

**Original Article**

# EVALUATION OF THE EFFICACY OF SODIUM DICHLOROISOCYANURATE AND CALCIUM HYDROXIDE AS INTRA-CANAL MEDICAMENTS ON MICROHARDNESS OF RADICULAR DENTIN: A COMPARATIVE IN-VITRO STUDY

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## Abstract

**Aim:** The aim of the present study was to evaluate the efficacy of Sodium dichloroisocyanurate (NaDCC) compared to Calcium hydroxide Ca(OH)<sub>2</sub> as intracanal medicaments in terms of superficial chemical structure and microhardness.

**Methods:** Sixty human dentin discs were obtained from single-rooted premolars, and randomly allocated into 4 groups (n=15); NaDCC group, Ca(OH)<sub>2</sub> group, N-S group (Saline control group), N-Na group (NaOCl control group). After 1 and 4 weeks, Phosphate/amide I ratio (PO<sub>4</sub>/amide I) was evaluated by FTIR then dentin microhardness was assessed.

**Results:** The results of PO<sub>4</sub>/amide I ratio showed a statistically significant difference between NaDCC, Ca(OH)<sub>2</sub> and NaOCl after 1 week ( $p < 0.001$ ) where the highest mean value was found in Ca(OH)<sub>2</sub> and the least mean value was found in NaDCC. Also, after 4 weeks the results of PO<sub>4</sub>/amide I ratio showed a statistically significant difference between NaDCC, Ca(OH)<sub>2</sub> and NaOCl ( $p < 0.001$ ) where the highest mean value was found in Ca(OH)<sub>2</sub> and the least mean value was found in NaDCC. The results of microhardness showed no statistically significant difference between all tested groups after 1 week ( $p = 0.337$ ) while, after 4 weeks, NaDCC showed a statistically significant difference when compared to Ca(OH)<sub>2</sub> and NaOCl groups ( $p < 0.001$ ) where the highest mean value was found in saline control group followed by NaDCC then NaOCl and the least mean value was found in Ca(OH)<sub>2</sub>.

**Conclusions:** The use of Ca(OH)<sub>2</sub> for 4 weeks reduced dentin microhardness, whereas NaDCC did not result in any change in the microhardness value. However, a change in PO<sub>4</sub>/amide I ratio was observed earlier after 1 week in both Ca(OH)<sub>2</sub> and NaOCl groups.

**Keywords:** EDTA; NaOCl; hardness tests; superficial chemical structure; FTIR.

## I. INTRODUCTION

The primary cause of pulp and periapical diseases is microorganisms. Infection, necrosis, and apical periodontitis occur if they invade the root canals. Therefore, the ability to eradicate these bacteria and avoid reinfection affects the outcome of the treatment (*Sjögren et al, 1999*).

The first step towards eradicating the intracanal bacterial invasion is considered to be the chemo-mechanical preparation of the root canal (*Hülsmann et al, 2005*). Despite meticulous cleaning and shaping, and the use of an antimicrobial irrigating solution, the majority of the root canals still harbored bacteria within the complicated anatomy of the root canal system. In order to completely disinfect the root canal system, a more rigorous root canal treatment with intracanal medications is the best additional step (*Kumar et al, 2019*).

Calcium hydroxide (Ca(OH)<sub>2</sub>) paste is frequently used as an intracanal medication. The bactericidal effect (*Siqueira Jr et al, 1999*), capability of detoxifying and denaturing bacterial products like lipopolysaccharide, the ability of dissolving tissue (*Kawashima et al, 2009*), hard tissue formation induction, and ability to prevent inflammatory root resorption (*Camargo et al, 2006*), make Ca(OH)<sub>2</sub> most commonly used as an intracanal dressing between visits.

Alternatives to Ca(OH)<sub>2</sub> should be taken into consideration though, as it has been reported that its prolonged use has been linked to dentin weakening that may encourage tooth fracture and that *Candida* and *Enterococcus faecalis* are both able to resist its antibacterial action clinically (*Sundqvist et al., 2003*). Additionally, the organic matrix in the dentin has likely been damaged as a result of the dentin's mechanical properties deteriorating following exposure to Ca(OH)<sub>2</sub> (*Batur et al, 2013*).

However, the use of intracanal medications has a detrimental effect on radicular dentin's chemical composition, which decreases its mechanical characteristics. Therefore, it is essential to choose an intracanal medicament with a strong antibacterial activity and little impact on the chemical structure (*Yassen et al, 2014*).

Commercially available sodium dichloroisocyanurate (NaDCC) tablets are used to disinfect swimming pools, household appliances,

baby feeding utensils, and other equipment. The World Health Organization also recommends using NaDCC to sanitize water supply (*World Health Organization, 2020*). NaDCC disinfects water when its free available chlorine (FAC) is partially released as hypochlorous acid (HOCl), while the remaining FAC remains attached to the NaDCC molecule, acting as a reservoir for additional HOCl release in case of chlorine diminution (*Clasen et al, 2006*). Additionally, NaDCC kills *E. faecalis* more efficiently than NaOCl whereas their cytotoxicity and other microbial species related to root canal infections is comparable (*Heling et al, 2001*).

Also, *Jacob Kurian et al, 2012* examined the ability of NaDCC and (Ca(OH)<sub>2</sub>) to combat *C.albicans*. After collecting the *Candida* stock cultures, they were subjected to various NaDCC and Ca(OH)<sub>2</sub> concentrations. Results showed that, NaDCC was efficient in killing *C.albicans* at all concentrations, but Ca(OH)<sub>2</sub> was completely unsuccessful. It was also discovered that both together were effective.

Additionally, *Shenoy et al, 2013* investigated the antimicrobial action of 0.6% NaDCC, 2% CHX and 2% NaOCl and a combination of 0.6% NaDCC and 0.2% CHX against *C.albican* and *E. faecalis* before and after instrumentation. Results showed that NaDCC 0.6% and CHX 2% demonstrated significantly bigger zones of microbial inhibition against *E.faecalis*. When NaDCC 0.6% and CHX were compared, NaDCC 0.6% showed bigger zones of inhibition than CHX 2% and NaOCl 2% against *C. albicans* while, the control group of normal Saline showed no inhibition.

The impact of NaDCC and Ca(OH)<sub>2</sub> as intracanal medications on the physical and chemical properties of dentin has not yet been compared in research. So, this study aimed to assess the effect of NaDCC and Ca(OH)<sub>2</sub> as intracanal medications on microhardness and superficial chemical structure of dentin in extracted single-rooted teeth. The null hypothesis tested was that there would be no difference between NaDCC and Ca(OH)<sub>2</sub> as intracanal medicaments regarding chemical structure and microhardness of radicular dentin when used as intracanal medications in single-rooted teeth.

## II. SUBJECTS AND METHODS

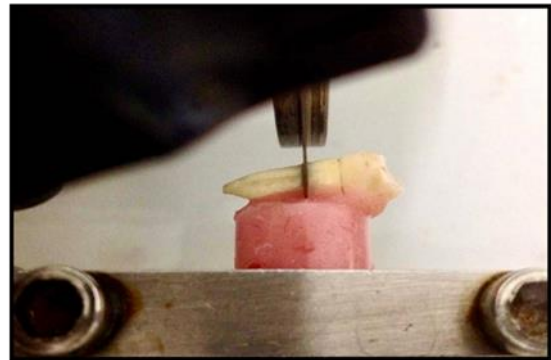
The Research Ethics Committee at the Faculty of Dentistry, Cairo University, granted ethical approval for this in vitro study and the approval number is 24121. Based on previous study performed by Naseri et al, (2019) on dentin microhardness, Sample size was calculated using the (PS software) with an alpha ( $\alpha$ ) level of 0.05 (5%) and beta ( $\beta$ ) level of 0.20 (20%) i.e power = 80% on estimated difference between the experimental and control means = 7.2. A total of 60 dentin discs per group (15 per group).

