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Original Research Article

Comparative Assessment of the Effect of EDTA, Lactic acid and Citric acid on Root Dentin Microhardness

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Abstract: ***Aim*:** To determine the effect of ethylenediaminetetraacetic acid (EDTA), lactic acid and citric acid on root dentin microhardness. ***Materials and Methods*:** In this in vitro study, twenty freshly extracted anterior teeth were transversely sectioned at the level of cementoenamel junction. The middle third of each root is then horizontally sectioned into slices that are 4 mm thick to obtain a total of 20 dentin discs. Based on the chelating agent used, the samples were randomly divided into four groups- saline (control group), 17% EDTA, 20% lactic acid and 10% citric acid. Vicker's indenter was used to test the microhardness of the dentin before and after treatment with the chelating agents for two minutes. Data was obtained and statistically evaluated using a one-way analysis of variance (ANOVA), paired – t test and Tukey's post hoc test. ***Results*:** The microhardness values varied significantly among the groups. Citric acid and and lactic acid were less effective at reducing dentin microhardness. EDTA led to a higher reduction in microhardness. ***Conclusion*:** All the chelating solutions reduced the microhardness. Lactic acid showed less alteration in the hardness of root dentin than EDTA.

Keywords: Chelation, dentin microhardness, smear layer, lactic acid, Vicker's indenter, EDTA.

INTRODUCTION

Chemomechanical debridement of root canals consists of the combined action of both endodontic instruments and irrigants. During debridement of root canal space, the dentinal tubules get occluded with smear layer thereby reducing dentin permeability. Chelating agents along with sodium hypochlorite aid in the complete removal of both the organic and inorganic components of the smear layer. Smear layer removal increases the sealing ability of root filling materials and reduces the potential for bacterial growth and multiplication [1]. Chelators have the ability to demineralize both the smear layer and root dentin. As a result, there is a decrease in the microhardness of dentin along with collagen exposure.

Chelating agents were first used in endodontics by Nygaard Ostby in 1957 to prepare calcified and narrow root canals [2]. Chelation is a physiochemical process that causes certain chemicals to take up multivalent positive ions. Phosphorous and calcium make up the majority of dentin's inorganic components. The dentin's microstructure and Ca: P ratio will alter due to the use of chelating solutions [3]. This affects the adherence of dental materials to root dentin by increasing the permeability and solubility of the root canal dentin. The two chelating agents that are most frequently used are 10% citric acid and 17% EDTA. In addition to these, the following solutions have also been used- EDTAC, EDTA-T, EGTA, hydrogen peroxide, 6% NaOCl, sodium citrate, phosphoric acid, MTAD, chlorhexidine

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digluconate, etidronic acid and smear clear (17% EDTA + cetrimide + surfactants) [4-18].

According to Ayad *et al*., lactic acid irrigation performed similarly to 15% EDTA and demonstrated greater bond strength due to the complete elimination of the smear layer [19]. So far, there are no studies evaluating the effect of lactic acid on the microhardness of root canal dentin. Bond strength results from micromechanical retention provided by the demineralized dentin surface in association with resin tag formation. Subsequently, in order to ensure a successful endodontic treatment, cleansing the root canal walls is an essential step. Panighi and G'Sell stated that a positive correlation exists between microhardness and the mineral content of teeth. Thus, evaluating microhardness helps in the determination of mineral loss or gain in the dentin [20]. The purpose of this in vitro study was to assess the effect of 17% EDTA, 20% lactic acid and 10% citric acid on the microhardness of root dentin.

MATERIALS AND METHODS

Twenty anterior teeth that were recently extracted for periodontal causes were collected. In order to establish uniformity, the teeth were chosen based on their similar morphology, dimensions, and absence of carious lesions, particularly in the root areas. A sharp scalpel was used to gently clean the debris and soft tissue remnants on the root surfaces. The crowns were sectioned transversely at the cementoenamel junction and disposed off. To facilitate metallographic preparation, each root was sectioned horizontally in the middle third into 4mm thick slices using a diamond disc, producing twenty dentin discs in all. The specimens were then mounted in acrylic resin with the dentin exposed and the dentin surface was ground smooth and flat using silicon carbide abrasive sandpaper discs under distilled water to remove nay scratches. Finally, the specimens were polished using a composite polishing kit.

Based on the chelating agents used, the specimens were randomly divided into 4 groups-

Group 1- Saline (control)
Group 2- 17% EDTA
Group 3- 20% lactic acid
Group 4- 10% citric acid

The indentations were created on each specimen using 50 grams load and 15 seconds dwell time. Each specimen had three indentations made close to the root canal lumen and care was taken to not overlap them. Using an optical microscope equipped with a digital camera, and image analysis software, the diamond shaped indentations were observed, permitting the precise digital measurement of their diagonals (Fig 1). The hardness value for each specimen was determined by averaging the results of the three indentations and the microhardness was calculated using the average length of the two diagonals. The microhardness values of the specimens were measured prior to their treatment with the chelating agents and noted. The specimens were then treated with the appropriate chelating solutions for 2 minutes and a second set of measurements were obtained.

