THE EFFECT OF CHLORHEXIDINE IRRIGATION ON ROOT DENTIN MICROHARDNESS IN REGENERATIVE ENDODONTICS USING DOUBLE ANTIBIOTIC PASTE (AN IN-VITRO STUDY)

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Abstract:

Aim: The aim of this study was to evaluate the effect of chlorhexidine (CHX) irrigation on the root dentin microhardness of single-rooted teeth compared to saline irrigation before the application of double antibiotic paste (DAP) for two weeks in regenerative endodontics. **Subjects and methods:** Thirty-two single rooted teeth were selected. Following decoronation and mechanical preparation, teeth were randomly assigned into two groups in accordance with irrigation technique as follows: the intervention group: irrigation with 20 ml of 1.5% sodium hypochlorite (NaOCl), then irrigation with 20 ml of saline, followed by irrigation with 2% CHX; and the control group: irrigation with 20 ml of 1.5% NaOCl, then irrigation with 20 ml of saline. After treatment, 1mg/mlof DAP was injected into the canal system for two weeks. Pre-treatment dentin microhardness was measured for a 2 mm cervical cut before the irrigation protocol. Post-treatment dentin microhardness was measured for remaining root section. Microhardness was measured by a vickers test machine. **Results**: The microhardness value before treatment was significantly higher than after treatment in both groups. However, there was no statistically significant difference in microhardness values between the intervention and control groups after treatment. **Conclusion**: Within the study limitations, 2%CHX irrigation did not affect the root dentin microhardness.

Keywords: endodontic regeneration, **c**hlorhexidine, immature teeth, microhardness.

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Introduction:

The blunderbuss apices and thin, brittle roots of young permanent teeth with necrotic pulp (immature teeth) complicate their endodontic therapy. Single-session apexification using mineral trioxide aggregate (MTA) or several-sessions apexification by calcium hydroxide ($Ca(OH)_2$) are the classical lines of treatment [(1)].Nevertheless, because the root width does not grow, the weak and thin roots of immature teeth that already have a danger of root fracture do not get stronger. Regenerative endodontics (RE) has been utilized for the past ten years. Since the root canal is not fully cleansed mechanically during this treatment process, disinfection of the root canal with intra-canal medications is a crucial need for RE [(2)].

The Association of American Endodontists (AAE) recommends irrigating the teeth for 5 minutes with 20 ml of 1.5% NaOCl during the first visit, followed by 5 minutes with 20 ml of saline, drying the canals with paper points, and applying 1mg/ml of double antibiotic paste (DAP) for 2 weeks instead of triple antibiotic paste to avoid discoloration. The following second visit after it has been confirmed that there are no clinical signs or symptoms: removal of DAP, irrigation with 30 ml of 17% ethylenediaminetetraacetic acid (EDTA), and induction of bleeding [(3)].

The root dentin's mechanical characteristics, such as micro-hardness and root fracture resistance, were shown to be dramatically reduced by the use of NaOCl, EDTA, and antibiotic paste [(1,2,4,5)]. Proteinases called matrix metalloproteinases (MMPs) are involved in the extracellular matrix's (ECM) breakdown [(6)].

A powerful MMP inhibitor, chlorhexidine (CHX), can thereby lessen the breakdown of collagen in demineralized dentin by blocking host-derived proteases. The greater the concentration of CHX, the greater the effect of MMP inhibition [(7)].Since no prior research had been done, the goal of this in-vitro study was to ascertain how CHX irrigation affected the root canal dentin wall's microhardness during RE procedures using DAP.

SUBJECTS AND METHODS:

This study was conducted in the Endodontics Department, Faculty of Dentistry, Cairo University, Cairo, Egypt. The university's Research Ethics Board had

approved this study protocol. The sample size was calculated using the PS soft ware and was found to be 16 extracted single-rooted teeth per group, making the total sample size 32 teeth (2 groups). Teeth were randomly assigned into 2 groups by using a Web program available at www. Randomizer.org.

1. Inclusion and Exclusion Criteria

The inclusion criteria were straight roots with single canals and completely formed apices or slightly curved freshly extracted teeth stored in normal saline solution [8].

The exclusion criteria were previously treated root canal teeth, fractured or cracked teeth, and carious teeth.

2. Preparation and classification of teeth

The crown of the teeth was cut using a water-cooled, high-speed diamond bur at the cement-enamel interface, leaving root samples with a standardized length of 14mm ±1mm. Two groups of root samples were randomly assigned based on the irrigation procedure utilized in endodontic regeneration.

A) <u>Intervention group</u>

Before chemo-mechanical preparation, 2 mm of cervical radicular part from each sample was sectioned with a diamond bur in a transverse direction, leaving 12 mm root length to determine the pre-treatment microhardness (Figure1).After assessing the apical patency with the ISO-standard k-file #15 (MANI, Inc., Japan), the working length of the remaining root sample was ascertained.

