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INFLUENCE OF NOVEL CHELATING SOLUTIONS ON THE MICROHARDNESS OF ROOT CANAL DENTIN

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ABSTRACT

Aim: To assess impact of two novel chelating agents; Silver-Citrate root canal irrigation solution (BioAkt), apple cider vinegar (ACV) in comparison to ethylenediaminetetraacetic acid (EDTA) on the microhardness of root canal dentin.

Methodology: Forty single-rooted teeth were gathered, decoronated, root canals were instrumented and roots were divided longitudinally. According to the chelating agent, the specimens were split evenly among four groups; Group 1: BioAkt, Group 2: ACV, Group 3: EDTA, and Group 4: distilled water as a control. At all root levels, the dentin microhardness was assessed both before and after chelating agents were applied. The percentage of microhardness reduction were calculated and the collected data were compared using Two-way Analysis of Variance (ANOVA) and Tukey's tests ($P \leq 0.05$).

Results: At all root levels, the microhardness of the radicular dentin was significantly reduced by all irrigating solutions as compared to the pre-immersion values ($p \leq 0.05$) except for group 4. 17% EDTA showed the highest percentage of microhardness reduction followed by BioAKT, ACV and the least values were recorded for distilled water. Group 3 was significantly different than group 2 and group 4 (control) was significantly different than all tested groups at all root canal levels ($P \leq 0.05$). Difference of percentage of microhardness reduction among root levels in all groups were insignificant ($p > 0.05$).

Conclusions All tested final irrigating solutions had negative effect on dentin microhardness. Both BioAKT and ACV can be used as safer alternatives to EDTA regarding their effect on microhardness of dentin.

KEYWORDS: Traditional and Digital Shade Selection, Shade Selection, Digital Shade selection

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INTRODUCTION

Root canal irrigation is one of the crucial stages of root canal therapy. It aims to eliminate microorganisms and their toxins from root canal, remove pulp remnants, and remove the smear layer[1]. The amorphous smear layer, which covers the dentinal surfaces that the file contacts, is created during root canal preparation when bacterial structures are added to the organic and inorganic detritus[2]. To guarantee a solid bond between sealers and dentin surface, this layer should be removed prior to the root canal obturation.

Root canals are disinfected and dentin debris are removed using a range of endodontic irrigants [3]. The most popular is sodium hypochlorite (NaOCL), as it has antibacterial and antifungal qualities as well as the capacity to breakdown any organic tissue that may still be present. However, NaOCL's capacity to eliminate the smear layer is restricted. [4]. Therefore, to get rid of the inorganic tissues in the smear layer, a different irrigation solution must be used as the last irrigation.

The last irrigation solution is a solution used to completely remove inorganic components of the smear layer [5]. Because it can react with calcium ions in dentin to generate calcium chelation. Ethylenediaminetetraacetic acid (EDTA) is the most frequently used chelating agent[6]. Nevertheless, EDTA lacks antimicrobial qualities [7], and extended contact to it may change the dentin's structural characteristics, compromising its mechanical integrity and causing erosion [8].

The limitations of EDTA as a chelating agent, necessitate the investigation of alternative final irrigating solutions that have the ability to remove the smear layer, possess antibacterial properties, and have chelation ability without negatively affecting the dentin properties.

Research is now being conducted to assess the advantages and disadvantages of BioAkt, a novel chemical compound made up of silver ions (0.003%) in citric acid (4.846%) along with detergents and water, as a chelating agent against microorganisms during endodontic treatment [9].

The lifetime of endodontically treated teeth depends on the discovery of a natural chelating agent that has a less negative effect on the properties dentin. In order to reduce the detrimental effects of EDTA on dentin and periapical tissues, apple cider vinegar (ACV) was administrated. Since apple vinegar was found to be effective as EDTA in the elimination of smear layer, its use as an irrigating solution has been suggested due to its encouraging findings [10]. Since malic acid, which accounts for the majority of its therapeutic effects, including anti-inflammatory, antibacterial, antioxidant, and antifungal, is present in considerable amounts, it has a high degree of biocompatibility. [11-14].