Subsequent to the collection of the 60 single rooted mandibular premolars, a random sequence was generated using the random sequence generator website ([http://: www.random.org](http://www.random.org)). According to the tested intracanal medicament, teeth were randomly divided into four equal groups (n=15). Allocation concealment was done by inserting each sample into a separate opaque sealed envelope, shuffling the envelopes then giving each envelope a number. Random allocation sequence and allocation concealment were done by the co-supervisor, and the technical procedures of the research method were carried out by the investigator.

### Sample preparation:

The external root surfaces of the collected teeth were cleaned with ultrasonic scaler to remove calculus and periodontal tissues, and then was immersed in 5.25% NaOCl (JK Dental vision, Mansoura; Egypt) for 30 min to remove soft tissue debris and finally stored in saline till use. Utilizing core building material, each tooth was set in a block of acrylic resin. Each block was placed on laser precision saw (isomet 4000, Bruehler, Lake Bluff, IL, USA). Decoronation of teeth was done using a low-speed diamond saw (CUTFLEX® diamond discs, Dental Future Systems DFS DIAMON, Germany ) under copious irrigation to obtain 14 mm uniform root lengths. Two coronal dentinal discs with a 4-mm (thickness x width x length) were cross sectioned using a hard tissue microtome (Buehler, London, UK) which accurately and repeatedly slices sections under deionized running water with minimal subsurface damage (**Figure 1**), then each disc was sectioned vertically along the root canal (four dentin half discs were yielded from each root) thus making a total of 60 samples of dentin half discs (**Figure 2**). Using an automatic polishing machine (Struers

Rotopol 31, Struers, Cleveland, OH, USA ) with 500 grit silicon carbide grinding paper, the non-pulpal surfaces of the dentinal discs were flattened to a uniform thickness while, the internal pulpal surface of each dentinal disc was polished with 1,200, 2,400 and 4,000 grit papers and was polished using a 1  $\mu$ m diamond polishing solution. Finally, the smear layer of each sample was removed completely by utilizing an ultrasonic cleaner (Codyson, GuangDong, China).



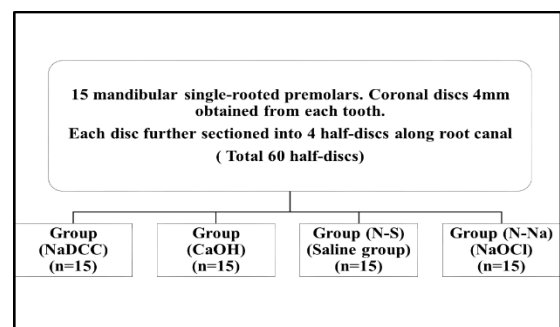
**Figure 1:** Two coronal dentinal discs 4-mm obtained from each root after decoronation.



**Figure 2:** Four Dentinal half discs were obtained from each tooth

### Intervention and Control:

60 half dentin discs were randomly divided into 4 groups according to the root canal medication used (**Figure 3**).



**Figure (3):** Schematic diagram representing the distribution of samples in experimental and control groups.

## Intracanal medicament preparation and application:

### Experimental groups

- **Group (NaDCC)**, NaDCC was used as an intracanal medication. NaDCC tablets (*Klorsept 25 disinfectant tablets, Medentech, Wexford, Ireland*) were crushed using a mortar and pestle, in order to make 5 g of powder then added to 5 ml of ready-made phosphate-buffered saline (PBS) (*Thermo Fisher Scientific, Waltham, MA*) to obtain a creamy mix. The mixture was created by stirring with a sterile spatula on a sterile glass slab.

- **Group (CaOH)**, A commercial Ca(OH)<sub>2</sub> (*UltraCal XS, Ultradent, South Jordan, UT, USA*) was used as an intracanal medication.

In both groups, the dentinal discs were irrigated with 2.5% NaOCl for 5 minutes, then they were placed into a 2 ml eppendorf and the experimental paste was injected by a modified plastic syringe into the eppendorf while being lightly tapped to release any trapped air bubbles (**Figure 4**).

Samples were preserved in an incubator for 1 and 4 weeks at 37° C and 100% relative humidity. After 1 and 4 weeks, the discs were rinsed with 17% EDTA solution (*EDTA®, Prevest Direct, India*) for 1 minute until no paste remnants were detected, then after 15 minutes of ultrasonic cleaning, air spray was used to completely dry the samples.

### Control groups

- **Group (N-S)**, No medicament was used. The discs were rinsed only with 0.9% normal saline (*saline (0.9% NaCl), FIPCO, Egypt*) and stored for 1 and 4 weeks in 100% relative humidity. After the desired time, the samples were rinsed with normal saline.

- **Group (N-Na)**, No medicament was used. The samples were first irrigated only with 2.5% NaOCl for 5 minutes, after preservation in an incubator for 1 and 4 weeks at 37° C and 100% relative, the discs were irrigated with 17% EDTA solution for 1 minute then after 15 minutes of ultrasonic cleaning, air spray was used to completely dry the samples.



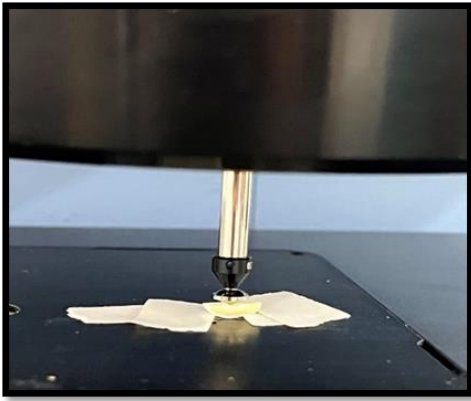
**Figure (4):** Loading of Eppendorf with 2 ml of intracanal medicaments.

### Outcome assessment:

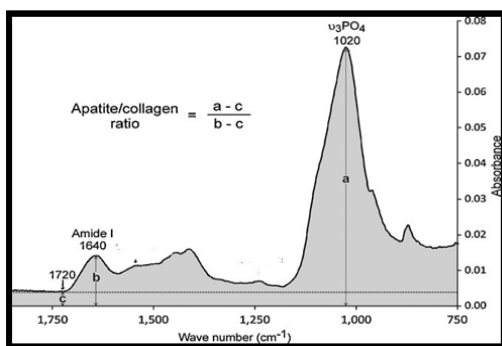
Fourier-transform infrared spectroscopy (FTIR)

The chemical composition of all specimens was analyzed using FTIR spectroscopy (Vertex70; Bruker, Berlin, Germany) between 800 and 2000 cm<sup>-1</sup> (the IR spectrum of human dentine) at 4 cm<sup>-1</sup> resolution by using 40 scans with a diamond ATR set-up (Smart OMNI-Sampler, Thermo Scientific Inc.). The specimens were positioned with the polished surface in contact with the diamond crystal of the ATR set-up. FTIR spectra were then collected from 3 selected areas in the pulpal side of each sample (**Figure 5**).

The infrared (IR) data set were imported into Origin Pro 2022. It was acquired using amide I courier for smoothing, baseline correction, and normalization. Using the mineral/ matrix ratio, the impact of different treatments on the organic and inorganic composition of dentin was assessed (the ratio of integrated areas of the phosphate peak to the amide I peak). The spectra of the mineral region (phosphate) (900–1200 cm<sup>-1</sup>) to the matrix region (amide I) (1590-1720 cm<sup>-1</sup>) is used to calculate the FTIR (**Figure 6**). Higher ratios of the PO<sub>4</sub>/amide I are associated with higher collagen deproteinization, whereas lower ratios are associated with higher dentin demineralization, when compared to untreated dentin samples.



