Journal of Applied Dental and Medical Sciences
NLM ID: 101671413 ISSN:2454-2288
Volume 7 Issue 4 Oct-Dec 2021
https://www.ncbi.nlm.nih.gov/nlmcatalog/101671413
www.joadms.org

Original Research

To compare the influence of different irrigation solutions on calcium ion loss and microhardness of root dentin- An in vitro study

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ARTICLE INFO

Keywords: calcium ion loss, spectrometer, Vickers microhardness test

ABSTRACT

Aim: To compare and evaluate the calcium ion loss and microhardness of root dentin using sodium hypochlorite, peracetic acid, and chitosan and apple cider vinegar.

Methods: GI-sodium hypochlorite 5%,GII- peracetic acid 1%, GIII- chitosan 2% and GIV-apple cider vinegar. Fourty single rooted freshly extracted premolars were decoronated at the cemento-enamel junction and at apical end of the root using a diamond disc to obtain 10mm length which was standardized for all the specimens in different groups. The pulp extirpation and root canal enlarged using K files up to ISO # 25. Then sectioned longitudinally into two halves making a total of 40 samples and other half was discarded. Then using magnetic stirrer test solutions were prepared for each group and then spectrometric analysis and microhardness test were done. Data collected was analyzed using SPSS software. Level of significance was fixed at p= 0.05

Result: Mean calcium ion loss was highest for Group III followed by Group IV, Group II and Group I. Mean microhardness was lowest for Group III followed by Group IV, Group II and Group I. Group III had the highest amount of calcium ion loss and lowest microhardness, which was significantly different from Group I, II and IV.

Conclusion: Chitosan 2% resulted in maximum sequestration of calcium ions present amongst the chelating solutions, followed by apple cider vinegar, peracetic acid 1% and sodium hypochlorite. The highest microhardness of root dentin was seen with sodium hypochlorite and peracetic acid.

Introduction

Root canal irrigation is one of the important phases in root canal treatment. The function of root canal irrigation is to remove the smear layers and the remnants of necrotic tissues, as well as to eliminate microorganisms and their products from root canal.[1] **Sodium hypochlorite (NaOCl)**, with a concentration of 2.5-5%, has been frequently employed in the clinic as an irrigation solution. However, the drawback of NaOCl as an irrigation solution is unable to remove inorganic tissues from the smear layers on the root canal walls. Therefore another irrigation solution needs to be employed as the final irrigation to eliminate inorganic tissues of smear layers. Ethylenediaminetetraacetic acid (EDTA) is often utilized in the clinic as the final irrigation solution because of its ability to react with calcium ions in dentin forming calcium chelation, thereby dissolving the inorganic tissues from smear layers. However, prolonged exposure of EDTA may alter the structural characteristic of dentin resulting in compromised mechanical integrity and erosion. Due to the drawbacks of EDTA as a final irrigation solution, hence, other final irrigation solutions need to be studied.[2]

Alternative irrigants, among them **peracetic acid (PAA)**, has been researched with the purpose of improving the cleaning and disinfection of the root canal system. A recent study showed that when used as a single endodontic irrigant, PAA 1% shows an antibacterial efficacy similar to that of 2.5% NaOCl and 2% chlorhexidine against Enterococcus faecalis. Another study showed that 4% PAA kills and dissolves significantly mixed biofilms in a manner similar to that of 2.5% and 5.25% NaOCl. In addition to its antibacterial effectiveness, PAA has the capacity to remove the smear layer when used as a final rinse after the use of NaOCl.[3]

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Chitosan may also use as a irrigant solution. **Alkaline deacetylation of insoluble chitin leads to formation of chitosan.** It is more useful than chitin as it possesses properties such as increased and enhanced biocompatibility and Biodegradability and is a safe antimicrobial agent.[4]

Vinegar is a natural food preservative that has been used for thousands of years, and it is of interest to the field of medicine due to its beneficial health effects. Since vinegar has antibiotic and antiseptic properties, it has been used for the treatment of infected wounds. Moreover, because it is bactericidal, vinegar could be an alternative root canal irrigant. **Apple cider vinegar**, which contains acetic and maleic acid, has been found to reduce approximately 30% of the Enterococcus faecalis cell population.