The rotary nickel-titanium (Ni-Ti) system M3 Pro Gold (United Medical Group, China) was then used to prepare each root canal, utilizing the crown-down approach till size 35 taper 4 until clean white chips of dentin were observed under the constant speed and torque of manufacturer instructions. Following full instrumentation, irrigation of the samples was done as follows: 5 minutes using 20 ml of 1.5% NaOCl, 5 minutes using 20 ml of saline, and 2 minutes using 5 ml of 2% CHX.

Fig. (1) The 2 mm cervical section

B) <u>Control group</u>

Before chemo-mechanical preparation, 2 mm of cervical radicular part from each sample was sectioned as mentioned in the intervention group to determine the pre-treatment microhardness (Figure1). After assessing the apical patency with the ISO-standard k-file #15 (MANI, Inc., Japan), the working length of the remaining root sample was ascertained.

The rotary nickel-titanium (Ni-Ti) system M3 Pro Gold (United Medical Group, China) was then used to prepare each root canal, utilizing the crown-down approach till size 35 taper 4 until clean white chips of dentin were observed under the constant speed and torque of manufacturer instructions. Following full instrumentation, irrigation of the samples was done as follows: 5 minutes using 20 ml of 1.5% NaOCl, and 5 minutes using 20 ml of saline.

3. Preparation and application of the intracanal medicament

Tablets of 500 mg ciprofloxacin (Ciprocin®Eipico EGY) and 500 mg metronidazole (Flagyl® Pfizer Medical Information – US) were grounded using a mortar and pestle. Then, using an analytical balance, equal volumes of powdered metronidazole and ciprofloxacin in a 1:1 ratio were determined. A 100-mg portion of this combination was combined with a 100-ml volume of sterile water to get 1 mg/ml of DAP. Considering liquid form is common for antibiotic pastes of low concentration, in order to obtain an antibiotic medicament that is clinically applicable to be injected, a methylcellulose vehicle was loaded[(1)].

After endodontic preparation was finished, all root canals in both groups received 1 mg/ml of DAP via a plastic syringe using disposable intra-canal tips. Flowable composite was used to seal the canals coronally. They were then placed in normal saline for a period of two weeks.

The flowable composite was taken out after two weeks, and the antibiotic paste was cleaned from both groups using 20 ml of saline for 5 minutes, followed by irrigation with 30 ml of 17% EDTA for 5 minutes, and a last flush with 5 ml of saline for one minute. All samples from both groups were prepared to determine the post-treatment microhardness.

4. Preparation and sectioning of the root samples

Cervical sections of the two groups that

were cut previously, before chemo-mechanical preparation, were longitudinally sectioned by a water-cooled precision saw into two equal halves, and one section was mounted on resin blocks, leaving their dentin exposed to allow the measurement procedure to determine the pre-treatment microhardness of root canal dentin.

The remaining radicular sections of the two groups were longitudinally sectioned into two equal halves using a water-cooled precision saw following chemo-mechanical preparation, antibiotic paste administration, and removal.

One section longitudinally was taken from each of the two groups to be mounted on acrylic resin blocks, leaving their dentin exposed to allow the measurement procedure to determine the post-treatment microhardness(Figure2).

Fig. (2) Specimens mounted on acrylic blocks

In order to eliminate any surface scratches, all mounted specimens had their root canal dentin ground flat, polished using a composite polishing kit, and smoothed with silicon carbide abrasive sheets utilizing discs.

5. Microhardness evaluation

Vickers microhardness tester was used to determine each sample's microhardness. Using a Vickers diamond indenter, three indentations were produced on top of each other coronally at a distance of 100 µm parallel to the long axis of the root canal lumen[(9)](Figure3). A 50-gram load was applied to each sample for 10 seconds [(10)]. For each specimen, the mean value of the three indentations was determined and represented as the Vickers Hardness Number (VHN).

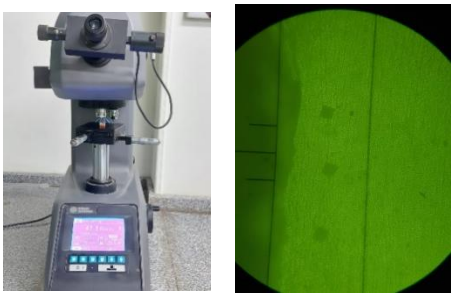

Fig. (3) Vickers microhardness testing machine making three indentations parallel to canal lumen

For statistical analysis, every data set was tabulated.

6. Statistical Analysis

Numerical data were presented as mean with 95% confidence intervals, standard deviation, and minimum and maximum values. The Shapiro-Wilk test was used to check the normality. The Data showed a parametric distribution and were analyzed using ANCOVA for intergroup comparisons and a paired t-test for intra-group comparison. The significance level was set at p ≤0.05 within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows.