**Figure (5):** The diamond crystal of the ATR set-up in contact with the polished surface of the specimen.



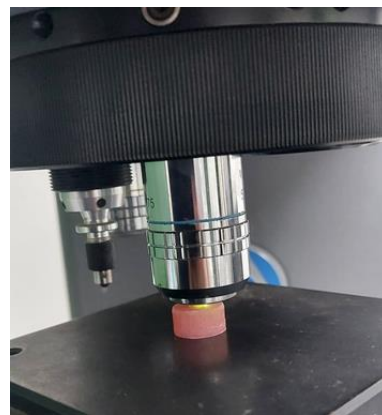
**Figure (6):** Schematic illustration of how the phosphate/amide I ratio was obtained from each FTIR spectrum using the peaks phosphate (a) and amide I (b)

#### - Microhardness Evaluation

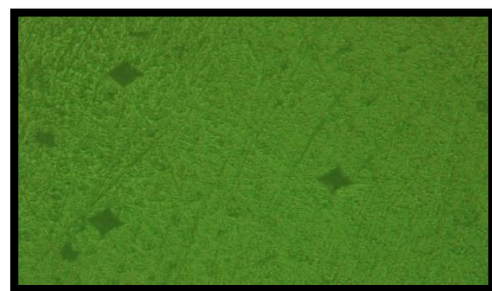
The samples were imbedded in acrylic resin blocks leaving their dentin surface exposed (**Figure 7**). Vickers microhardness test (HM-102, Mitutoyo Corporation, Yokohama, Kanagawa, Japan ) was used and the samples were subjected to 50-g load for 10 seconds smoothly and without impact (**Figure 8**). The physical quality of the indenter and the accuracy of the applied load must be controlled in order to get accurate results. After the load is removed, the indentation is focused with the magnifying eye piece and the two impression diagonals are measured (**Figure 9**), usually to the nearest 0.1- $\mu\text{m}$  with a micrometer, and averaged. The Vickers hardness number (VHN) is calculated using:  $HV = 1854.4L/d^2$ , Where the load L is in gf and the average diagonal d is in  $\mu\text{m}$  (this produces hardness number units of  $\text{gf}/\mu\text{m}^2$ . in practice the numbers are reported without indication of the units). Three readings for each specimen were performed 0.5 mm from the root canal wall, the mean value was calculated from the three measurements from each specimen.



**Figure (7):** The samples were imbedded in acrylic resin blocks leaving their dentin surface exposed.



**Figure (8):** 50-g load that were administered for 10 seconds smoothly without impact to the dentin specimen.



**Figure (9):** Vickers diagonal indentations.

### III. RESULTS

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests, data showed parametric (normal) distribution. Paired sample t-test was used to compare between two groups in related samples. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two

groups in non-related samples. Two-way ANOVA was used to test the interactions between different variables. Pearson correlation was used to test relationship between different parameters.

The significance level was set at  $P \leq 0.05$ . Statistical analysis was performed with IBM® SPSS® (SPSS software, IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp) Statistics Version 20 for Windows.

## 1. Fourier-transform infrared spectroscopy (FTIR)

Mean, Standard deviation (SD) values of the PO4/amide I ratio at different observation periods within each group are summarized in Table (1) and presented in Figure (10, 11).

### 1.1 Comparison among observation periods for each treatment protocol

#### A) Group NaDCC:

The mean and SD values of the PO4/amide I ratio after 1 week was  $(5.34 \pm 1.15)$ , which decreases to  $(4.84 \pm 0.63)$  after 4 weeks. Paired t-test showed no statistically significant difference among the two observational periods where ( $p=0.101$ ,  $p > 0.05$ ). The value of PO4/amide I ratio was highest after 1 week.

#### B) Group CaOH:

The mean and SD values of the PO4/amide I ratio after 1 week was  $(7.88 \pm 2.26)$ , which increases to  $(8.13 \pm 0.35)$  after 4 weeks. Paired t-test showed no statistically significant difference among the two observational periods where ( $p=0.693$ ,  $p > 0.05$ ). The value of PO4/amide I ratio was highest after 4 weeks.

#### C) Group N-S:

The mean and SD values of the PO4/amide I ratio after 1 week was  $(5.50 \pm 1.29)$ , which decreases to  $(5.02 \pm 0.47)$  after 4 weeks. Paired t-test showed no statistically significant difference among the two observational periods where ( $p=0.283$ ,  $p > 0.05$ ). The value of PO4/amide I ratio was highest after 1 week.

#### D) Group N-Na:

The mean and SD values of the PO4/amide I ratio after 1 week was  $(6.31 \pm 0.60)$ , which increases to  $(6.50 \pm 0.44)$  after 4 weeks. Paired t-test showed no statistically significant difference among the two observational periods where ( $p=0.166$ ,  $p > 0.05$ ). The value of PO4/amide I ratio was highest after 4 weeks.

### 1.2 Comparison of different treatment protocols among observation periods

#### A) After 1-week:

The mean and SD values of PO4/amide I ratio for NaDCC, CaOH, N-S and N-Na were  $(5.34 \pm 1.15)$ ,  $(7.88 \pm 2.26)$ ,  $(5.50 \pm 1.29)$  and  $(6.31 \pm 0.60)$  respectively. One-way ANOVA test showed a statistically significant difference between NaDCC, CaOH, N-S and N-Na where ( $p < 0.001$ ,  $p < 0.05$ ). The highest mean value was found in CaOH, followed by N-Na then N-S and finally, the least mean value was found in NaDCC. Tukey post-hoc test revealed that there was a statistically significant difference between CaOH and each of NaDCC, N-S and N-Na ( $p < 0.001$ ,  $p < 0.001$ , and  $p = 0.024$  respectively,  $p < 0.05$ ), while no statistically significant difference existed between NaDCC and each of N-S and N-Na ( $p = 0.991$  and  $p = 0.272$ ,  $p > 0.05$ ) respectively as well as between N-S and N-Na ( $p = 0.427$ ,  $p > 0.05$ ).

#### B) After 4-weeks:

The mean and SD values of PO4/amide I ratio for NaDCC, CaOH, N-S and N-Na were  $(4.84 \pm 0.63)$ ,  $(8.13 \pm 0.35)$ ,  $(5.02 \pm 0.47)$  and  $(6.50 \pm 0.44)$  respectively. One-way ANOVA showed a statistically significant difference between NaDCC, CaOH, N-S and N-Na where ( $p < 0.001$ ,  $p < 0.05$ ). The highest mean value was found in CaOH, followed by N-Na then N-S and finally, the least mean value was found in NaDCC. Tukey post-hoc test revealed that there was a statistically significant difference between CaOH and each of NaDCC, N-S and N-Na where ( $p < 0.001$ ,  $p < 0.05$ ), as well as between N-Na and each of NaDCC and N-S where ( $p < 0.001$ ,  $p < 0.05$ ), while there was no statistically significant difference existed between NaDCC and N-S where ( $p = 0.740$ ,  $p > 0.05$ ).

### 1.3 Two-way ANOVA

According to Two-way ANOVA analysis for the interaction of different variables **Table (2)**. There was statistically significant effect of different treatment protocols on mean PO<sub>4</sub>/amide I ratio where ( $p=0.000$ ,  $p<0.05$ ). While, the observation periods had no statistically significant effect on mean PO<sub>4</sub>/amide I ratio ( $p=0.505$ ,  $p>0.05$ ). The interaction between the two variables (treatment protocol x observation periods) showed no statistically significant effect ( $p=0.358$ ,  $p>0.05$ ).

## 2. Microhardness evaluation

Mean, Standard deviation (SD) values of VHN at different observation periods within each group are summarized in **Table (3)** and presented in **Figure (12, 13)**.