Previous researchers have found that chelating drugs likely affects the characteristics of the root canal dentin, resulting in surface roughness and a decrease in microhardness. Because of its accuracy and dependability, Vickers hardness number (VHN) has been utilized for testing the microhardness. Moreover, it uses pyramidal indentation technique which allows for analysis of the mechanical properties of a very thin disc [15-17].

It's interesting to note that recent research evaluating the effectiveness of modern chelating agents have focused mostly on the microhardness of root dentin. Therefore, the effects of BioAkt, ACV, and 17% EDTA using a Vickers tester was compared in this ex-vivo study. The zero hypothesis is that the three tested final irrigating solutions have negative impact on the radicular dentin microhardness

MATERIALS AND METHODS

Preparation of tooth specimens

The sample size was determined using power calculations (G Power version 3.1.9.; Franz Faul, University of Kiel, Kiel, Germany), a minimum sample size of 36 would be required to demonstrate results with a 5% significance level, more than 80% power and confidence interval of 95%. The required sample size was increased to be 40 to increase the validity of results.

The formula of sample size

Sample size = Z2 P (1-P)/ C2

Where:

Z = Z value (1.96 for 95% confidence level)

p = percentage picking a choice, expressed as decimal

c = confidence interval, expressed as decimal

Forty single-rooted, single canal, intact, and nearly straight teeth were selected from the outpatients of the clinic of Oral Surgery Department, Faculty of Dentistry, Tanta University. Care has been taken to ensure that the teeth are of similar morphology,and that their roots are of similar length and root canal width. Prior to extraction, patients gave their informed consent. Tap water was used to rinse the teeth as soon as they were extracted, and they were subsequently submerged and cleaned for an hour in 2.5% NaOCL[16]. The teeth were kept in distilled water and used nearly within one month after extraction. To rule out any teeth having cracks, fractures, cavities, or enamel defects, the teeth were inspected under 2.5x magnification loupes. A safe-sided diamond disc (Komet, Brasseler, Lemgo, Germany) was used to decoronate teeth. It was fixed to a straight handpiece under water cooling.

K-file #10 (Dentsply Maillefer, Ballaigues, Switzerland) was used to calculate the working length. After insertion inside the root canal, the file was moved forward until it emerged from the apex. The file's working length was determined by taking this measurement and subtracting 1 mm. Root canals were prepared using Protaper Universal system (Dentsply Maillefer, Ballaigues, Switzerland) at 250 rpm, up to F3 file reached the working length. Following each file, a 27-gauge side-vented needle was used to irrigate the canals in each group with a standardized volume of 3 mL of distilled water.

The sound section of the roots was chosen for the study after they were divided longitudinally. For easier handling, each root half was horizontally embedded in auto polymerized acrylic resin (Figure 1), and a series of increasing grades of carbide abrasive papers (500, 800, 1,000, and 1,200 grit; BIGO, Dent Product, Germany) were used to grind the dentin surface flat and smooth, this was done under distilled water irrigation, and a 0.1 mm alumina suspension was then used on a rotating felt disc (Microdont LDA, Brazil) to create a smooth, mirror-like surface [17].

Fig. (1)

Forty samples were randomly assigned to four tested groups according to the chelating solution. Group 1: BioAkt, Group 2: apple cider vinegar (ACV), Group 3: 17% EDTA and group 4: distilled water (control group).

Microhardness measurement

The three root thirds of each specimen's dentin surface were subjected to a Vickers microhardness test both before and after 3 minutes immersion in 10 mL of the specific irrigating solution. On each third of the root surface, three testing sites were chosen, each was 0.5 mm lateral to the canal lumen.

The test was performed in Faculty of Dentistry, Tanta University using a digital microhardness testing machine (ZwicRoell, west Midland, England) with a Vickers' diamond indenter using 300 gm force for 20 seconds with 20x objective length to form a rhomboid impression monitored on the computer screen attached to the microhardness tester. Using the resulting rhomboid diagonal (Figure 2), the microhardness was electronically calculated.

Two-Way ANOVA was used to statistically evaluate the data, and Tukey's test was run using a 95% significant threshold.

