	10	61.2700	1.31417	60.01	69.05
17% EDTA@1min	10	57.3200	2.76622	52.64	63.19
17%EDTA @3min	10	44.7470	2.32756	40.05	48.24
17% EDTA @5min	10	40.2660	2.68809	36.09	44.27
17% EDTA @7min	10	37.2350	2.38757	33.64	40.11
17% EDTA @10min	10	31.4190	2.15305	28.40	35.19
17% EDTA @12min	10	26.6100	2.69084	22.22	30.03
17% EDTA @15min	10	23.0310	2.76053	18.94	26.03
Total	80	38.8351	12.35055	18.94	63.19

Graph I: Comparison of calcium loss from the root canal dentin of control and 17% EDTA groups at different time intervals

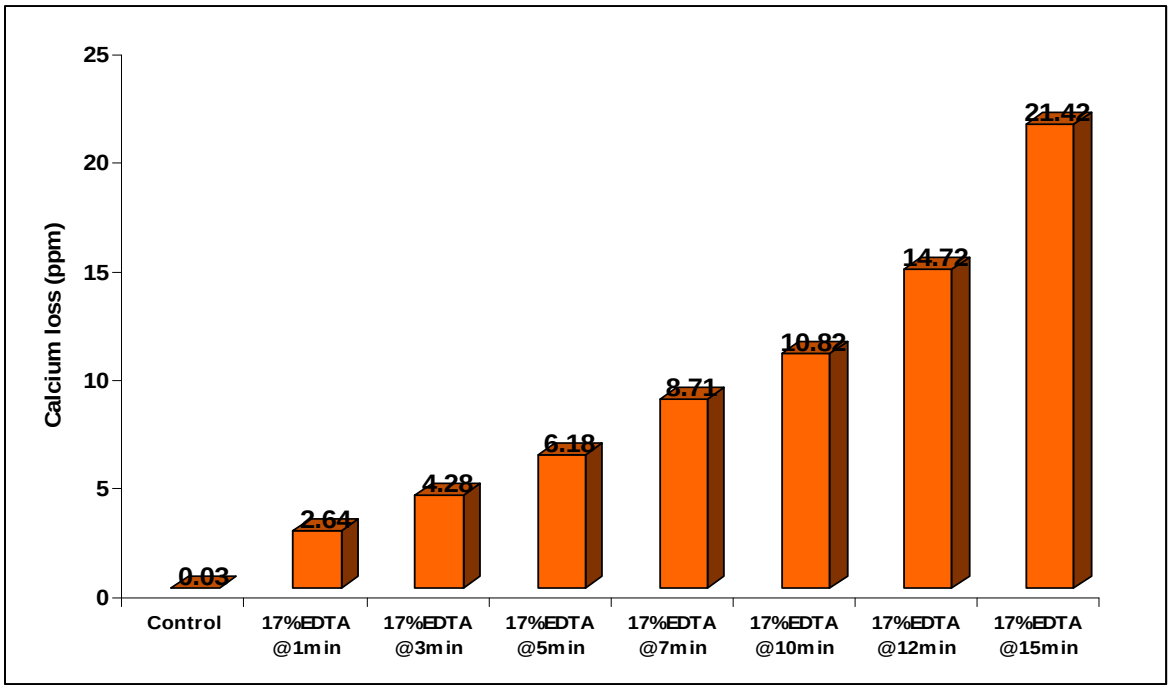
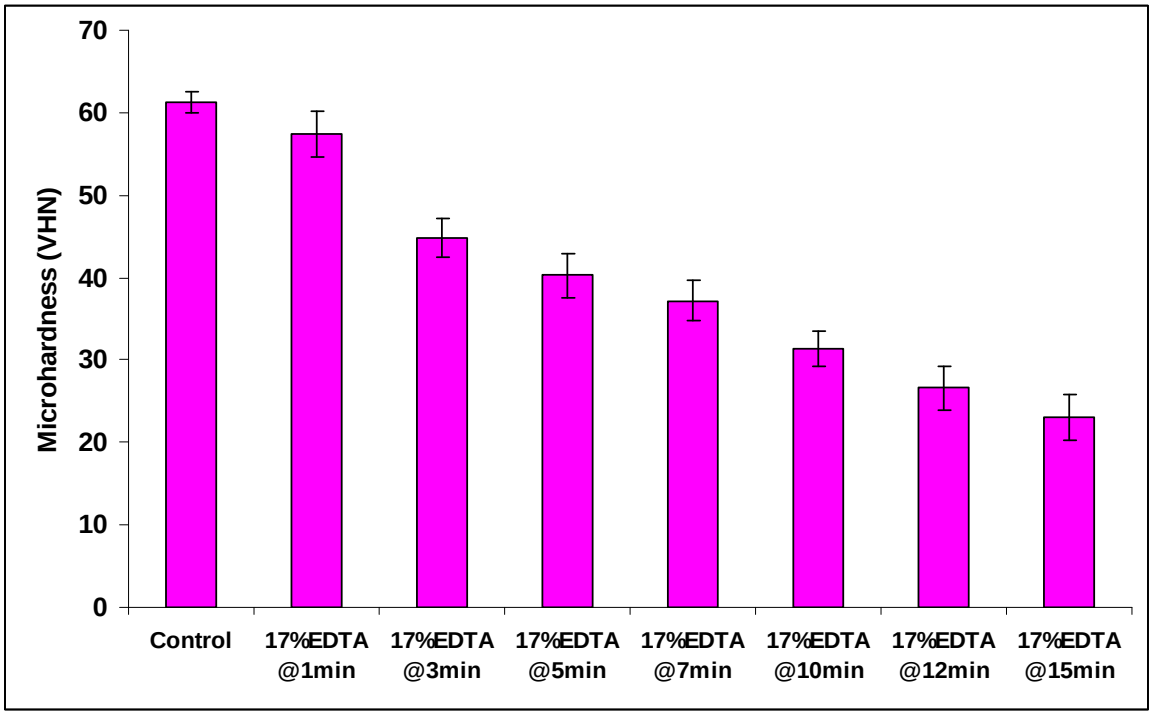


