ORIGINAL RESEARCH

Comparative Assessment of Different Concentrations of Sodium Hypochlorite and Calcium Hypochlorite on Microhardness of Root Canal Dentin - An *In Vitro* Study

Sai Anil Babu Duvvi[1], M S Adarsha[2], H L Usha[3], P Ashwini[4], Chethana S Murthy[5], A K Shivekshith[6]

ABSTRACT

Introduction: The study aimed to assess the effect of different concentrations of sodium hypochlorite and calcium hypochlorite on micro hardness of root canal dentin.

Methodology: The seventy five root halves were prepared by longitudinal splitting of seventy five extracted mandibular premolars and embedded in autopolymerizing acrylic resin, leaving the dentin surface exposed After polishing, the groups were then divided into five groups of 15 samples each through random sampling the microhardness values of the untreated dentin surfaces were recorded by using Vickers tester at the mid-root level. Then samples were surface treated for five minutes with five ml of one of the following irrigants Saline (control group), 2.5% NaOCl solution, 5% NaOCl solution, 5% CaOCl2 solution, 10% CaOCl2 solution. After surface treatment,dentin microhardness values were recorded at close proximity to the initial indentation areas. Experimental data were statistically analyzed by using one way ANOVA test followed by post hoc tuckey's honest significant test and comparison between pretreatment and post treatment groups by student pair T test.

Results: All irrigating solutions showed reduction in microhardness of root canal dentin except saline. 5% NaOCl and 10% $CaOCl_2$ showed maximum reduction in microhardness. 2.5% NaOCl shown least reduction in microhardness followed by 5% $CaOCl_2$ ($P < 0.05$).

Conclusions: 2.5% NaOCl and 5% $CaOCl_2$ shown less reduction in microhardness of root canal dentin when compared to 5% NaOCl and 10% $CaOCl_2$.

Keywords: Calcium hypochlorite, Microhardness, Root canal dentin, Sodium hypochlorite.

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[1]Post Graduate Student, [2,5]Professor, [3]Professor and HOD, [4]Reader, [6]Senior Lecturer

[1-6]Department of Conservative Dentistry and Endodontics, V.S Dental College, Bengaluru, Karnataka, India

Corresponding Author: Dr. Duvvi Sai Anil Babu, Post Graduate Student, V.S Dental College and Hospital, K.R Road, V.V Puram, Bengaluru, Karnataka, India. e-mail: saianilbabuduvvi@gmail.com

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INTRODUCTION

Irrigation is one of the most important aspects of root canal preparation. It helps to clean the complex root canal system that could not be directly planed by instruments. An ideal irrigant should have these requisite functions: Lubrication, debridement, antimicrobial effect, and dissolution of organic and inorganic material.[1] Unfortunately, there is no single irrigant to date that fulfills all these ideal requisites.

Sodium hypochlorite (NaOCl) is a commonly used irrigation solution, and it is used in several concentrations, in temperature and agitation. It has been recommended for the debridement of the root canal due to its effective antimicrobial action against a broad spectrum of bacteria and tissue solvent activity.[2] Cytotoxic activity is another well-known shortcoming of NaOCl that may cause acute injuring effects, if it reaches the periapical area. NaOCl extrusion during root canal therapy is commonly referred to as "the hypochlorite accident;" it causes acute immediate symptoms and potentially serious sequelae.[3] Hence, there is a need to find a new effective and safer irrigant for the root canal treatment.

Calcium hypochlorite ($Ca[OCl]_2$) is a white powder used for industrial sterilization, bleaching, and purifying water treatment. It is relatively stable and has greater available chlorine compared with NaOCl. Its incorporation in water can be more accurate than preparations by dilution of a more concentrated solution, which can be an advantage for clinical use. De Almeida *et al*. showed that $Ca(OCl)_2$ associated with ultrasonic irrigation is efficient to reduce root canal contamination and can aid in chemomechanical preparation, and it was as effective as NaOCl.[4]

Microhardness is considered as an indirect evidence of mineral changes in root canal dentin; such changes could affect the adhesive properties of the dentin surface. The effect of irrigation solutions on dentin should be evaluated as the irrigation solutions come in contact with dentin during irrigation procedures, which might alter dentin and enamel surfaces, affecting their interactions with obturation and coronal restorative materials. Garcia *et al*. reported that different concentrations of NaOCl decreased the microhardness of root canal dentin in cervical and apical thirds. Studies have shown a decrease in microhardness of radicular dentin exposed to NaOCl and 2% CHX as root canal irrigation solutions.[5,6]

However, there are not many studies on the effect of different concentrations of calcium hypochlorite on root canal dentin microhardness. Therefore, the purpose of this study was to evaluate *in vitro* different concentrations of NaOCl and CaOCl2 on microhardness of root canal dentin.

METHODOLOGY

A total of 75 mandibular premolars extracted for orthodontic reasons from the subjects referred to the Department of Oral and Maxillofacial Surgery, Vokkaligara Sangha Dental College and Hospital, Bengaluru, were collected. Teeth which were carious, with resorption or with vertical fracture, were excluded from the study. The extracted teeth were washed in normal saline until they were clear of blood and debris. After cleaning the collected samples, they were stored in an aqueous solution of 0.5% chloramine-T until further processing.