### 2.1 Comparison among observation periods for each treatment protocol

#### A) Group NaDCC:

The mean and SD values of VHN after 1 week was ( $50.70\pm 3.30$ ), which decreases to ( $46.39\pm 4.29$ ) after 4 weeks. Paired t-test showed a statistically significant difference among the two observational periods where ( $p=0.005$ ,  $p < 0.05$ ). The value of VHN was highest after 1 week.

#### B) Group CaOH:

The mean and SD values of VHN after 1 week was ( $50.10 \pm 4.95$ ), which decreases to ( $33.46\pm 5.67$ ) after 4 weeks. Paired t-test showed a statistically significant difference among the two observational periods where ( $p<0.001$ ,  $p < 0.05$ ). The value of VHN was highest after 1 week.

#### C) Group N-S:

The mean and SD values of VHN after 1 week was ( $50.84\pm 5.07$ ), which decreases to ( $50.35\pm 4.69$ ) after 4 weeks. Paired t-test showed no statistically significant difference among the two observational periods where ( $p=0.139$ ,  $p > 0.05$ ). The value of VHN was highest after 1 week.

#### D) Group N-Na:

The mean and SD values of VHN after 1 week was ( $47.79\pm 6.58$ ), which decreases to ( $43.95\pm 6.52$ ) after 4 weeks. Paired t-test showed a statistically significant difference among the two observational periods where ( $p=0.039$ ,  $p > 0.05$ ). The value of VHN was highest after 1 week.

### 2.2 Comparison of different treatment protocols among observation periods

#### A) After 1-week:

The mean and SD values of VHN for NaDCC, CaOH, N-S and N-Na were ( $50.70\pm 3.30$ ), ( $50.10\pm 4.95$ ), ( $50.84\pm 5.07$ ) and ( $47.79\pm 6.58$ ) respectively. One-way ANOVA showed no statistically significant difference between NaDCC, CaOH, N-S and N-Na where ( $p=0.337$ ,  $p>0.05$ ). The highest mean value was found in N-S, followed by NaDCC then CaOH and finally, the least mean value was found in N-Na.

#### B) After 4-weeks:

The mean and SD values of VHN for NaDCC, CaOH, N-S and N-Na were ( $46.39\pm 4.29$ ), ( $33.46\pm 5.67$ ), ( $50.35\pm 4.69$ ) and ( $43.95\pm 6.52$ ) respectively. One-way ANOVA showed a statistically significant difference between NaDCC, CaOH, N-S and N-Na where ( $p<0.001$ ,  $p<0.05$ ). The highest mean value was found in N-S, followed by NaDCC then N-Na and finally, the least mean value was found in CaOH. Tukey post-hoc test revealed that there was a statistically significant difference between CaOH and each of NaDCC, N-S and N-Na ( $p<0.001$ ,  $p<0.05$ ), as well as between N-S and N-Na ( $p=0.010$ ,  $p<0.05$ ), while there was no statistically significant difference existed between NaDCC and each of N-S and N-Na ( $p=0.192$  and  $p=0.601$ ,  $p>0.05$ ) respectively.

### 2.3 Two-way ANOVA

According to Two-way ANOVA analysis for the interaction of different variables **Table (4)**. There was statistically significant effect of different treatment protocols on mean microhardness (VHN) where ( $p=0.000$ ,

$p < 0.05$ ). Also, the observation periods had a statistically significant effect on mean microhardness (VHN) where ( $p = 0.000$ ,  $p < 0.05$ ). The interaction between the two variables (treatment protocol x observation periods) showed a statistically significant effect ( $p = 0.000$ ,  $p < 0.05$ ).

### 3. Correlations between Phosphate/amide I ratio and Microhardness

#### 3.1 After 1-week

Pearson correlation detected a negative relationship between Phosphate/amide I ratio

Microhardness. There is no correlation between negative relationship between Phosphate/amide I ratio and Microhardness ( $p$ -value  $> 0.05$ ) **Table (5)** and **Figure (15)**.

#### 3.2 After 4-weeks

Pearson correlation detected a negative relationship between Phosphate/amide I ratio and Microhardness **Table (6)** and **Figure (16)**.

**Table (1):** The mean, standard deviation (SD) values of phosphate/amide I ratios of different treatment protocols in different observation periods.

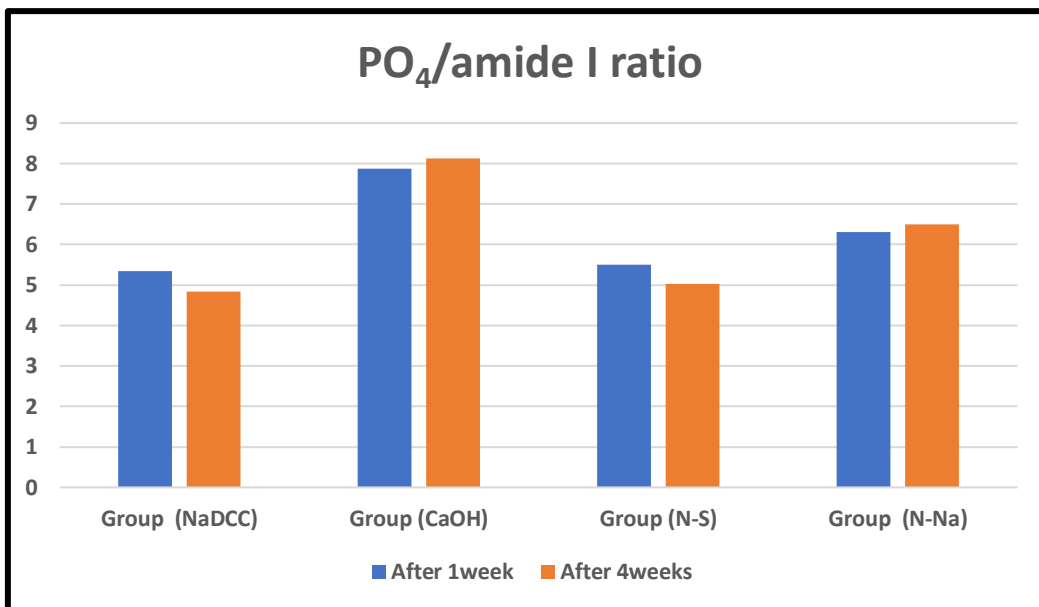
Variables	PO <sub>4</sub> /amide I ratio				p-value
	After 1week		After 4weeks		
	Mean	SD	Mean	SD	
NaDCC	5.34 <sup>ba</sup>	1.15	4.84 <sup>ca</sup>	0.63	0.101ns
CaOH	7.88 <sup>aA</sup>	2.26	8.13 <sup>aA</sup>	0.35	0.693ns
N-S	5.50 <sup>ba</sup>	1.29	5.02 <sup>ca</sup>	0.47	0.283ns
N-Na	6.31 <sup>ba</sup>	0.60	6.50 <sup>ba</sup>	0.44	0.166ns
<b>p-value</b>	<0.001*		<0.001*		

\*; significant ( $p < 0.05$ )