It is important to assess the effects of irrigation solutions on the dentin surface because they stay in contact with the dentin during irrigation. Irrigants may alter the physical and chemical structure of dentin, and they may inhibit interactions with obturation materials and coronal restorations. The microhardness and calcium ions, as physical indicators of dentin, are sensitive to the composition and surface changes of the tooth structures, which could be influenced by irrigation solutions.Any change in the calcium ion ratio may change the original proportion of the organic and inorganic components.[5]

Therefore, the present study has been undertaken to evaluate the effectiveness of removal of calcium ion loss and its effect on the microhardness of the root dentin after using different irrigants during endodontic procedure.

Materials And Method

Fourty single rooted freshly extracted premolars for Orthodontic purpose were used for the study. All teeth were stored in normal saline containing 0.1% thymol upto the period of the study. The soft tissue covering the root surface was removed using gauze piece and a fine brush. Later, the teeth were decoronated at the cemento-enamel junction and at apical end of the root using a diamond disc to obtain 10mm length which was standardized for all the specimens in different groups.

The pulp extirpation was done and root canal was enlarged using K files up to ISO # 25 with intermittent use of distilled water for irrigation to remove the debris and each tooth was sectioned longitudinally into two halves using a low-speed diamond disc making a total of 40 samples and other half was discarded. It exposed the root dentin surfaces for evaluation.

The samples were then divided into 4 groups randomly according to irrigation solution to be used. (n=10)

Group I: Sodium Hypochlorite(5%)

Group II:Peracetic Acid (1%)

Group III:Chitosan (2%)

Group IV:Apple Cider Vinegar

The study has 2 parts calcium ion loss and microhardness of root dentin

Calcium ion loss:

In each group, the specimen was individually immersed in a **magnetic stirrer bath** containing 10ml of test solution for 2 minutes. Accordingly, 1ml of solution was aspirated using pipette from bath after 2 minutes and collected in eppendorf tube. The Calcium ion level of each group was determined by ICP-Optic Emission spectrometer analysis. The readings were expressed in parts per million (ppm).(fig no.1)

Root dentin microhardness:

The longitudinally sectioned teeth which were previously used for preparation of test solution were collected in petri dish and then mounted horizontally on an acrylic block and smoothened with varying grits of silicon carbide papers and finely finished with a composite finishing kit from coarse to fine.[6]

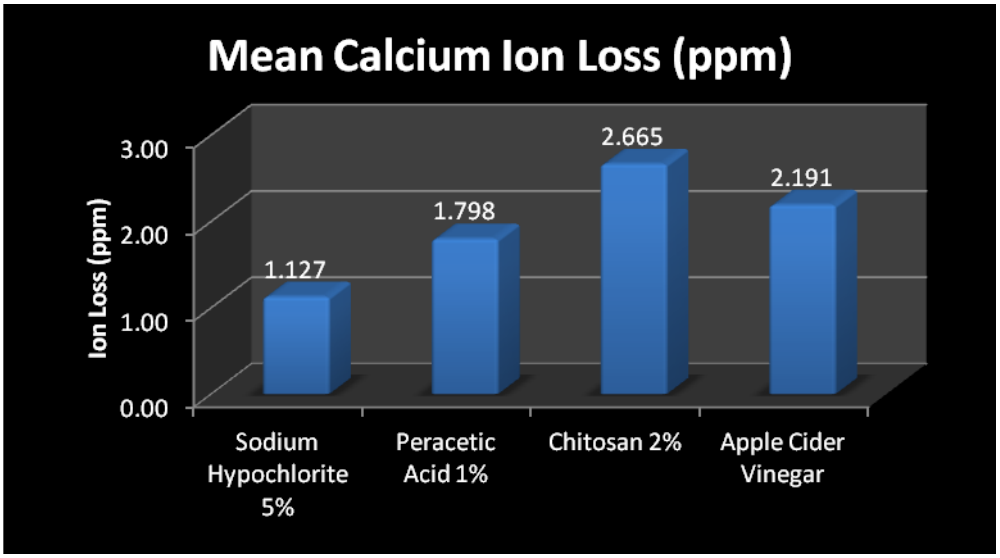
They were then polished with a polishing paste to give them a uniform and polished surface for the microhardness test. The specimens were then used for the Vickers microhardness test. Hardness was measured in dentin areas on the middle third of the root canal of each specimen for each sample, three indentations were made, and hardness value was obtained by calculating the average of three indentation values. Hardness was measured under the load of 300 g with duration of 15 sec.[7](fig.no.2)