Figure 1: Diamond shaped indentation on the root canal surface

RESULTS

The mean microhardness before and after treatment in each group are shown in tables 1 and 2 and in figure 2. The comparison of mean microhardness between before and after treatment in each group using Student paired t test is listed in table 3. Mean microhardness values before and after treatment for group 1 and group 2, 3 and 4 showed statistically significant results with alteration in hardness values. Saline showed the highest mean microhardness value,

EDTA showed the least and lactic acid and citric acid showed similar microhardness values.

Multiple comparison of mean difference in the microhardness between groups after treatment using Tukey's Post hoc test is listed in Table 4. Multiple comparison of Group 1 with Group 2, 3 and 4 showed P <0.001. Multiple comparison of Group 2 with Group 3 and 4 showed P = 0.02. However, no significant difference was demonstrated between Group 3 and Group 4 (P = 1.00).

Table 1: Comparison of mean Micro Hardness before treatment between four groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group 1	5	82.39	1.11	81.1	83.8	0.99
Group 2	5	82.37	1.36	80.7	84.0	
Group 3	5	82.27	1.10	80.6	83.2	
Group 4	5	82.30	0.89	80.9	83.2	

Table 2: Comparison of mean Micro Hardness after treatment between four groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group 1	5	81.67	0.76	80.7	82.6	<0.001*
Group 2	5	77.77	0.50	77.0	78.3	
Group 3	5	79.07	0.49	78.3	79.6	
Group 4	5	79.11	0.71	78.0	79.9	

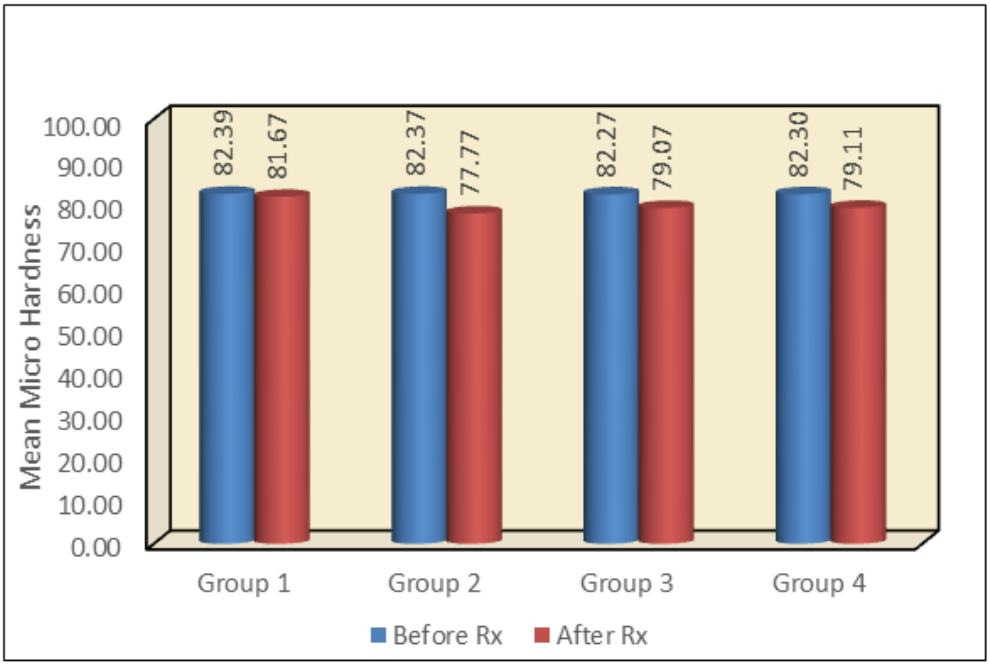

Figure 2: Mean microhardness between before and after treatment in each group

Table 3: Multiple comparison of mean difference in the Micro Hardness between groups after treatment using Tukey's Post hoc Test

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
Group 1	Group 2	3.90	2.76	5.03	<0.001*
	Group 3	2.60	1.46	3.73	<0.001*
	Group 4	2.56	1.43	3.70	<0.001*
Group 2	Group 3	-1.30	-2.44	-0.17	0.02*
	Group 4	-1.34	-2.47	-0.20	0.02*
Group 3	Group 4	-0.03	-1.17	1.10	1.00

Table 4: Comparison of mean Micro Hardness between Before & after Treatment in each group using Student Paired t Test