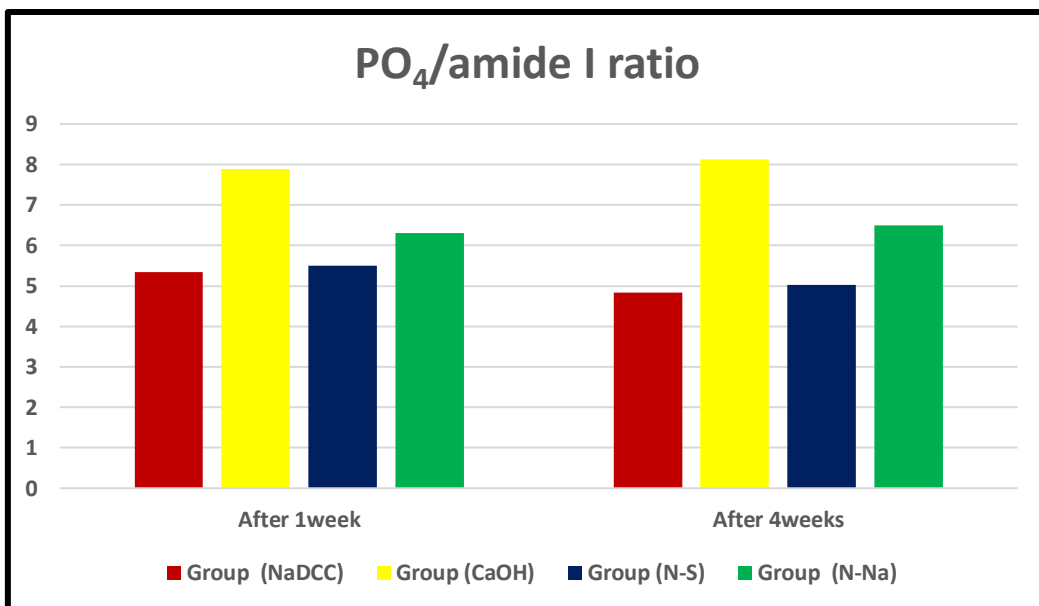
ns; non-significant ( $p > 0.05$ )

Groups identified with different lower-case letters in the same column indicates significant difference and different upper-case letters in the same row indicates significant difference.





**Figure (10):** Bar Chart representing effect of the observation periods on phosphate/amide I ratio within each treatment protocol.



**Figure (11):** Bar Chart representing the effect of different treatment protocols on the phosphate/amide I ratio at each observation periods.

**Table (1):** Results of Two-way ANOVA for the effect of different variables on mean PO<sub>4</sub>/amide I ratio.

Source	Sig. <i>p</i> -value
Groups	0.000
observation periods	0.505
Groups * Periods	0.358

\* Significant at  $p \leq 0.05$

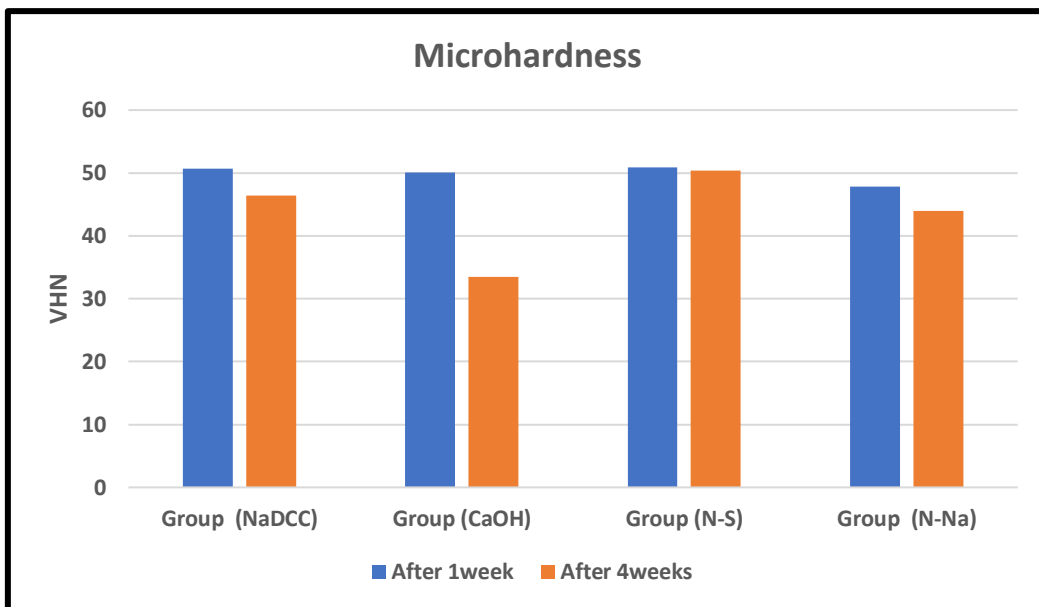
**Table (2):** The mean, standard deviation (SD) values of microhardness (VHN) of different treatment protocols in different observation periods.

Variables	VHN				<i>p</i> -value
	After 1week		After 4weeks		
	Mean	SD	Mean	SD	
NaDCC	50.70 <sup>aA</sup>	3.30	46.39 <sup>abB</sup>	4.29	0.005*
CaOH	50.10 <sup>aA</sup>	4.95	33.46 <sup>cB</sup>	5.67	<0.001*
N-S	50.84 <sup>aA</sup>	5.07	50.35 <sup>aA</sup>	4.69	0.139ns
N-Na	47.79 <sup>aA</sup>	6.58	43.95 <sup>bB</sup>	6.52	0.039*
<i>p</i> -value	0.337ns		<0.001*		

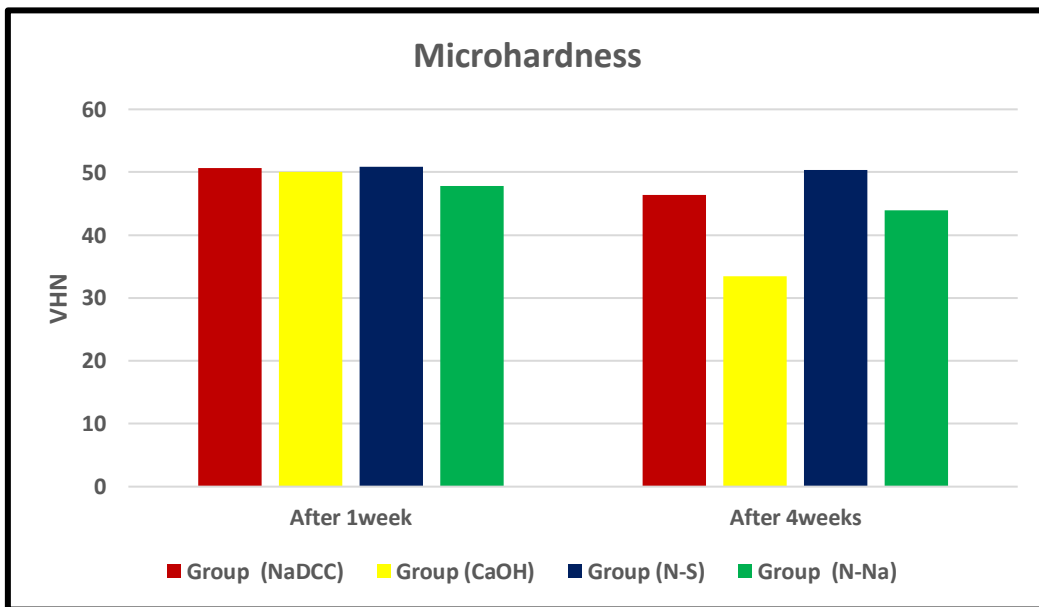
\*: significant ( $p < 0.05$ )

ns: non-significant ( $p > 0.05$ )

Groups identified with different lower-case letters in the same column indicates significant difference and different upper-case letters in the same row indicates significant difference.



**Figure (12):** Bar Chart representing effect of observation periods on microhardness (VHN) within each treatment protocol.

