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EVALUATION OF THE EFFICACY OF DUAL RINSE AND GLYCOLIC ACID AS ROOT CANAL IRRIGATION ON SUPERFICIAL CHEMICAL STRUCTURE AND MICROHARDNESS OF RADICULAR DENTINE

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ABSTRACT

Aim to analyze the impact of various irrigating solutions on the superficial chemical structure and microhardness of the root dentine.

Methodology Fifty-two dentin half discs were distributed randomly into four groups (n=13); Control groups: distilled water was used to treat the samples, NaOCl/EDTA group: 2.6% NaOCl was applied to the samples, followed by distilled water and then 17% EDTA, NaOCl/Dual Rinse group : was irrigated with 2.6% NaOCl/9% Dual Rinse, NaOCl/Glycolic acid group : 2.6% NaOCl was applied to the samples, followed by distilled water and then 17% glycolic acid (PH 2). Utilizing Fourier Transform Infrared Spectroscopy (FTIR), the ratio of phosphate to amide I ratio was calculated, and then Vickers hardness test was employed to gauge the microhardness of the dentine. One-way ANOVA was used to compare between groups, followed by Tukey post-hoc analysis.

Results: Phosphate/Amide ratio and microhardness evaluation revealed statistically significant differences (p<0.05) among groups, with no difference demonstrated between the Control, NaOCl/Dual Rinse or NaOCl/Glycolic acid groups (p>0.05).

Conclusion: Date revealed that sequential treatment of dentine with NaOCl and different decalcifying affected the chemical composition and microhardness, where NaOCl/Dual Rinse and NaOCl/Glycolic acid caused less depletion of the apatite and changes in dentine microhardness compared to NaOCl/EDTA group.

KEY WORDS: NaOCl/EDTA; NaOCl/Dual Rinse; NaOCl/Glycolic acid; FTIR; Vickers hardness

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INTRODUCTION

The main purpose of endodontic treatment is to provide effective chemo-mechanical preparation to eliminate bacteria that cause infections and to avoid reinfections (1). Irrigation is essential as it removes necrotic tissue, bacteria and the smear layer present inside the root canal, where complex root canal anatomy makes thorough disinfection impossible with instruments alone (2). Smear layer is a 1-2 µm layer of organic and inorganic material, formed on the root canal walls during chemo-mechanical preparation (3). The use of irrigating solutions has been suggested as a way to remove this layer since it serves as a physical barrier that prevents, intracanal medications and root canal sealers from penetrating into the dentinal tubules (4,5) .

To achieve this, two or more irrigating solutions can be utilized in a specific order to achieve proper disinfection of the root canal. The two most frequently used irrigating solutions are sodium hypochlorite (NaOCl), an oxidizing solution recognized for its efficacy in removing tissue debris and, 17% ethylenediaminetetraacetic acid (EDTA) which acts on inorganic content. However, several investigations have reported surface dissolution of intertubular and peritubular dentin with widened dentinal tubules, as well as a reduction in the mechanical properties of this regime-treated dentin (6,7) .

Type 1 collagen, which is considered the primary constituent of organic dentin, is essential for maintaining its structural integrity, in addition, hydroxy apatite and other mineral salts are included in the inorganic components (8). Consequently, any operation that modifies the fundamental ratio of organic and inorganic parts in dentin microstructure negatively reduces both flexural strength and microhardness, which could lead to dentin fracture (9,10) . As a result, the dentin's ultrastructural features are crucial to the mechanical integrity of the tooth.

To ensure the effectiveness of root canal therapy, it is necessary to investigate alternative irrigating solution that are biocompatible, efficient in removing the smear layer, and free from damaging the structure of root dentin. Etidronic acid, also known as 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), is a "soft" chelator that can be employed together with NaOCl directly and could potentially be used during the root canal preparation. The term "continuous chelation" refers to the process of continuously applying a solution of HEDP with NaOCl to prevent the formation of smear layers during instrumentation (11) .