RESULTS:

Comparison between pre-treatment and post-treatment of both groups (intragroup comparisons)

Intra-group comparisons, mean and standard deviation (SD) values of micro-hardness were measured by paired t-test and presented in **table (1)** and **figure (4)**.

Table (1): Intragroup comparisons, mean and standard deviation (SD) values of microhardness

Group	Microhardness (mean±SD)		p-value
	Pre-treatment	Post-treatment	
Intervention	46.03±1.65	44.32±0.03	**<0.001***
Control	46.79±1.94	44.34±0.02	**<0.001***

*; significant ($p \leq 0.05$) ns; non-significant (p>0.05)

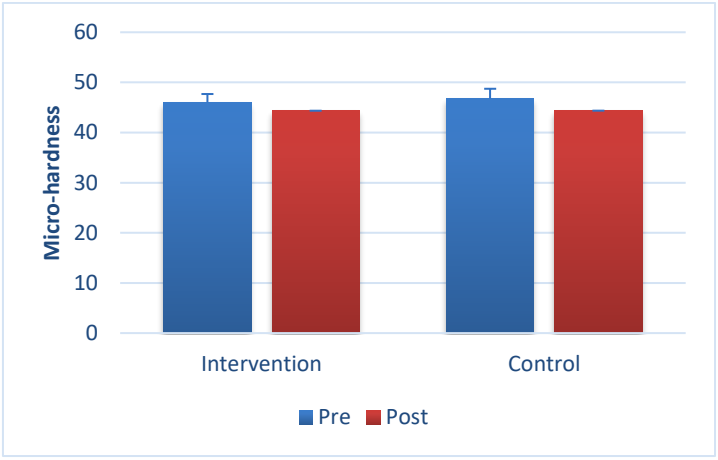

Fig. (4) Bar chart showing mean and standard deviation values for microhardness

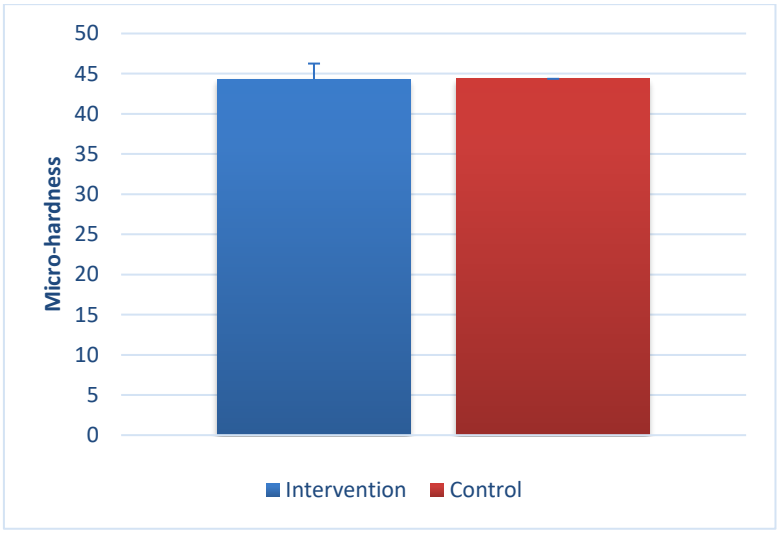

Fig. (5) Bar chart showing mean and standard deviation values for post treatment microhardness in both groups

Intervention group:

The microhardness value of thepost-treatment (44.32±0.03) was significantly lower than the microhardness value of the pre-treatment (46.03±1.65) ($p<0.001$).

Control group:

The microhardness value of the post-treatment (44.34±0.02) was significantly lower than the microhardness value of the pre-treatment (46.79±1.94) ($p<0.001$).

Comparison of post-treatment between both groups with pre-treatment micro-hardness measurements as covariate (Intergroup comparisons)

Intergroup comparisons, mean and standard deviation (SD) values of microhardness were presented in **figure (5)**.

This is a comparison to compare the change in microhardness value of the post-treatment between the control and intervention groups. While adjusting the pre-treatment microhardness measurements of both groups as it was used as a covariate, the control group had a higher mean value of microhardness (44.34±0.02) than the intervention group (44.32±0.03); however, this difference was not statistically significant ($p=0.057$).

Discussion:

Infected pulp does not create an environment favorable to host tissue regeneration, in addition to the complexity of the root anatomy of teeth with an open apex that requires little or no shaping. Hence, effective disinfection is a prerequisite to pulpal revascularization[11, 12].