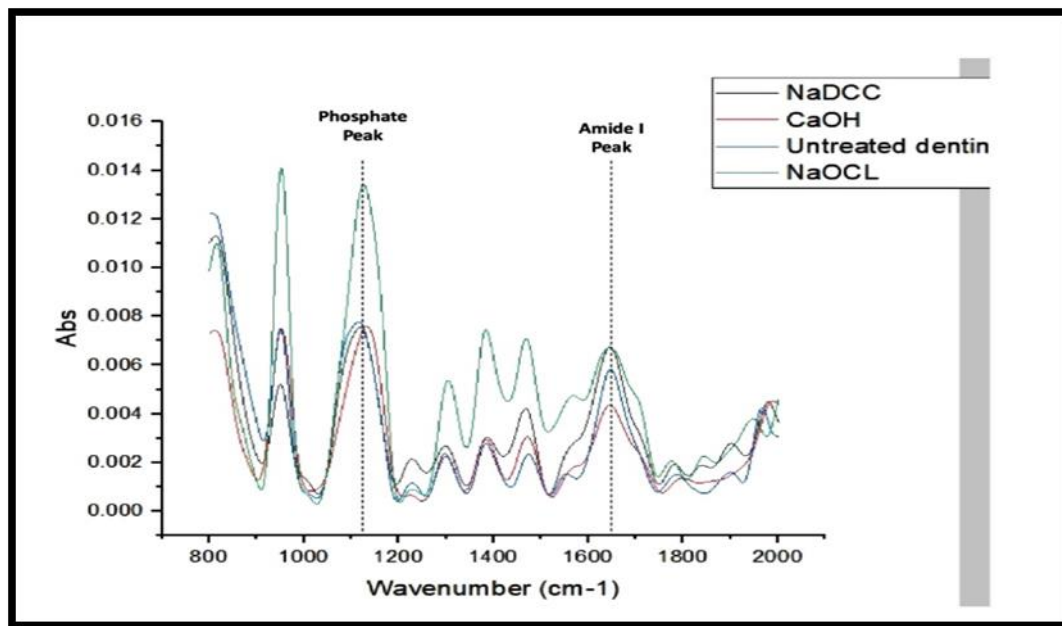


**Figure (13):** Bar Chart representing the effect of different treatment protocols on microhardness (VHN) at each observation periods.

**Table (4):** Results of Two-way ANOVA for the effect of different variables on mean VHN.

Source	Sig. <i>p</i> -value
Groups	0.000
Storage time	0.000
Groups * Time	0.000

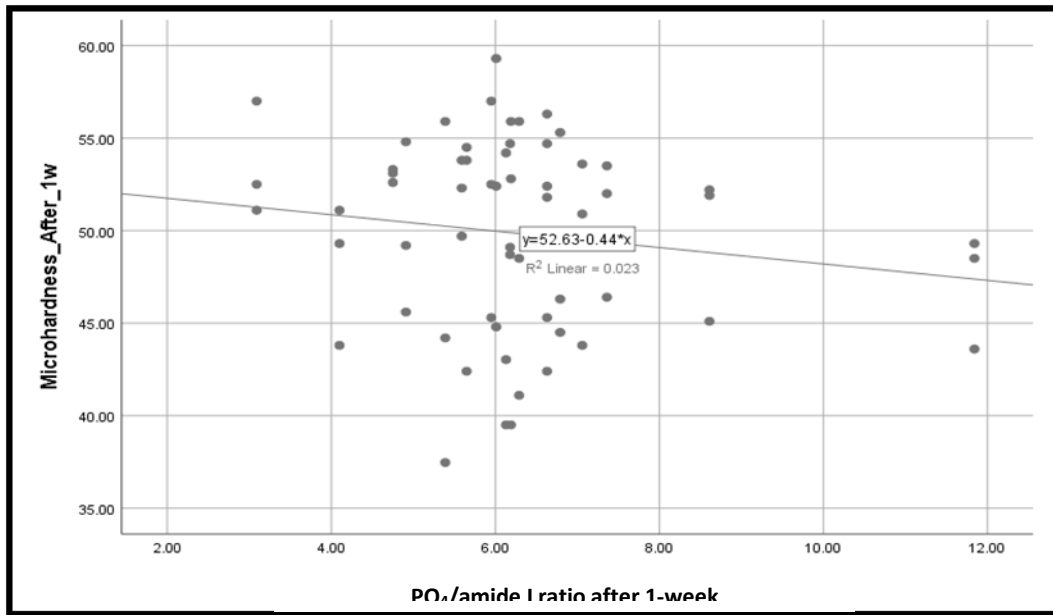
\* Significant at  $p \leq 0.05$



**Figure (14):** Representative FTIR spectra of each group. Group (NaDCC), Group (CaOH), untreated dentin Group and Group (NaOCl) after 4-weeks.

**Table (5):** Pearson correlation between PO<sub>4</sub>/amide I ratio and Microhardness after 1 week.

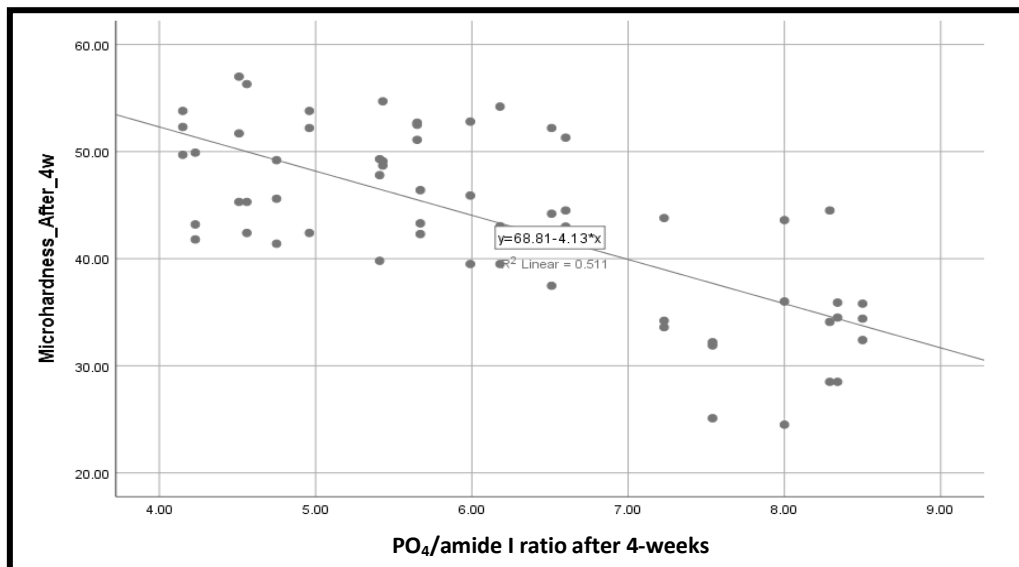
Variables	Pearson correlation	
PO <sub>4</sub> /amide I ratio and Microhardness	Correlation coefficient	-0.151
	Sig. (2-tailed)	0.250



**Figure (15):** Scatter plot representing correlation between Microhardness and phosphate/amide I ratio after 1-week.

**Table (3):** Pearson correlation between PO<sub>4</sub>/amide I ratio and Microhardness and after 4 weeks.

Variables	Pearson correlation	
PO <sub>4</sub> /amide I ratio and Microhardness	Correlation coefficient	-0.715
	Sig. (2-tailed)	<0.001



**Figure (16):** Scatter plot representing correlation between Microhardness and phosphate/amide I ratio after 4-weeks.

#### IV. DISCUSSION

Root canal infections is a polymicrobial environment that contains both aerobic and anaerobic germs (*Vijayaraghavan et al, 2012*), the removal of bacteria as much as possible from root canals during root canal therapy becomes essential and of greatest importance. The complex anatomy of root canals render the ability to reduce bacterial counts through cleaning and shaping only (*Yaduka et al, 2014*), thus the usage of root canal medications is one of the crucial stages for further suppressing the bacteria count (*Yoldas et al, 2004*). A combination of instrumentation, irrigation, and intracanal medications was advocated (*Yaduka et al, 2014 and Bansal et al, 2014*).