Table IV: Tukey's HSD Multiple Comparisons between the groups

Group		Mean Difference	P
Control	17% EDTA@1min	3.95000	0.090ns
	17%EDTA @3min	16.52300	<.0005
	17% EDTA @5min	21.00400	<.0005
	17% EDTA @7min	24.03500	<.0005
	17% EDTA @10min	29.85100	<.0005
	17% EDTA @12min	34.66000	<.0005
	17% EDTA @15min	38.23900	<.0005
17% EDTA@1min	17%EDTA @3min	12.57300	<.0005
	17% EDTA @5min	17.05400	<.0005
	17% EDTA @7min	20.08500	<.0005
	17% EDTA @10min	25.90100	<.0005
	17% EDTA @12min	30.71000	<.0005
	17% EDTA @15min	34.28900	<.0005
17% EDTA @3min	17% EDTA @5min	4.48100	0.004
	17% EDTA @7min	7.51200	<.0005
	17% EDTA @10min	13.32800	<.0005
	17% EDTA @12min	18.13700	<.0005
	17% EDTA @15min	21.71600	<.0005
17% EDTA @5min	17% EDTA @7min	3.03100	0.135ns
	17% EDTA @10min	8.84700	<.0005
	17% EDTA @12min	13.65600	<.0005
	17% EDTA @15min	17.23500	<.0005
17% EDTA @7min	17% EDTA @10min	5.81600	<.0005
	17% EDTA @12min	10.62500	<.0005
	17% EDTA @15min	14.20400	<.0005
17% EDTA @10min	17% EDTA @12min	4.80900	0.001
	17% EDTA @15min	8.38800	<.0005
17% EDTA @12min	17% EDTA @15min	3.57900	0.040

Graph II: Comparison of microhardness reduction of root canal dentin with control and 17%EDTA groups at different time intervals



Discussion:

The concept of chemomechanical preparation implies that chemicals have to be used during shaping and cleaning procedure because of variation of the root canal system internal anatomy where instrumentation is impaired due to difficulty of penetration of these instruments.[13] The use of chemicals has also been recommended on instrumented root canal surfaces in order to remove the smear layer[5] which is considered detrimental as it prevents the penetration of irrigants, intra canal medicaments and filling materials into the dentine tubules or it may even obstruct their contact with the canal wall[17]. Although various chelating agents have been tried, 17% EDTA has been popular as the most effective chelating agent for the removal of smear layer.[9,10] The Ca^{2+}: PO_4^{3-} ratio of root dentin is significantly reduced when EDTA is used as irrigating solution[13]. Different methods such as atomic absorption spectrometry, Flame photometry, complexometric titration with EDTA, Scanning electron microscope, Energy dispersive spectrometer, Fourier Transform Infrared (FTIR) or inductively coupled plasma–atomic emission spectroscopy (ICP-AES) are used to evaluate the demineralization effect of different chemicals, provided that calibration is accomplished precisely[18].

This study quantified the calcium ions chelated by 17% EDTA solution from the root canal dentin by atomic absorption spectroscopy at different time intervals. For evaluating the amount of particular metal element in a sample Atomic absorption spectroscopy is used. This procedure is based on absorption spectrometry to calculate the amount of required element in a sample. In the atomizer, the electrons of the atoms can be moved to higher orbital level for a moment by absorbing a set quantity of energy (i.e. light of a given wave length). The amount of energy (or wavelength) is precise to an exacting electron transition in a particular element. In general, each wavelength corresponds to only one

element, which gives the technique its elemental selectivity. Since the amount of energy /the power put into the flame is known, and the amount remaining at the detector side can be measured, it is possible to calculate the exact transitions that have taken place, and thus detect the quantity of the element being used.

Surface changes evaluation of dental hard tissues for alteration in Ca^{2+}: PO_4^{3-} ratio can be done by many techniques, such as microhardness measurements, micro radiographic assessment, SEM methods, energy dispersive spectrometric analysis[19]. Also, Micro-multiple Internal Reflectance Fourier Transform Infrared Spectroscopy (micro-MIRFTIS) and surface roughness testing.[20]

The amount of calcified matrix per mm^2 indicates the Dentin Microhardness and its assessment provides indirect proof for mineral loss or gain in the dental hard tissues.[21] The suitability and practicality of Vickers microhardness test for assessing dentin surface changes that has been treated with chemical agents has been proved by previous investigations.[2, 22-28] hence, this technique was adopted in this study.

Dentin hardness is related with location, and its value decreases as the indentations tested were made closer to the pulp. Pashley et al[21] reported that the microhardness of dentin decreased when dentin was tested from superficial to deep regions. The increased number of widely opened dentinal tubules, free of peritubular dentin near the pulp, offered little resistance to the testing indenter. It was also noted in all the samples that there was a variable increase in the microhardness from coronal to apical third of root canal dentin irrespective of treatment with any test agent. This may be attributed to the histology of the root canal dentin. Carrigan et al[30] showed that tubule density decreased from cervical to apical dentine and Pashley et al[21] reported an inverse correlation between dentine microhardness and tubular density. This histological pattern probably contributes to the hardness reduction at the cervical region of the root.[29]

Conclusion:

Results obtained within the experimental conditions of the present study indicates that, root canal irrigation with 17% EDTA solution is time dependent; increased irrigation time leads to the structural changes, as evidenced by reduction of dentin microhardness compared with the control group.

Chemical solutions which have softening effects on root canal dentin may be helpful in the clinical use as it possibly allows rapid negotiation of calcified root canals and also in preparation of narrow, constricted root canals. Yet, the degree of softening and decalcification may influence the physical and chemical properties of the root canal dentin which needs auxiliary investigations.

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Conflicts of interest- Nil
Acknowledgements- Nil

Date of submission: 29-11-2014
Date of acceptance: 15-12-2014

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