Groups	Time	N	Mean	SD	Mean Diff	p-value
Group 1	Before Rx	5	82.39	1.11	0.72	0.01*
	After Rx	5	81.67	0.76		
Group 2	Before Rx	5	82.37	1.36	4.60	0.001*
	After Rx	5	77.77	0.50		
Group 3	Before Rx	5	82.27	1.10	3.20	0.005*
	After Rx	5	79.07	0.49		
Group 4	Before Rx	5	82.30	0.89	3.20	0.001*

DISCUSSION

Some of the factors that affect the efficiency of a chelating agent include the root canal length, penetration depth of material, dentin hardness, duration of application, pH and concentration. Saline does not have any demineralizing or chelating effect [21]. Therefore, in comparison to the chelating agents, it had little effect on the microhardness of root dentin. In accordance with multiple studies, chelating agents were found to decrease the microhardness of root dentin. This is due to their chelating action. Phosphate and calcium found in dentin are soluble in an aqueous medium. The solution looses calcium ions when a chelator is added. Subsequently, more ions from the dentin dissolve so that the solubility product remains consistent [22, 23]. This is the mechanism used by chelators to cause dentin decalcification.

Dentin hardness is location-dependent, the closer the indentations were to the pulp, the lower the values. According to Pashley *et al*., dentin microhardness decreased when examined from the superficial to deep areas. Little resistance was provided to the indenter by the greater number of opened dentinal tubules free of peritubular dentin close to the pulp. Density of dentinal tubules was found to decrease from cervical to apical dentin by Carrigan *et al*., while microhardness of dentin and tubule density were found to be inversely correlated by Pashley *et al*., [24, 25]. This histological pattern most likely plays a role in the hardness reduction at the cervical portion of the root.

All solutions reduced the microhardness of root canal dentin, although EDTA irrigation produced a greater reduction, according to Saleh and Ettman's evaluation of the effects of hydrogen peroxide/ sodium hypochlorite and EDTA as irrigants [26]. Cruz Filho *et al*., assessed the effect of chelating agents such as EGTA, CDTA and EDTAC on the microhardness of radicular dentin and found that they demonstrated a significant reduction in microhardness [6]. The degree of mineral content and the amount of hydroxyapatite present in the intertubular substance are important variables that impact the intrinsic hardness of dentin. The considerable change in dentin hardness following the irrigation treatment suggests that chelators have strong effects on the components of dentin structure.

The strong demineralizing effect of EDTA causes softening of the dentin and a decrease in root canal dentin microhardness. Studies show that the smear layer can be effectively removed by EDTA irrigation for 1 minute. It is effective at neutral pH (7.3 – 8) and lower concentration (15%-17%). Lactic acid cause dentin decalcification, according to Ayad *et al*., lactic acid removed the smear layer, leaving dentin surfaces smoother and cleaner. The smear layer removal helps in better penetration of intracanal medicaments or filling materials. Wayman *et al*., found that sodium hypochlorite and saline control both removed less calcium than 50% lactic acid [27]. The ideal pH is 1.5 and the optimum concentration is between 20% and 50%. Lactic acid is produced by the body spontaneously during anaerobic muscle exertion and is believed to be biologically acceptable. It may not irritate or damage the periapical tissues. However, higher concentration of EDTA and citric acid solutions exhibit more cytotoxicity and can impede cell growth. Additionally, lactic acid possess chelating action that can be useful in negotiating calcified and narrow canals. Thus, the results of this study suggest that lactic acid can be used as an effective chelating agent. Further research can be done to find out more about the properties of lactic acid and its potential as a root canal irrigant. Citric acid is a weak organic acid. Calcium is lost as a result of the chelating action, demineralization of the calcified components of dentin.it acts better at a pH of 1.2 and a concentration of 25-5%. It is utilized for dentin conditioning, improved smear layer and smear plug removal in operative dentistry. Wayman reported that 50% citric acid irrigation efficiently removed the smear layer, producing clean root canal walls with patent dentinal tubules [27].

The differences in Vicker's hardness may be due to the demineralizing effect of the solutions on the root canal dentin. The chemical irrigants are clinically beneficial as it helps in the negotiation of tight, small canals due to its softening effect on the walls of dentin. Erosion of root dentin is caused by some chelating agents [28]. This results in changes in the enamel and dentin surface which may influence their interaction with root canal fillings and restorative materials used for coronal seal and lower the resistance to bacterial penetration and microleakage [29, 30].

CONCLUSION

The results revealed that microhardness decreased after treatment with the chelating solutions. 20% lactic acid and 10% citric acid showed similar values in the dentin microhardness. Lactic acid showed less alteration in the hardness of root dentin when compared to EDTA.

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