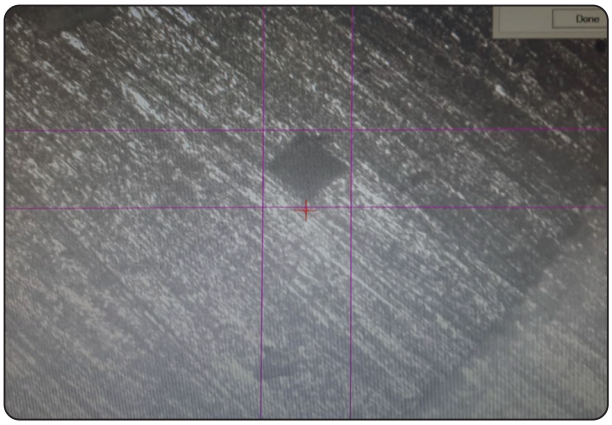

Fig. (2)

RESULTS

Every specimen served as its own control in this investigation. The Vickers microhardness values (mean± standard deviation) pre and post application of the various final irrigating solutions are enumerated in Table 1. Each table cell's mean is equivalent to the average of three measurements over ten distinct specimens, for a total of thirty measurements.

There were no statistically significant differences between the mean of pre-treatment VHN values for all tested groups at all root levels ($p>0.05$), the microhardness of the radicular dentin surface was considerably reduced by all irrigating solutions, as assumed in zero hypothesis, after the specimens were submerged in them, as compared to the pre-immersion values at every root level ($p\leq0.05$) except for group 4.

Percentage of microhardness reduction was calculated and compared among different groups at each root level (Table2). 17%EDTA showed the highest percentage of microhardness reduction followed by BioAKT, ACV and the least value was recorded for the control group at all canal levels. High statistically significant differences among tested groups at each root level was found using Two-Way ANOVA test. So, Tuckey pairwise comparisons were done and it showed that group 3 (17% EDTA) was significantly different than group 2 (ACV), group 4 (control) was significantly different than all tested groups at all root canal levels ($P \leq 0.05$). On the otherhand, differences among other groups were insignificant ($p>0.05$).

In comparing the percentage of microhardness reduction among root levels in each group, it was found that percentage of microhardness reduction was higher in cervical level followed by middle and apical one but without statistically significant differences in all groups ($p>0.05$).

TABLE (1). Mean values ±SD of microhardness at different levels of radicular dentin before and after irrigation in all study groups:

Groups	Pre-treatment			Post-treatment		
	Cervical Mean±SD	Middle Mean±SD	Apical Mean±SD	Cervical Mean±SD	Middle Mean±SD	Apical Mean±SD
1	56.376±6.802	45.452±3.780	35.100±3.062	39.062±2.016	31.995±1.114	25.776±1.902
2	57.095±5.196	46.976±1.776	35.762±2.953	41.505±1.328	34.438±1.933	27.148±2.432
3	56.195±7.462	45.438±3.669	34.671±2.890	36.524±2.125	30.190±1.676	24.129±1.847
4	57.924±4.875	43.743±1.344	34.505±2.645	54.057±4.910	40.857±1.086	32.281±2.451

TABLE (2). Percentage of microhardness reduction in different groups at each root level and their statistical analysis:

Groups	Cervical Mean±SD	middle Mean±SD	Apical Mean±SD	P value
1	29.557^{ab}±10.471	29.089^{ab}±6.995	26.208^{ab}±6.452	0.3615
2	26.848^{b}±5.561	26.650^{b}±3.943	24.00^{b}±4.310	0.0952
3	33.727^{a}±10.709	33.083^{a}±7.076	30.094^{a}±6.291	0.3208
4	6.694^{c}±2.563	6.566^{c}±1.958	6.429^{c}±1.411	0.9148
P value	0.0001	0.0001	0.0001	

Different small letters denote significance between groups.