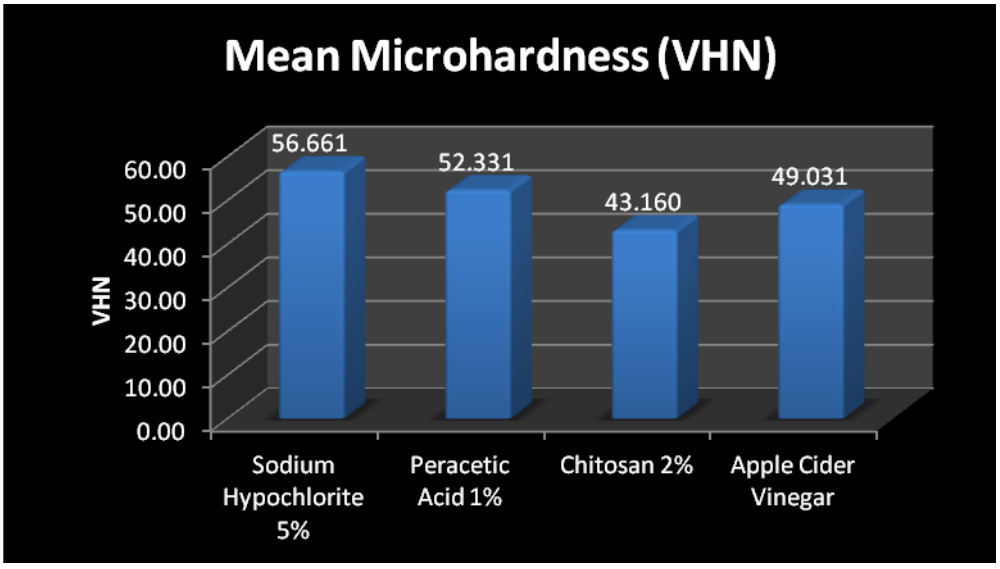
Group	*Mean*	*Std. Deviation*	*Minimum*	*Maximum*
Sodium Hypochlorite 5%	*1.1265*	*.36529*	*.71*	*1.93*
Peracetic Acid 1%	*1.7980*	*.33297*	*1.02*	*2.39*
Chitosan 2%	*2.6645*	*.32513*	*2.09*	*3.22*
Apple cider vinegar	*2.1905*	*.25136*	*1.87*	*2.89*

Table 1: Mean Calcium Ion Loss (ppm)

Group	*Mean*	*Std. Deviation*	*Minimum*	*Maximum*
Sodium Hypochlorite 5%	*56.6605*	*2.11974*	*52.39*	*59.43*
Peracetic Acid1%	*52.3310*	*3.00940*	*47.44*	*57.39*
Chitosan 2%	*43.1595*	*2.48182*	*39.87*	*47.02*
Apple Cider Vinegar	*49.0310*	*2.23394*	*45.03*	*53.56*

Table 2: Vickers microhardness


Mean Calcium Ion Loss (ppm)
Ion Loss (ppm)
3.00
2.00
1.00
0.00
1.127
1.798
2.665
2.191
Sodium Hypochlorite 5%
Peracetic Acid 1%
Chitosan 2%
Apple Cider Vinegar


Mean Microhardness (VHN)
VHN
60.00
50.00
40.00
30.00
20.00
10.00
0.00
56.661
52.331
43.160
49.031
Sodium Hypochlorite 5%
Peracetic Acid 1%
Chitosan 2%
Apple Cider Vinegar