Intracanal medication has been advocated for many purposes in infected root canals, including the elimination of any remaining bacteria after canal instrumentation, the reduction of inflammation of periapical tissues and pulp remnants, neutralize tissue debris and help in drying persistently wet canals. Intracanal medication is a part of controlled disinfection of infected root canals along with root canal cleaning and shaping (*Chong et al, 1992*). These medicaments can be applied for different periods varying from 1 week to several months (*Alsubait et al, 2014*). However, the long-term exposure to interappointment medicament has been reported to affect the mechanical properties of root dentin by either collagen degradation in the case of  $\text{Ca(OH)}_2$  or excessive demineralization in the case of antibiotic pastes that might affect the root fracture resistance (*Yassen et al, 2013*).

The chemical integrity of dentin after root canal medicament application was investigated in this study. Dentin is hard and comprising the greatest part of the dental tissue that contains approximately 70% minerals, 20% organic matrix, and 10% fluids by weight (*Xu et al, 2012 and Deng et al, 2013*). The major component of dentin organic matrix is type I collagen (90%) that serves as a scaffold for minerals deposition and packing of non-collagenous matrix and this deposition of minerals into and between collagen fibrils leads to the construction of a hard, resilient and supported matrix (*Beniash et al, 2000*). Within the organic component of dentin, amide is a protein that forms part of collagen and provides dentin with a superior fracture toughness, since

mineral dentin (hydroxyapatite) is a brittle structure. The removal of tough organic phases can have a direct impact in the microhardness and fracture toughness (*Gúzman et al, 2022*).

The mechanical and physical characteristics of radicular dentin may be adversely affected by root canal irrigants and intracanal medications used during endodontic therapy procedures. The microhardness, root resistance to fracture and dentin flexure strength were shown to be dramatically decreased by the use of NaOCl, EDTA, TAP, or  $\text{Ca(OH)}_2$  (*Yassen et al, 2013, Zhang et al, 2010 and Grigoratos et al, 2001*). Alterations in the ratio of the minerals reduce the microhardness and increase the solubility and permeability of radicular dentin (*Zhang et al, 2014*).

For decades,  $\text{Ca(OH)}_2$  has been widely utilized as an intracanal medicament in mature teeth because of its bactericidal activity depending on its long-lasting alkalinity and antibacterial activity related to the hydroxyl ions release into an aqueous environment (*Siren et al, 1997*). However, substitutes to  $\text{Ca(OH)}_2$  should be considered as *C. albicans* and *enterococcus species* have the ability to clinically resist its antibacterial effect (*Sundqvist et al, 2003*) and its prolonged use has been associated to dentin degradation, which could cause tooth fracture.

To ensure the effectiveness of root canal treatment, it was necessary to investigate alternative intracanal medication that maintain the chemical integrity and microhardness of human dentin as well as total or almost complete eradication of bacteria present in the root canals (*Yaduka et al, 2014*). Therefore, the Aim of the present study was to compare the effect of NaDCC and  $\text{Ca(OH)}_2$  as intracanal medicaments on superficial chemical structure and microhardness of radicular dentin.

Chlorine-releasing agents (CRA) are commonly used for the purpose of eradication of bacteria and necrotic tissue from the root canal system and ensure a good outcome of therapy, particularly NaOCl in concentrations ranging from 0.5% to 5.25% (*Heling et al, 1998*). NaOCl is a potent antibacterial and efficiently dissolves pulpal remnants, however it negatively affects the organic dentine

components (Siqueira et al, 1997). Recently, NaDCC is a sodium salt of dichloroisocyanuric acid (pH 6.6) (Naenni et al, 2004 and Sequeira et al, 1999). When compared to other chlorine donors, NaDCC has a greater antibacterial effect, which is explained by the instantly release of "reservoir" chlorine (Coates et al, 1985 and Siquera et al, 2002). It also explains why NaDCC is less inactivated by organic matter (Ingle et al, 2008 and Coates et al, 1985). Additionally, *E. faecalis* was shown to be more easily killed by NaDCC than by NaOCl (Esterla et al, 1999). Also, an in-vitro investigation shown that NaDCC alone can effectively inhibit the growth of *C. albicans* at all concentrations (Kurian et al, 2012).

Medications were applied on each dentin disc for 1 week or 4 weeks, this was in accordance to the guidelines of *American Association of Endodontics (2016)* which states that; the period between the first and second appointments might range from 1 to 4 weeks. Where, some cases were successful after 1 week (Jung et al, 2008), while it was advised that a 2- to 4-week treatment course is adequate to produce the therapeutic impact (Cehreli et al, 2011 and Chen et al, 2011).

To evaluate the samples by FTIR, the samples were thoroughly dried with air spray to reduce the confounding effect of water ( $1640\text{ cm}^{-1}$ ) (Ramirez-Bommer et al, 2018). In addition, it is widely employed in spectroscopic studies of calcified tissues to analyse the mineral content distribution with the band ratio between phosphate ion ( $900\text{--}1200\text{ cm}^{-1}$ ) and collagen amide I ( $1590\text{--}1720\text{ cm}^{-1}$ ) (Gourion-Arsiquaud et al., 2008) which was used in this study.

In the present study, results showed that the  $\text{PO}_4/\text{amide I}$  ratio was higher in the  $\text{Ca}(\text{OH})_2$  and NaOCl groups when compared with the untreated control group after 1 and 4 weeks of observation. The higher  $\text{PO}_4/\text{amide I}$  ratio after the 2.25% NaOCl treatment of the dentin discs may be attributed to that the organic phase of mineralized dentin superficial surface can be removed by NaOCl (Haapasalo et al, 2010 and Marending et al, 2007). The results confirm previous research findings that the usage of NaOCl irrigant resulted in increased minerals and degradation of collagen of dentinal subsurface (Di Renzo et al, 2001, Dogan & Calt et al, 2001 and Driscoll et al,

2002) that is expected to be more brittle than untreated mineralized dentin (Marending et al, 2007). Also, results were in consistent with Mountouris et al, 2004 that found that NaOCl treatment reduced organic matrix (amide I, II, III peaks), but did not affect carbonates and phosphates.

Yassen et al, 2013 reported that the samples treated with  $\text{Ca}(\text{OH})_2$  had higher  $\text{PO}_4/\text{amide I}$  ratios than the untreated control group, which was in consistent with the findings of this study. This could be explained by the highly alkaline characteristics of  $\text{Ca}(\text{OH})_2$ , which have a significant denaturing impact on the organic matrix of dentin that caused by breakdown of protein structure (white et al, 2002). Furthermore, Yassen et al, 2013 found that when compared to a 1-week application, a 4-week treatment with  $\text{Ca}(\text{OH})_2$  paste medication considerably increased the  $\text{PO}_4/\text{amide I}$  ratio resulting in superficial collagen degradation. Those results agreed with the present study in which an increase in the  $\text{PO}_4/\text{amide I}$  ratio was observed over time after treatment with  $\text{Ca}(\text{OH})_2$ .

Also, Verma et al, 2020 evaluated the effect of  $\text{Ca}(\text{OH})_2$  on chemical arrangement of radicular dentin after 1 and 4 weeks and showed an increase in  $\text{PO}_4/\text{amide I}$  ratio and no sign of demineralization at all observation periods which was in line with the results of the present study that justify the previous in vitro study by Leiendecker et al, 2012 who reported that  $\text{Ca}(\text{OH})_2$  has a higher phosphate amide ratio corresponding to a greater dentine collagen deproteinization and less demineralization.