Recently glycolic acid; an alpha hydroxy acid (AHA), derived from sweet plants like sugar cane, with only two carbons in its chemical structure, no color, no odour, and readily soluble in water was investigated. Studies demonstrated its suitability for etching enamel and dentin during restorative procedures and its efficiency in removing the smear layer from root canal walls when used at low pH without compromising dentin characteristics (12,13) .

Several techniques, such as energy-dispersive X-ray spectroscopy (EDS), microhardness, roughness tests (14) and Attenuated Total Reflectance in Fourier Transform Infrared Spectroscopy (ATR-FTIR), have been used to directly and indirectly analyze the changes irrigating solutions promote in the composition of dentine (15,16) .

As per our knowledge, no studies in literature evaluated the impact of Dual Rinse HEDP and glycolic acid as root canal irrigation on the physical and chemical characteristics of dentine. Thus, this study compared the impact of EDTA, Dual Rinse HEDP, and glycolic acid on the superficial chemical structure of dentin and the microhardness on extracted single-rooted. The null hypothesis assumed that there is no difference among the effects of the different groups on the dentin chemical structure and microhardness.

MATERIALS AND METHODS

Ethical approval

This comparative in vitro planned trial's conformity with the regulations governing the use of human subjects was reviewed and approved by the research ethics committee at Cairo University's Faculty of Dentistry (Ethics Committee approval number 27522).

Calculated sample size:

Based on the finding of Zhang et al.[17], the minimum estimated sample size using PS version 3.1.2, with an alpha (a) level of 0.05 (5%) and a beta (b) level of 0.20 (20%), i.e., power = 80% and an estimated difference between the experimental and control means of 0.9 is 13 dentine discs per group to reject the null hypothesis.

Sample description:

Thirteen human mature single-rooted, single canalled lower premolars teeth extracted due to periodontal or prosthodontic reasons were collected from the Oral and maxillofacial department after their approval. Pre-operative radiographs were taken from both the buccolingual and mesiodistal directions to rule out those with root curvature or complex anatomy, as well as previous endodontic treatment, decay, cracks, fractures or calcified canals and to demonstrate the presence of a single patent canal. According to Schneider, teeth with root curvature 0-10^{o} were chosen and included in the study. Flowing water was utilized to carefully and thoroughly wash the chosen teeth and then calculus or soft tissue remnants were removed using an ultrasonic scaler. The length of the root left after the removal of the crowns was at a distance of 14mm1 (± 1mm) from the apex to the cement-enamel junction.

Sample preparation:

Using a hard tissue microtome (Buehler, London, UK), two coronal dentin cylinders with 4x4 x0.8mm (length X width X thickness) were cross-sectioned. Then each cylinder was then split along the root canal to produce four dentinal discs per tooth, for a total of 52 dentin half discs.

Silicon carbide grinding paper (Buehler, Lake Bluff, IL, USA) with grit range 500–4000 grit was used to flatten the non-pulpal external sides of the dentin discs to a consistent thickness. Each dentin disc had its deep pulpal side polished using 1,200, 2,400, and 4,000 grit papers (Struers). This process creates a flat, smooth surface that favors infrared radiation absorption. To get rid of the polishing residues, the specimens were submerged in distilled water and agitated using an ultrasonic cleaner for 5 minutes.

According the irrigating solution employed, the specimens were randomly divided into four groups (n=13).

Control group: for 1 minutes, distilled water was used to treat samples of dentine.

NaOCl/EDTA group: dentine samples were treated with 5 mL 2.6% NaOCl for 1min, then placed in a microtube containing distilled water for 1 min. Afterward, they were placed in a microtube containing 5 mL 17% EDTA for 1 min, and finally placed in a microtube containing contain distilled water for 1 min.

NaOCl/Dual Rinse HEDP group: dentine samples were treated by 5 mL 2.6% NaOCl/9% Dual Rinse HEDP for 1 min inside a microtube, then transferred to another microtube contain distilled water for 1 min.