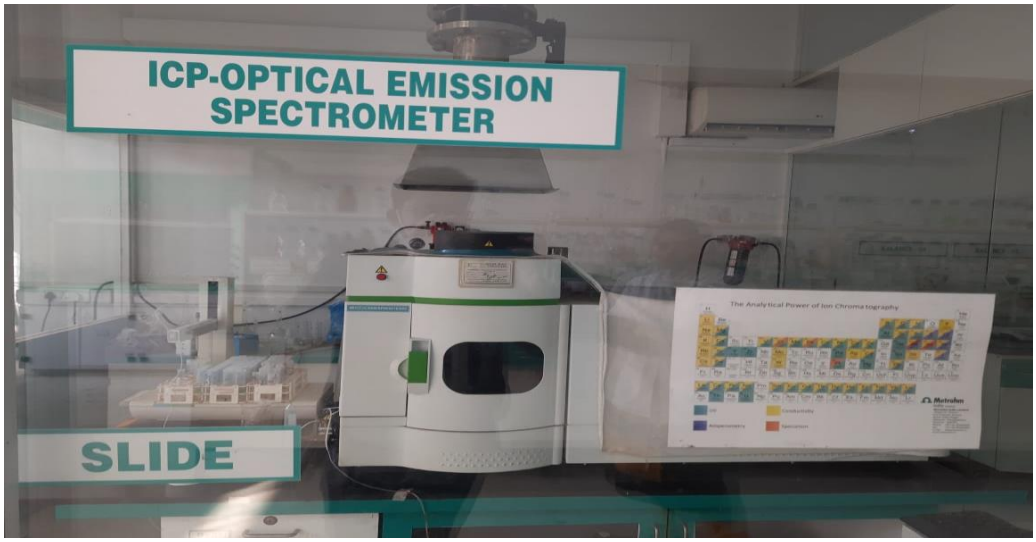


Fig no.1 ICP-OES

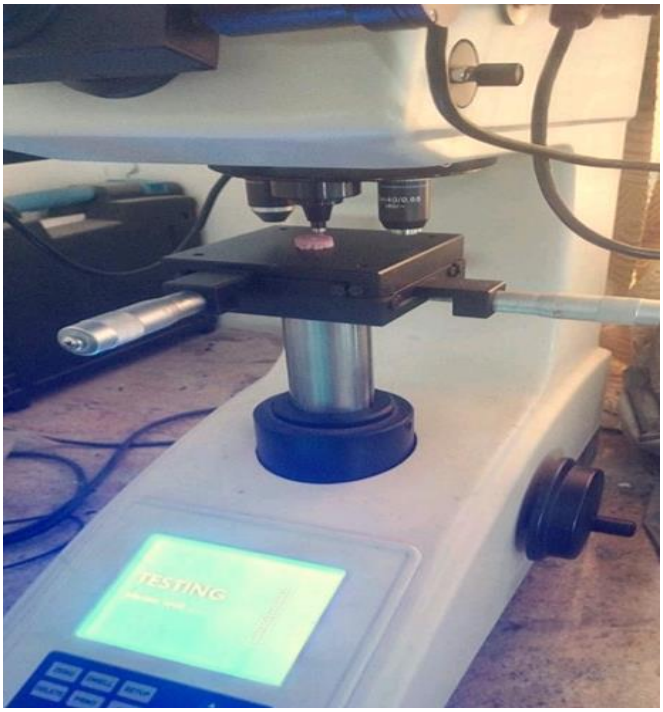


Fig no.2 Vickers microhardness test

Results

This in vitro study was divided into two parts: First part was to evaluate the calcium ions loss from root dentin and the second part was to evaluate the microhardness of root dentin after agitation in magnetic stirrer bath with irrigant solutions. The specimens to be evaluated were broadly divided into 4 group according to type of irrigation solution used.