DISCUSSION

Solutions with both organic and inorganic tissue dissolving properties are required to eliminate the smear layer [18]. Chelating chemicals have the ability to disintegrate inorganic tissue by dissolving the calcium present in dentin's hydroxyapatite crystals. Both organic and inorganic structures of the dentin differ significantly when the ratio of dentin calcium changes, which alters the dentin's permeability, solubility, and microhardness[3]. Measurements of hardness can be linked to other mechanical and adhesive characteristics including yield strength, fracture resistance, and the corresponding bond strength. Consequently, a first step in forecasting dentin and restoration surfaces is provided by microhardness[19,20]. The impact of chelating compounds on dentin microhardness must thus be investigated, and this was done in this study.

This study utilized EDTA, a widely recognized chelating agent in clinical applications. When combined with NaOCl, it was recorded to be successful in taking off the smear layer [21, 22]. Recently, a lot of research has been done to try to create a more biocompatible and efficient chelating agent than EDTA [23,24]. BioAkt is a novel endodontic irrigant; silver ions primarily contribute to its bactericidal properties, while citric acid provides its chelating impact [25]. The dual function of BioAkt during endodontic therapy is specified by this special composition [26, 27]. In order to evaluate its impact

on dentin microhardness, this novel chelating agent was used for this investigation.

Because of its many benefits, including its chelating ability, antibacterial activity, biocompatibility, low cost, and potential anti-inflammatory function, apple vinegar was chosen for this investigation [28]. Furthermore, it was discovered to have an equivalent impact to EDTA in the elimination of smear layers [23,29,30]. Since prolonged exposure times can result in dentinal erosion and adversely alter the mechanical qualities of radicular dentine, 10 mL of chelating agents were chosen for a 3-minute exposure. Additionally, apple vinegar contains malic acid, which is extremely acidic and has a better demineralizing impact in a shorter period [31].

Cruz-Filho et al. [32] pointed out that cutting the roots longitudinally instead of transversally into discs can result in more realistic depictions of clinical conditions, which is why this approach was selected for the current study. The most superficial layer of root canal dentin that comes into initial contact with irrigants in the root canal is located 0.5 mm lateral to the canal lumen, where the microhardness was measured in this study.

Vicker's hardness number is based on the mean of two diagonals, which yields more accurate findings than the knoop microhardness test, which only uses one diagonal. For small specimens, the Vicker's tiny indenter tip is perfect [33, 34] . Furthermore, the Vickers microhardness test was simpler and more accurate for evaluating dentin variations brought on by chelating solution-induced mineral loss [2, 35].

The current study's findings showed that, with the exception of the distilled water control group, all chelating agents significantly reduced the microhardness of the radicular dentin surface as compared to the pre-immersion values at all root levels. These findings may be explained by earlier studies using Atomic Absorption Spectrophotometry, which shown that calcium ions may be eliminated from the root canals by irrigation with chelation agents [33]. By dissolving the calcium hydroxyapatite matrix from dentin as well as the inorganic structure in the smear layer, chelation and demineralization agents cause collagens to open and microhardness to decrease [36, 37].

The results of this study recorded that EDTA possessed the highest reduction effect on microhardness of radicular dentin, this could be explained by the fact that EDTA is a chelating agent, which means that it ables to form chelates with both transition and main groups and four or six bonds with metal calcium ions. Consequently, dentin properties such as hardness can be impacted by any alteration or flaw in the initial concentration of calcium ions [38].

Additionally, this demineralization impact may cause erosion, particularly in peritubular and intertitular dentin, which would weaken dentin and enlarge dentinal tubules. It may also change the surface of dental hard tissues [39]. This occurrence was brought on by a shift in the calcium/phosphorus ratio in dental tissue [40], which caused the root canal dentin's microhardness to diminish and its surface roughness to increase. Furthermore, EDTA can decalcify dentin up to 20–50 μm. This process takes two to three minutes [28, 41]. The diminishing impact of EDTA on dentin microhardness has been reported by several authors as; Ratih et al. [28], Cruz-Filho et al. [32], and Sayin et al [42].