In accordance with the manufacturer's instructions, 10 ml of the 2.5% sodium hypochlorite solution were combined with one Dual Rinse HEDP capsule containing 0.9 g of powder to create NaOCl/Dual Rinse HEDP (1-Hydroxyethylidene-1, 1-Bisphosphonate) mixes.

NaOCl/Glycolic acid: dentine samples were treated by 5 mL 2.6% NaOCl for 1 min, then one minutes of distilled water in a different microtube, afterwards, transferred to another microtube contain 5 mL 17% glycolic acid (PH 2, El Ezapy Pharmacy, Cairo,Egypt) for 1 min and a then placed in another microtube contain distilled water for 1min.

Outcome assessment :

FTIR Analysis (Fourier-transform infrared spectrometry test)

All specimens were analyzed for composition using an FTIR spectrometer (Vertex 70 FTIR spectrometer, Bruker, Germany) between 500 and 4000 cm-1, with 32 scans at 4 cm-1 resolution and a diamond ATR set-up (Smart OMNI-Sampler, Thermo Scientific Inc.). The specimens were dried with absorbent paper before being placed with the polished surface in contact with the diamond crystal of the ATR setup. FTIR spectra were then collected from three different areas of each specimen's pulpal surface. Each collected spectra was smoothed, baseline adjusted, and normalized to the amide I peak using the Spectra Manager program (Jasco Inc.).

The FTIR calculation is based on the ratio of the mineral spectral region (900-1000 cm-1) to the matrix region (1600–1700 cm-1). To calculate the relative contents of these inorganic and organic components, the ratio of integrated areas of the phosphate peaks to the amide I peak was quantified. The mean of the ratios obtained from the three single scans was used to calculate the final ratio assigned to each sample. Greater Phosphate/Amide I ratios in comparison to untreated dentin samples correspond to more collagen deproteinization, while smaller Phosphate/Amide I ratios corresponding to more demineralization.

Microhardness Measurement (Vickers hardness test)

In order to conduct a microhardness test, a Vickers' microhardness tester was employed on the samples after they had been placed in acrylic resin blocks exposing only the dentin surface (HM-102, Mitutoyo Corporation, Yokohama, Kanagawa, Japan).

In the Vickers test a steady, impact-free 50 gf load is delivered to push the indenter into the test specimen. For 10 sec, the indenter is held in place. In order to obtain accurate results, the indenter's physical quality and the precision of the applied load must be under control. The indentation is focused with the magnifying eye piece once the load has been removed, and the two diagonals imprints are measured and averaged with a micrometer to the nearest 0.1-μm. Vickers hardness number (VHN) was calculated as follows: HV = 1854.4L/d2, where L is the load in gf and d is the average diagonal in μm (this produces hardness number units of gf/m2). Three readings were taken at 0.5 mm from the root surface for each specimen, and the mean value was calculated from the three measurements.

Statistical analysis of the Data

Shapiro-Wilk and Kolmogorov-Smirnov tests was used to check whether the data were normal, the data showed a parametric (normal) distribution. For each category, the mean and standard deviation were determined. To compare more than two groups, a one-way ANOVA was performed. This was followed by a Tukey post hoc analysis, and Pearson correlation was used to examine the relationship between various parameters. IBM SPSS Statistics Version 20 for Windows (IBM Corporation, New York, USA) was used for the statistical analysis. The outcome assessor was blinded in this trial.

RESULTS

The means and standard deviations (SD) of the Phosphate/ Amide I ratio and microhardness of the experimental groups are shown in (Table 1-2 and Figures 1-4)

Phosphate/Amide I ratio varied significantly ($p<0.05$) among the four groups. NaOCl/EDTA demonstrated lowest Phosphate/Amide I ratio, while there was no statistically significant difference between Control and either NaOCl/Dual Rinse HEDP or NaOCl/Glycolic acid group ($p>0.05$). Moreover, there was no statistically significant difference between NaOCl/Dual Rinse HEDP and

Glycolic Acid (p>0.05). A statistically significant difference occurred between the groups in terms of microhardness (p<0.05), NaOCl/EDTA group had the lowest mean microhardness value, while Control had the highest mean microhardness value with no difference between Control and NaOCl/Dual Rinse HEDP or NaOCl/Glycolic acid group and no difference exist between NaOCl/Dual Rinse HEDP and Glycolic Acid. Phosphate/Amide I ratio and microhardness were shown to be positively correlated, according to Pearson correlation (0.700).