Mean calcium ion loss was highest for Group III (2.6645 ± 0.32513 SD) followed by Group IV (2.1905 ± 0.25136 SD), Group II (1.7980 ± 0.33297 SD) and Group I (1.1265 ± 0.36529 SD). Statistical analysis indicated that Group III had the highest amount of calcium ion loss, which was significantly different from Group I, II and IV. (table no.1)

Mean microhardness was lowest for Group III (43.1595 ± 2.48182 SD) followed by Group IV (49.0310 ± 2.23394 SD), Group II (52.3310 ± 3.00940 SD) and Group I (56.6605 ± 2.11974 SD). Statistical analysis indicated that Group III had the lowest microhardness, which was significantly different from Group I, II and IV.(table no.2)

To observe statistical analysis of data among all groups, One Way ANOVA test was applied and it was statistically significant ($p \leq 0.05$)**.** The mean difference is significant at the 0.05 level.

Intergroup comparison of the calcium ion loss values among the different groups was done with Tukey's Post Hoc test. There was statistically significant difference between the groups ($p \leq 0.05$). The mean difference is significant at the 0.05 level.

Discussion

Cleaning and shaping of the root canal system are considered the key requirements for success in root canal treatment. Irrigation of root canal system provides gross debridement, lubrication, destruction of microbes, dissolution of tissues and help in cleaning areas that are inaccessible for mechanical cleansing. During irrigation, radicular and coronal dentin is exposed to irrigating solution deposited in the pulp chamber, which may cause alteration on dentin and effect their interaction with material used for obturation.[8] Dentin is composed of the various inorganic component of dental hard tissue, present as hydroxyapatite crystals in the form of calcium and phosphorous. In hydroxyapatite crystals Ca/P ratio has been established at 1.67 approximately.[9]

This study was conducted to test the calcium uptake by 5% sodium hypochlorite (Group I), 1% peracetic acid (Group II), 2%chitosan (Group III), apple cider vinegar (Group IV) sequestrated from the root dentine, and its effect on dentin microhardness.

In first part of the study calcium ion loss from specimen for each group of irrigating solution was calculated. The Calcium ion level of each group was determined by ICP-Optic emission spectrometer. Different methods such as atomic absorption spectrometry, Flame photometry, Energy dispersive spectrometer, Fourier Transform Infrared (FTIR) or inductively coupled Plasma optic emission spectroscopy (ICP-OES) are used to evaluate the demineralization effect of different chemicals, provided that calibration is accomplished precisely. ICP- OES (inductively coupled plasma – optic emission spectrometry) technique is one of the most attractive detection systems for determination of trace elements in dentin.[10,11] In the second part of the study, the longitudinal sections of the samples were subjected to Vickers microhardness test.

According to study performed by **Maria del Pilar Gutiérrez-Salaza et al(2003)** Vickers microhardness values were in the range from 270 to 360VHN for enamel and from 50 to 60 VHN for dentin.[12]

This study suggested inverse proportional relation between calcium ion loss and microhardness value i.e as amount of calcium ion loss increase, the microhardness decreases.

1. Peracetic acid and sodium hypochlorite had minimum ion loss, so there microhardness values were greater than apple cider vinegar and chitosan.

2. Whereas, chitosan and apple cider vinegar had maximum ion loss, so there microhardness values were less than sodium hypochlorite and peracetic acid.

This study shows accordance with previous studies

Katia C. Keine et al 2019, assess the effects of 1% peracetic acid (PAA) as a single endodontic irrigant on

microhardness, roughness, and erosion of root canal dentin, compared with 2.5% sodium hypochlorite (NaOCl) and with 2.5% NaOCl combined with 17% EDTA and normal saline and concluded that both groups showed higher microhardness reduction than NaOCl and normal saline groups.[13]

Suparna Ganguly Sah et al 2017, evaluated the effect of various endodontic irrigants on the micro-hardness of the root canal dentin using 3% Sodium Hypochlorite (3% NaOCl), 17% Ethylene Dioxide Tetra Acetic Acid (17% EDTA), 0.2% Chitosan and 6% Morindacitrifolia Juice (MCJ) for 15 minutes each. They concluded that 17% EDTA and 0.2% Chitosan, significantly decreased the microhardness of root dentin whereas 6% MCJ and 3% NaOCl had no significant effect on the microhardness before and after immersing in the irrigants.[14]