Sample Preparation

The crowns of 75 mandibular premolars were decoronated at the level of cementoenamel junction under water cooling using low-speed diamond disk. The roots were then sectioned along the long axis in a buccolingual direction with a low-speed diamond disk. One half of each root was selected, and the specimens were then embedded into acrylic resin blocks of 5 cm × 5 cm leaving the dentin surface exposed. The 75 root halves were then ground and polished with 500 and 600 grit sandpapers under distilled water to remove surface scratches. The groups were then divided into five groups of 15 samples each through random sampling, and microhardness was checked.

Pretreatment Microhardness Measurement

A total of 75 root halves were subjected to microhardness testing in Vickers microhardness tester (Raghavendra Spectro Metallurgical Laboratory, Peenya Industry, Bengaluru). The dentin microhardness of each sample was measured at a magnification of ×250 and a depth of 300 µm from the pulp dentin interface by Vickers microhardness tester. Three separate indentations were made using a 300 g load and a 20 s dwell time at the mid-root level of root dentin samples. The average of three indentations was made to get the final value of microhardness of each sample.

Surface Treatment of Samples

The surface treatment was done for the 75 samples which were divided into five groups, each group of 15 samples 5 min with 5 mL of one of the following irrigants.

Group 1	Saline (control group)
Group 2	3% NaOCl solution
Group 3	5% NaOCl solution
Group 4	5% $CaOCl_2$ solution
Group 5	10% $CaOCl_2$ solution

After the surface treatment, the samples were dried with sterile blotting paper.

Post-treatment Microhardness Measurement

All the 75 samples were subjected for post-treatment microhardness testing in Vickers microhardness tester. Three separate indentations were made using a 300 g load, and a 20 s dwell time was recorded at close proximity to the initial indentation areas using Vickers microhardness tester. The average of three indentations were made to get the final value of microhardness of each sample.

STATISTICAL ANALYSIS

Experimental data were statistically analyzed by using one way ANOVA test followed by post hoc tuckey's honest significant test and comparison between pretreatment and post treatment groups by student pair T test. Significant difference test at a = 0.05.

RESULTS

In the present study, there was no significant difference of mean microhardness between the samples in pretreatment groups [Graph 1].

The statistical comparison of pre- and post-treatment microhardness values within each study group demonstrated that treatment with 2.5% NaOCl, 5% NaOCl, 5% $CaOCl_2$, and 10% $CaOCl_2$ significantly decreased the microhardness of root canal dentin, whereas in saline Group (Group 1 control), the decrease in dentin microhardness was statistically insignificant [Table 1].

Comparison of post-treatment microhardness showed a significant ($P < 0.05$) decrease in microhardness of all the groups compared to control (Group 1 saline) [Figure 1].

Among the test groups, decrease in microhardness was least with 2.5% NaOCl followed by 5% $CaOCl_2$ and 5% NaOCl and was highest in 10% $CaOCl_2$. The decrease in dentin microhardness between 2.5% NaOCl and other test groups was statistically significant ($P < 0.005$).

Table 1: Comparison of mean microhardness between pre- and post-treatment periods within each study group using student paired *t*-test

Group	Time	*n*	Mean	SD	SEM	Mean different	*t*	*P* value
Saline	Pre-Rx	15	56.95	3.40	0.88	3.03	3.159	0.007*
	Post-Rx	15	53.91	2.56	0.66			
2.5% NaOCl	Pre-Rx	15	56.50	2.54	0.66	10.81	12.259	<0.001*
	Post-Rx	15	45.69	1.49	0.39			
5% NaOCl	Pre-Rx	15	56.71	2.31	0.60	16.78	22.015	<0.001*
	Post-Rx	15	39.93	2.73	0.70			
5% CaOCl	Pre-Rx	15	57.06	2.66	0.69	14.41	15.743	<0.001*
	Post-Rx	15	42.65	1.45	0.37			
10% CaOCl	Pre-Rx	15	56.96	1.84	0.48	17.93	25.453	<0.001*
	Post-Rx	15	39.03	2.17	0.56			

* Statistically significant

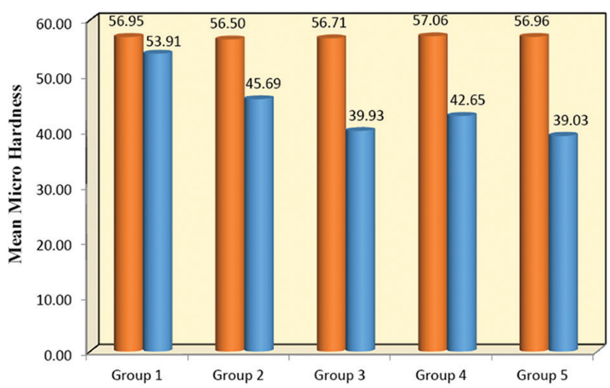

Figure 1: Comparison of mean microhardness between pre- and post-treatment periods within each study group

Nearly 5% NaOCl treatment significantly decreased the microhardness of root canal dentin when compared with 5% $CaOCl_2$. 10% $CaOCl_2$ treatment showed a decrease in dentin microhardness when compared to 5% $CaOCl_2$ and was statistically significant. However, there was no statistically significant difference between 5% NaOCl and 10% $CaOCl_2$ when post-treatment microhardness values were compared.