TABLE (1) Descriptive statistics and comparison between groups for Phosphate/ Amide I ratio values for the Control, NaOCl/EDTA, NaOCl/Dual Rinse HEDP and NaOCl/Glycolic acid group.

Variables	Phosphate/ Amide I ratio	
	Mean	**SD**
Control	11.46 ^{a}	1.50
NaOCl/EDTA	6.92 ^{b}	0.87
NaOCl/Dual Rinse HEDP	10.69 ^{a}	1.06
NaOCl/Glycolic acid	10.99 ^{a}	1.46
p-value	**<0.001***	

Significance level P<0.05, *significant

Means with different superscript letters are significantly different.

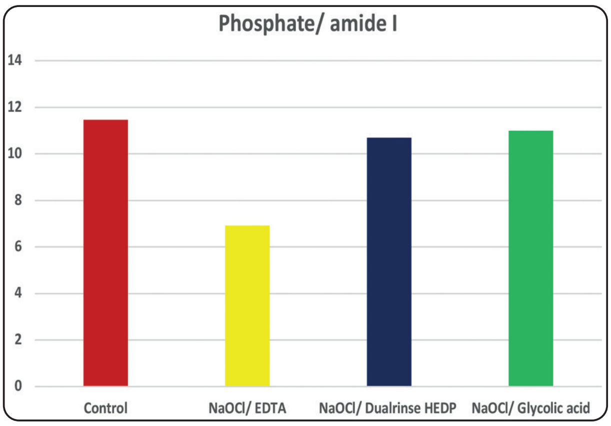

Fig. (1) Bar chart viewing Phosphate/ Amide I ratio mean values for the four tested groups.

TABLE (2) Descriptive statistics and comparison between groups for Microhardness values of Control, NaOCl/EDTA, NaOCl/Dual Rinse HEDP and NaOCl/Glycolic acid group.

Variables	Microhardness	
	Mean	**SD**
Control	59.07 ^{a}	3.07
NaOCl/EDTA	44.77 ^{b}	2.73
NaOCl/Dual Rinse HEDP	56.18 ^{a}	4.07
NaOCl/Glycolic acid	57.68 ^{a}	1.84
p-value	**<0.001***	

Significance level P<0.05, *significant

Means with different superscript letters are significantly different.

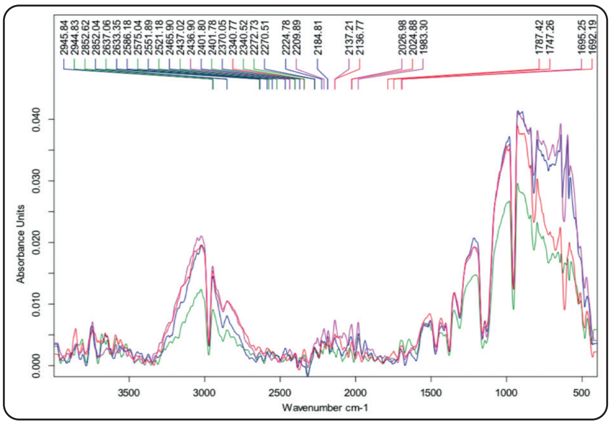

Fig. (2): Illustration of the FTIR spectra of dentin samples following irrigation with the experimental solutions; Dark blue represents Control, green represents NaOCl/EDTA, pink represents NaOCl/Dual Rinse HEDP, and red represents NaOCl/Glycolic Acid)]. The Phosphate/Amide I ratio was calculated using the peaks for amide I (1600–1700 cm1) and phosphate (900–1000 cm1).