On the contrary of the present study results, Yassen et al, 2015 examined the impact of  $\text{Ca}(\text{OH})_2$  on the chemical structure of radicular dentine after 4 weeks and found that the ratio of  $\text{PO}_4/\text{amide I}$  was reduced. This could be attributed to a different irrigation protocol, in which they used 20 ml of 17% EDTA for 10 minutes after a month where prolonged EDTA irrigation for 10 minutes has been attributed to significant dentine demineralization (Ozdemir et al 2012 and Zehnder et al, 2006). While, in the present study the samples were irrigated with 17% EDTA for only 1 minute before application of  $\text{Ca}(\text{OH})_2$  and after each observation period which didn't affect the mineralized dentin.

On the other hand, it was observed in this study that PO<sub>4</sub>/amide I ratio of dentin treated with NaDCC was similar to that of the untreated control group at 1-week and 4-week observation periods which could be assigned to the release of FAC into water in the form of HOCl which is a weak acid (pH 4-6) therefore, NaDCC is unlikely to adversely affect the organic matrix of the dentin (*Rossi-Fedele et al, 2011*). However, the results of this study showed that dentine discs treated with NaDCC showed a slight reduction in PO<sub>4</sub>/amide I ratio between 1-week and 4-week time periods owing to its acidic nature that has a demineralization effect on dentine (*Yassen et al, 2013*).

Regarding the microhardness, when compared to untreated control dentin, all experimental groups resulted in a reduction in microhardness at all observation periods. The most significant effect was seen after the irrigation with 2.5% NaOCl after 1 week observation period. *Baumgartner et al, 1992* suggested that the use of NaOCl alone as an irrigant may expose the inorganic material that prevents further dissolution of the dentin or it may dissolve the organic components so, the apatite-rich, collagen-sparse dentin matrix that remained after NaOCl treatment is more brittle which in turn reduced the mechanical properties of dentin, such as microhardness (*Goldberg et al, 2004, Oliveira et al, 2007, Garcia et al, 2013 and Aslantas et al, 2014*).

Additionally, the combination of chelating agents with NaOCl is known to reduce the microhardness of both the coronal and root dentin (*Dotto et al, 2020; Yassen et al, 2015*). NaOCl degrades organic tissue during endodontic therapy while EDTA eliminates the inorganic part (*Prada et al, 2019; Chandler et al, 2019*). The use of an irrigation protocol with NaOCl and EDTA alternatively is also recommended to eliminate Ca(OH)<sub>2</sub> residues and establish a conditioned root canal surface, particularly after one week, resulted in a reduction in microhardness. The results were in agreement with *Yassen et al, 2013 and Ari et al, 2004* that reported both NaOCl and EDTA irrigants had a detrimental effect on dentin microhardness. Also, *Yassen GH et al, 2014* reported that dentin treated with NaOCl + EDTA prior to Ca(OH)<sub>2</sub> application caused reduction in microhardness ranging from 20% to 27% compared to untreated dentin that

supported this study results and could be explained by the denaturation effect of NaOCl and the negative effect of both irrigants on dentin microhardness (*Zhang et al, 2010*).

After one week, there was no difference between the Ca(OH)<sub>2</sub> group and the control group, however after four weeks, there was a reduction in dentin microhardness. This was in agreement with *Yilmaz et al, 2016* where this revealed that the prolonged exposure to the highly alkaline nature of Ca(OH)<sub>2</sub> may cause denaturation of the phosphate and carboxylate groups of the dentin resulting in dentine structure collapse and reduction in microhardness (*Rosenberg et al, 2007*). Another explanation for this reduction in microhardness of the dentin can be the penetration of Ca(OH)<sub>2</sub> molecules into the intrafibrillar structure of mineralized collagen fibrils due to their minute molecular size, which cause changes in the 3-D conformation of tropocollagen (*Leiendecker et al, 2012 and Yassen et al, 2013*).

Also, *Naseri et al, 2019* assessed the impact of Ca(OH)<sub>2</sub> as an intracanal medication on radicular dentin microhardness in which the Ca(OH)<sub>2</sub> group resulted in higher dentin microhardness after 1 week while there was a reduction after 4 weeks and this also supports the results of our study and might be attributed to the residues of NaOCl in the dentinal tubules that could react with Ca(OH)<sub>2</sub> forming Calcium chloride salt which crystallizes upon drying and expands inside the dentinal tubules leading to an increase in brittleness and reduction in microhardness of dentin (*Pu et al, 2011*).

On the contrary, *Yoldas et al, 2004* assessed dentin microhardness after 1 week after Ca(OH)<sub>2</sub> application compared to the untreated group and concluded that the use of Ca(OH)<sub>2</sub> causes reduction in dentin microhardness. This difference could be attributed to the medicament preparation where they combined Ca(OH)<sub>2</sub> with glycerine. *Alacam et al, 1998, and Seyed et al, 2009* suggested that when glycerin is used as the vehicle, the reduction of dentin microhardness associated with Ca(OH)<sub>2</sub> can be increased.

On the other hand, this study results showed no reduction in microhardness of dentin treated with NaDCC compared to untreated control dentin among the observation periods



and this might be attributed to that NaDCC (pH 6.6) is a weak acidic material that releases FAC into water in the form of HOCl that has similar chemical structure to that of water and electrically neutral so, it may not show any detrimental effect on dentin microhardness (Coates et al, 1985). However, there is insufficient evidence in literature about the effect of NaDCC on microhardness and superficial chemical structure of radicular dentin.

Correlation study, revealed negative correlation between  $PO_4$ /amide I ratios and microhardness values of dentin where higher  $PO_4$ /amide I ratios correspond to reduction in microhardness. This denotes that the reported reduction in microhardness of radicular dentin among all treatment groups could be explained by the superficial collagen denaturation effect (Higher  $PO_4$ /amide I ratios) that occurred after the routine endodontic procedural protocols. These results are in agreement with the studies by Yassen et al, 2015 and Naseri et al, 2019 which also suggested that radicular dentin microhardness depends on composition and dentin surface structure.

Collectively, the present study showed that  $Ca(OH)_2$  had collagen denaturing effect (High  $PO_4$ /amide I ratio) on the chemical structure of radicular dentin at different time periods which can be correlated to the effect of the routinely used intracanal medicament on the dentin microhardness. On the other hand, NaDCC showed no effect on both superficial chemical structure of radicular dentin ( $PO_4$ /amide I ratio similar to that of untreated dentin) and microhardness in comparison to  $Ca(OH)_2$ . However, additional mechanical and chemical structure tests should be conducted in further studies to confirm the results of this study.

## V. CONCLUSIONS

Within the limitations of this in-vitro study, it could be concluded that the use of  $Ca(OH)_2$  as an intracanal medicament showed detrimental effect on microhardness and chemical structure

of radicular dentin after 4 weeks while, the least changes in microhardness and chemical structure of treated dentin were observed in the NaDCC group, which suggests a possible replacement of NaDCC as an intracanal medicament for the preservation of intact dentin structure.

## VI. RECOMMENDATIONS

Under the condition of the current study, we can recommend that:

- NaDCC could be an alternative to  $Ca(OH)_2$  as it has the least destructive effect on the microhardness and superficial chemical structure of radicular dentin.
- Further studies need to be undertaken to establish the possible application of NaDCC in clinical endodontic therapy.
- Further researches are needed to investigate the systemic effect, biocompatibility, allergic potential and relieving of pain of NaDCC.
- Further studies are needed to evaluate the effectiveness of NaDCC as an intracanal medicament on multi-rooted teeth with curvatures.
- Further researches evaluating the whole chemical change across the total thickness of the dentin specimens rather than the superficial dentin after various treatment protocols are needed.
- In the future, in vivo studies with large number of sample size should be conducted to further validate the results.

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