Compared to EDTA, BioAKT demonstrated insignificantly smaller decrease in radicular dentin microhardness. This could be explained by BioAKT's chemical makeup, which primarily relies on citric acid, a potent chelating agent that can react quickly with calcium to form calcium citrate [43]. While the chelate created by the union of the EDTA ion with calcium happens at a 1:1 ratio, the salt that results from the interaction of citrate with calcium under normal reactive conditions is formed at a 1:1.5 ratio. Citric acid would potentially remove more

calcium ions if both solutions were used at the same concentration, which would aid in reducing dentin microhardness more [44]. While, in the current study, 17% EDTA was used and citric acid used in 4.846% in BioAKT that might explain the lower effect of BioAKT than EDTA on dentin microhardness.

It was found that 5% citric acid at PH of 1.9, could eliminate the smear layer, but at PH of 6, it was unsuccessful [45]. When compared to 17% EDTA, which had a more neutral PH but was utilized at a significantly higher concentration than citric acid, BioAKT's acidic PH of 1.5–2.5 demonstrated a strong chelating activity and subsequently reduced dentin microhardness. The practically same outcome for both compounds may also be explained by the bioavailability of calcium. As in dentin, calcium is present in the hydroxyapatite crystals as a compound rather than as an ion, which prevents the acid from reacting completely [32].

The current study's findings concurred with those of Alyahya et al. [46], who discovered that the four chelating solutions that were tested BioAkt, 40% citric acid, 10% citric acid, and 17% EDTA, all significantly reduced dentin microhardness. This outcome is consistent with the investigation of Scelza et al. [3], Cruz-Filho et al. [32], Jaiswal et al. [47] and Ballal et al. [48].

The findings of this investigation, however differ from those of a study by Eldeniz et al.[49] which found that citric acid was significantly more effective than EDTA at lowering dentin microhardness. Compared to BioAKT, which has less than 5% citric acid, they used 19% citric acid, which was a larger concentration. It has been demonstrated that the chelating action of an agent increases with its concentration [50].

However, in the work by De Deus et al. [51], they discovered that 10% citric acid decreased microhardness much less than 17% EDTA. This could be because the pH of the citric acid employed was nearly neutral. Calcium ion removal from dentin may be favored by a solution's more acidic PH. Compared to 1% citric acid with pH = 7.4, Sousa and Silva, [52] demonstrated that 1% citric acid with pH = 1.0 eliminated noticeably more calcium ions from dentin.

The current study found a substantial difference between EDTA and ACV, which was consistent with Cruz-Filho et al. [32], who found that EDTA significantly reduced microhardness more than ACV. One explanation for this could be that EDTA has a stronger chelating effect than ACV, as demonstrated by Spanó et al. [53], who found that the EDTA group was much more effective at removing smear layers.

This was corroborated by another study that found that ACV was superior to 17% EDTA in removing the smear layer without changing the intraradicular dentine's calcium content. The research revealed that the ACV samples had a higher calcium content than the EDTA group. This is because the ACV eliminated the calcium ions by acetification, whereas the EDTA group removed them through chelation [38].

This outcome, however, was at odds with that of Mahmoud et al. [54], who discovered that the impact of ACV on microhardness was equivalent to that of 17% EDTA. Since they utilized a 200 g load and the indentation points were 200um from the canal lumen, that could be related to the difference force used in this investigation and the varied positions of the detected indentations.

In the current study, the cervical thirds had slightly higher percentage of microhardness reduction in comparison with other root segments in all groups. This result is consistent with a number of researches[55-57]. The relative nature of dentin in the apical area and the histological pattern of the root canal dentin may be responsible for this. According to earlier studies, there are significant structural differences in the apical region such as accessory root canals, irregular secondary dentine, low levels of non-collagenous proteins, and even dentin sclerosis [58].

The microhardness reduction across the various root thirds of any group was found to be statistically insignificant (P>0.05). This is explained by the study's treatment strategy. Unlike in clinical settings, specimens immersed in irrigating solutions enable the fluid to reach the dentin surface of each root third with a consistent volume for the same time which was consistent with findings by Nikhil et al [59] and Adel et al. [60]

CONCLUSIONS

Within the limitations of the this study, it was concluded that:

1. All tested chelating agents had adverse effect on microhardness of radicular dentin
2. Both BioAKT and ACV can be used as safer alternatives to EDTA regarding their effect on microhardess of dentin

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