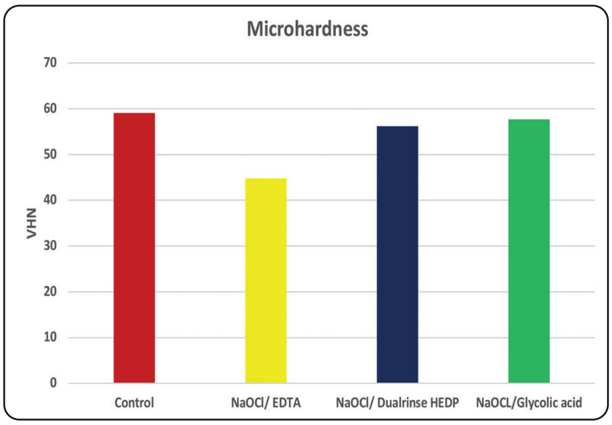

Fig. (3) Bar chart viewing the mean Vickers hardness number for the four tested groups.

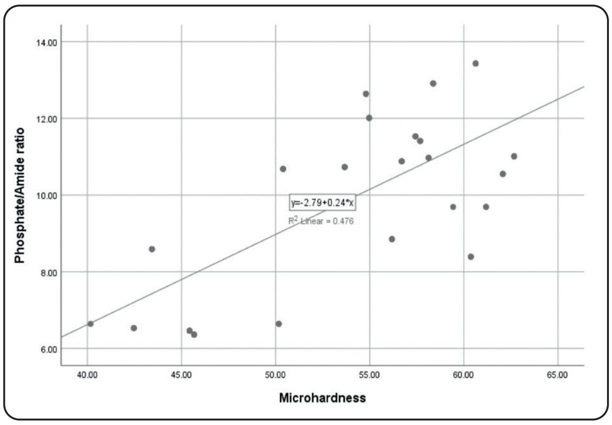

Fig. (4) Scatter correlation plot representing the total correlation between Phosphate/ Amide I ratio and microhardness.

DISCUSSION

Several irrigation protocols have been proposed to maximize instrument lubricant, tissue dissolution, and antimicrobial effects[18]. The chemical and structural makeup of human dentin is altered by various irrigants, though, which promotes dentin erosion and microhardness reduction[19,20], in addition, the sealing capacity and dentin adherence of materials like resin-based cements and root canal sealers may be negatively impacted by these modifications [21] .

The purpose of this study was to assess and compare the effect of Dual Rinse HEDP and Glycolic acid as root canal irrigating solutions on the chemical structure and microhardness of dentin.

Smear layer can be removed efficiently by combining the application of Dual Rinse HEDP and NaOCl without significantly harming the root dentin wall and without impairing the proteolytic and antibacterial activities of the NaOCl [22] . Moreover, Glycolic acid is a biocompatible material; demonstrated pH stability over a 90-day when stored at 4, 25, and 37 °C, which facilitate its use in endodontics with little adverse mechanical or biological impact [12,13, 22] .

Attenuated Total Reflection Fourier Transform Infrared Spectrophotometer (ATR FT-IR) was employed to measure the amounts of phosphate, organic matter, carbonate and water in dentine as they are chemical components of dentine that strongly absorb infrared radiation. ATR-FTIR method is advantageous for its simplicity, sensitivity, non-destructivity and it also requires little sample preparation with flat, polished surface [17-18] .

One of the drawbacks of using FTIR to assess the chemical structure of dentin, is that it can only measure changes in the chemical structure of superficial dentin due to infrared's limited depth of penetration [23] . Additionally, samples were cleaned and allowed to "air dry" before evaluation because testing wet samples was not possible.

In the present study, the impact of various irrigation solutions on the inorganic and organic components of root dentin structure was analyzed by FTIR[17,18,20,24,25]. A strong signal produced by proteins called the amide I, peak may be seen between 1600 and 1700 cm-1, while signals related to the phosphate group (V3PO4) can be seen between 900 and 1000 cm-1. widely used in the spectroscopic studies of calcified tissues to analyze the distribution of mineral content[17] .