Ju´lio Ce´sar Emboava Spano et al 2009, evaluate the concentration of calcium ions and smear layer removal by using root canal chelators like 15% ethylenediaminetetraacetic acid (EDTA), 10% citric acid, 10% sodium citrate, apple vinegar, 5% acetic acid, 5% malic acid, and sodium hypochlorite and concluded that 15% EDTA resulted in the greatest concentration of calcium ions followed by 10% citric acid, apple vinegar, 5% acetic acid, 5% malic acid, and 10% sodium citrate; 15% EDTA and 10% citric acid were the most efficient solutions for removal of smear layer with minimum calcium ion loss with sodium hypochlorite.[15]

Anika Mittal, Shifali Dadu et.al 2018, assessed the effectiveness of smear layer removal from the root canal wall using various final irrigating solutions like 0.2% chitosan, apple cider vinegar, and 15% ethylenediaminetetraacetic acid (EDTA), and concluded that the highest calcium ion concentrations was observed with apple vinegar followed by chitosan and EDTA.[16]

Result of our study are in contradiction with study of **O.I.Ulusoy et al 2020**, evaluate the nanohardness reduction and erosion in root canal dentine after application of 17% ethylenediaminetetraacetic acid (EDTA), 9% etidronic acid (HEBP), and 2% peracetic acid (PAA) either alone or combined with sodium hypochlorite (NaOCl) and concluded that Application of 2% PAA and 17% EDTA, either alone or coupled with NaOCl did not cause any erosive changes or severe alterations in the nanohardness of root canal dentine. Also reported Peracetic acid to exhibit a smear layer removal capacity comparable to EDTA when it was used as a final rinse in the root canals.[17,18,19]

The use of chelating agents will lead to increased Ca++ ions removal, greater depth of demineralisation zone, more dentin surface roughness with decreased dentin microhardness, larger erosive effects. Stronger the chelating solution, greater the decrease in dentin microhardness and higher the erosion of peritubular and intertubular dentin.[20]

Shortcomings of this study could be that, the activation of irrigants in this study was done during the agitation of specimen in magnetic stirrer bath, which would affect the calcium ion removal and microhardness levels Compared with routine irrigation during root canal treatment.

In this study, all the parameters were standardised but a clinical scenario will affect the calcium ions sequestration and the microhardness of the root canal wall dentin.

Conclusion

This study evaluated the effect of different final irrigating solutions on microhardness and calcium ions eluted from root dentin, in- vitro, with the help of Vickers Microhardness Test and ICP-OES, respectively.

Under the experimental conditions and within the limitations of this study, the following can be concluded,

1. Chitosan 2% resulted in maximum sequestration of calcium ions present amongst the chelating solutions, followed by apple cider vinegar, peracetic acid 1% and sodium hypochlorite.

2. The highest microhardness of root dentin was seen with sodium hypochlorite and peracetic acid.

A positive correlation was found between the removal of calcium ions from root dentin by the different solutions and the subsequent decrease in microhardness of root dentin at all levels.

It is important to emphasize that the results of this in vitro study cannot be directly extrapolated to all clinical conditions where the volume, exposure time and fluid dynamics of the irrigating solutions in the root canal may result in a different outcome. Also the concentration of different irrigants may result in different findings. So further

studies are required for these irrigants to emphasize the outcome with different conditions.

References

1.Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dental Clinics. 2010 Apr 1;54(2):291-312.

2.Ratih DN, Enggardipta RA, Kartikaningtyas AT. The effect of chitosan nanoparticle as a final irrigation solution on the smear layer removal, micro-hardness and surface roughness of root canal dentin. The Open Dentistry Journal. 2020 Feb 14;14(1).