The recent recommendations of AAE guidelines suggest using 1.5% NaOCl as a root canal irrigant and a low concentration of antibiotic combinations (1 mg/ml) as a medication to be taken in between appointments to avoid toxic effects on stem cells [13]. The administration of intra-canal medications often produces the therapeutic effects of antibiotics on teeth within two to four weeks [14].EDTA is an essential tool in regenerative endodontics, as it is thought to release growth factors from the dentin matrix at a concentration of 17%, which then causes odontoblastic differentiation [15].

CHX has been investigated in vivo and in vitro for use as an irrigant and intra-canal medicament in endodontics. It was widely effective against both Gram-positive and Gram-negative bacteria, as well as yeast. As in a previous study by **Nagata et al. (2014)**[16]in traumatized immature teeth to evaluate and assess the microbial composition and their reduction during different stages of the revascularization procedures, it was found that there was no significant difference between NaOCl and 2% CHX irrigating solutions.

Based on the results of the present study, there was a significant reduction in the microhardness value between the pre-treatment and post-treatment of the remaining radicular section of the two groups, indicating that the radicular dentin mechanical properties were adversely affected by the use of antibiotic paste, EDTA, and NaOCl irrigant during RE treatments. These chemicals were found to significantly reduce the fracture resistance of the root, dentin strength of flexure, and microhardness through interaction with mineral and organic contents. Alterations in the ratio of mineral content may negatively affect the microhardness, permeability, and solubility of the root canal dentin [1,2,4,5]. As regards effects on microhardness, **Slutzky Goldberg et al. (2004)** [17] reported changes in the biomechanical properties of dentin as a result of instrumentation and irrigation with NaOCl, indicating that these chemical solutions have direct effects on dentin organic and inorganic contents. Results from **Zaparolli et al. (2012**) [18] indicated that17% EDTA solution and 1% NaOCl individually and NaOCl/EDTA in alternation, have lowered dentin microhardness.

However, the difference in the microhardness values regarding the post-treatment readings of the two groups, which was the aim of this study, was of no significant difference. CHX is an efficient inhibitor of matrix metalloproteinase (MMPS); MMPs are proteinases that can be beneficial during tissue remolding and dentin mineralization, or they can be detrimental to dentin by contributing to the extracellular matrix's (ECM) breakdown.It has been demonstrated that when dentin is exposed to acids, these harmful enzymes are released, further dissolving and deteriorating the collagen matrix[6,19]. By acting as a

proteinase inhibitor, CHX inhibits and blocks the release of MMPs, improving the preservation of the dentinal surface's collagen fibrils [20,21]. Various studies have demonstrated that after CHX treatment, it may have an improving effect on dentin microhardness [6,22,23].

This study resultswere in-agreement with previous studies; the root canal dentin microhardness was assessed by **Aslantas et al. (2014)**[24], who discovered that treating the dentin for 5 minutes with 2% CHX and 2% CHX-Plus (including surfactant) had no influence on the microhardness of the dentin. Dentin remineralization may occur in demineralized lesions due to the ability of CHX to inhibit protease activity in demineralized dentin through adequate mineral supplementation from necessary sources, as reported in a study by **Kim et al. (2012)**[7]. In their study**, Asghari et al. (2018)**[8] examined the effects of 15 minutes of irrigation with 0.005% triphala, 2% CHX, and 5.25% NaOCl on the microhardness of root canal dentin. They discovered that, whereas triphala and 2% CHX had no discernible impact on dentin microhardness reduction, NaOCl greatly reduced it.

Dentin hardness was shown to have risen with the use of 2% CHX for 2 minutes as a final flush following irrigation with 2.5% NaOCl and 17% EDTA [6]**.** According to **Haralur et al. (2022)**[23], CHX has a negligible detrimental impact on dentin microhardness and is a safe alternative for dental cleaning while preserving bonding to dentin. Studies done by **Abdelrhman et al. (2023)**[25]**,** using CHX+ chitosan nanoparticles (CSNPs), as well as **Sahebi et al. (2023)**[26]**,** using imidazolium-based silver nanoparticles (Im-AgNPs) and zinc oxide nanoparticles (ZnONPs), had shown that nanoparticles together with CHX had improved the microhardness of root dentin.

In contrast to our results, Calcium and phosphorus levels significantly decreased following a 15-minute treatment with 0.2% CHX, according to research by **Ari et al. (2005)**[22]and this may be attributed to using different testing techniques as they used the Inductively Coupled Plasma Atomic Emission Spectrometry technique (ICP-AES). (**Oliveria et al.**

2007)[10]concluded that after 15 minutes of irrigation with CHX to the cervical, middle, and apical transverse sections, the root canal dentin microhardness was reduced at 500 μm and 1000 μm from the pulp-dentin interface by using a vickers microhardness tester, and this reduction may be related to different irrigation times.

Conclusions:

2% CHX did not affect the microhardness.

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Conflict of interest and source of funding

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Ethics

The ethical committee of the faculty of dentistry-Cairo University had approved this study protocol on: 27/9/2022.

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