The changes in the chemical integrity of dentin was also investigated by microhardness test to determine the mineral loss of root canal dentine and to indicate its overall strength and resistance to deformation . Although its reduction facilitates the instrumentation of the root canal but, it may also damage the root structure, increase root canal dentin permeability, and impair the sealers' ability to adhere to and seal the root dentin wall [25,26,27] . It was advised to use the Vickers microhardness test (VHT) to evaluate the hardness of brittle materials, such as tooth structure. The Vickers indenter was more frequently recommended than the Knoop indenter, as the used for deep dentin evaluation rather than the superficial one. Additionally, the Vickers tester uses the mean of two diagonals, whereas the Knoop tester uses only one [26]. VHT has been utilized in numerous research to assess the impact of irrigation solutions, root canal medicaments and root canal sealers on the root canal dentine microhardness[27-29].

Following investigation into the chemical integrity of dentin using various irrigation procedures, all experimental solutions should less value of Phosphate/Amide ratio compared to the control group, thus the null hypothesis was rejected. NaOCl/EDTA irrigation regime produced the lowest Phosphate/Amide ratio, this may be attributed to the fact that administration of NaOCl depletes the organic components of root canal dentine, which is then followed by the application of EDTA which depletes the hydroxyapatite component[30]. The outcomes of the present study were supported by the findings of Zhang et al.[17], who found that treatment with 17% EDTA for 2 minutes decreased the apatite/collagen ratio and coinciding with the studies of Doğan and Qalt S[19] and Ari and Erdemir[30] who demonstrated a considerable reduction in Calcium and Phosphorous after a final flush with 2.5% NaOCl and 17% EDTA when compared to the control group (distilled water).

Group NaOCl/Dual Rinse HEDP showed similar results to NaOCl/Glycolic acid group and to the control group regarding Phosphate/amide ratio, results are in line with those of Lottani et al. [22] and Tartari et al. [31] ,who reported slight alterations or demineralization of dentine with the weak chelating agent HEDP, in accordance with Barcellos et al. [25], who reported that applying GA at low pH caused less change in the collagen/apatite ratio because of its beneficial properties and its capacity to remove the smear layer without adversely impacting the dentin when applied at low pH. (around 2) and inconsistent with Bello et al.[13,27], who recommended its use as a last irrigant during root canal therapy.

Microhardness results revealed less value with all the irrigating solutions compared to the control, where the lowest value was detected in EDTA group, this was is in consistence with Barcellos et al.[25], who reported decrease in dentin microhardness by EDTA in comparison with the control (saline), Sayin et al.[7], also observed that EDTA, with and without subsequent NaOCl treatment significantly reduced dentin microhardness, which may be attributed to the chelating effect of 17% EDTA on the dentin minerals, making it much weaker than normal.

Tartari et al.[31], claimed that HEBP produces less reduction in the microhardness compared to EDTA. This may highlight the fact that the chelating power of the chemical is directly related to the decrease in hardness. Although Grinkevičiūtė et al.[32] showed higher reduction with dual rinse, which oppose our findings, which may be attributed to the different methodology and irrigation protocol utilized. Moreover, Souza et al.[33], revealed that low concentrations of glycolic acid did not significantly alter dentinal micro-hardness when compared to control.

The overall correlation analysis revealed a strong correlation between Phosphate/Amide I ratios and dentin microhardness values, which may help to explain the reported decrease in dentin microhardness across all experimental groups compared to the control group, which may be related to the superficial demineralization effect after the different irrigating solutions. These findings are consistent with previous researches, which also suggested that dentin microhardness is influenced by mineral concentration [33,34] .

Despite the limitations of the present investigation, it can be concluded that DualRinse HEDP and Glycolic acid can be utilized as an alternative irrigating solution in endodontics with further clinical research as they preserve dentine microhardness and produce less demineralization of the dentine surface.

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