3.Keine KC, Kuga MC, Tormin FB, Venção AC, Duarte MA, Chávez-Andrade GM, Faria G. Effect of peracetic acid used as single irrigant on the smear layer, adhesion, and penetrability of AH Plus. Brazilian oral research. 2019 Jul 29;33.

4.Singh G, Jamwal U. Chitosan hydrogel: Its applications in medicine and dentistry. International Journal of Preventive and Clinical Dental Research. 2018 Oct 1;5(4):71.

5.Akbulut MB, Guneser MB, Eldeniz AU. Effects of fruit vinegars on root dentin microhardness and roughness. Journal of conservative dentistry: JCD. 2019 Jan;22(1):97.

6.Baldasso FE, Roleto L, Silva VD, Morgental RD, Kopper PM. Effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. Brazilian oral research. 2017 May 15;31.

7.Taneja S, Kumari M, Anand S. Effect of QMix, peracetic acid and ethylenediaminetetraacetic acid on calcium loss and microhardness of root dentine. Journal of conservative dentistry: JCD. 2014 Mar;17(2):155.

8.Mishra L, Kumar M, Rao CS. Calcium loss from root canal dentin following EDTA and Tetracycline HCl Treatment with or without subsequent NaOCl irrigation and evaluation of microhardness of dentine. Intern JAdvancem in Rese and Tech. 2012 Jul;1(2):45-50.

9.Jain A, Waghmare P, Gandi P, Nigam N. Comparative Evaluation of Calcium Ion Loss and Microhardness using Different Irrigants -An In Vitro Study. Int J Oral Health Med Res 2016;3(2):13-16.

10.Thangaraj D, Ballal VA, Acharya S. Determination of calcium loss and its effect on microhardness of root canal dentin following treatment with 17% ethylenediaminetetraacetic acid solution at different time intervals-An in vitro study. Endodontology. 2009;21(1):7-13.

11.Ari H, Erdemir A. Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique. Journal of Endodontics. 2005 Mar 1;31(3):187-9.

12.Gutiérrez-Salazar MD, Reyes-Gasga J. Microhardness and chemical composition of human tooth. Materials Research. 2003 Jun;6(3):367-73.

13.Keine KC, Kuga MC, Coaguila-Llerena H, Palma-Dibb RG, Faria G. Peracetic acid as a single endodontic irrigant: effects on microhardness, roughness and erosion of root canal dentin. Microscopy Research and Technique. 2020 Apr;83(4):375-80.

14.Saha SG, Sharma V, Bharadwaj A, Shrivastava P, Saha MK, Dubey S, Kala S, Gupta S. Effectiveness of various endodontic irrigants on the micro-hardness of the root canal dentin: An in vitro study. Journal of clinical and diagnostic research: JCDR. 2017 Apr;11(4):ZC01.

15.Spanó JC, Silva RG, Guedes DF, Sousa-Neto MD, Estrela C, Pécora JD. Atomic absorption spectrometry and scanning electron microscopy evaluation of concentration of calcium ions and smear layer removal with root canal chelators. Journal of endodontics. 2009 May 1;35(5):727-30.

16.Mittal A, Dadu S, Yendrembam B, Abraham A, Singh NS, Garg P. Comparison of new irrigating solutions on smear layer removal and calcium ions chelation from the root canal: An in vitro study. Endodontology. 2018 Jan 1;30(1):55.

17.Ulusoy ÖI, Mantı AŞ, Çelik B. Nanohardness reduction and root dentine erosion after final irrigation with ethylenediaminetetraacetic, etidronic and peracetic acids. International Endodontic Journal. 2020 Nov;53(11):1549-58.

18. Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. International endodontic journal. 2009 Apr;42(4):335-43.

19. De-Deus G, Souza EM, Marins JR, Reis C, Paciornik S, Zehnder M. Smear layer dissolution by peracetic acid of low concentration. International endodontic journal. 2011 Jun;44(6):485-90.

20. Bedir SS, Mossa H, Hassan AM. Etidronate as A Weak Chelating Agent on Root Canal Dentin: An Update Review. Journal of Clinical & Diagnostic Research. 2017 Dec 1;11(12).