DISCUSSION

Chemical substances used during endodontic treatment are of great importance for infection control and to remove the smear layer. They should present a number of physiochemical and biological properties to be effective. However, the substances used in clinical practice may cause changes in the physical and mechanical properties of dentin. These substances modify the inorganic component of dentin and consequently its microhardness.[7] The decrease in mineral content and the amount of hydroxyapatite in the intertubular substance is important factors in the intrinsic hardness profile of a dentin structure. There is a positive correlation between hardness and mineral content in teeth. Hence, the measurement of hardness can provide indirect evidence of mineral loss or gain in dental hard tissue.[5]

The present study investigated the effect of 2.5% NaOCl, 5% NaOCl, 5% $CaOCl_2$, and 10% $CaOCl_2$ on the microhardness of root canal dentin.

After the use of chemical irrigants, which are capable of altering the proportion of organic and inorganic components of root canals, the structural properties of dentin such as permeability, solubility, and microhardness may change. Changes in microhardness, an indicator of mineral changes in root canal dentin, may affect the adhesive properties of root canal dentin surface and sometimes may decrease the strength of root and cause root fracture. Fusayama and Maeda reported a decrease in the dentine microhardness value of pulpless teeth compared to that of vital teeth. Moreover, the biomechanical properties of dentine have been shown to be altered after the loss of tooth vitality. As microhardness is sensitive to composition and surface changes of tooth structure, the effects of some chemicals such as fluorides, trichloroacetic acid, and bleaching agents on dentine hardness have been previously evaluated.[8]

The assessment of the microhardness of a material is one of the simplest nondestructive mechanical characterization methods. Hardness is measured as the resistance to the penetration of an indenter that is harder than the sample to be analyzed. Although the laboratory test does not represent real clinical relevance, it is possible to indirectly evaluate the impact of a relatively large amount of the substances in the dentin.[9,10]

In this study, microhardness measurement was performed at three points in the middle-third of the root canal dentin. Mean Vickers hardness number was calculated for each specimen.

Biological materials such as dentin may vary considerably between teeth and are far less homogenous, with dentin tubule density increasing from cervical to apical dentin, resulting in an inverse correlation between dentin microhardness and tubule density. This may lead to deviations in the results because of differences in adjacent regions of the dentin tissue.[11]

Hence, in the present study, the microhardness measurement was performed in the middle-third of the root for each sample at baseline and also post-treatment with irrigation solutions to establish a reasonable evaluation of the effect of the irrigant solutions on the dentin surface. Post-treatment indentations were performed on each sample at same areas but on the other side of dentin surface.

The longitudinal sectioning of the roots was preferred in this study which is in accordance with Cruz-Filho *et al*. who observed that it can show accurate representations of clinical situations. In addition, the irrigants first contact the most superficial layer of dentin in the root canal lumen and so the microhardness of the most superficial layer of root canal dentin was measured.[12]

The present study showed that there was more reduction in dentin microhardness in 5% NaOCl compared to 2.5% NaOCl. Slutzky-Goldberg *et al*. showed that instrumentation and irrigation with 2.5% NaOCl change the biomechanical properties of dentin. Dogan and Qalt also verified that the use of 2.5% NaOCl as irrigant for 15 min significantly altered the mineral content of root dentin. Driscool *et al*. also demonstrated that, when dentin immersed in 0.5% NaOCl and 5% NaOCl solutions, the weight loss of dentin was greater when immersed in 5% NaOCl solution than 0.5% NaOCl solution. According to Zhang *et al*., the superficial destructive effect on mineralized dentin with 5.25% NaOCl is irreversible and irrespective of whether EDTA is subsequently employed. Slutzky-Goldberg *et al*. showed that, at a depth of 500 µm from the lumen, 6% NaOCl had a greater effect on dentin microhardness than 2.5% NaOCl. Therefore, it is advisable not to use higher concentrations of NaOCl, which would otherwise alter the physical properties of dentin and jeopardize the tooth.[13-15]

Kinney *et al*. suggested that the decrease in hardness is caused by a decrease in stiffness of intertubular dentin matrix caused by heterogeneous distribution of the mineral phase within the collagen matrix. NaOCl is an efficient organic tissue solvent that causes dissolution of collagen by the breakdown of the bonds between carbon atoms and disorganization of the protein primary structure and change in magnesium and phosphate ions.[12]

In the present study, 5% NaOCl showed reduced microhardness when compared with 5% $CaOCl_2$, and similar result was found by Reddy *et al*. who showed increased reduction in flexural strength and modulus of elasticity of root dentin bars with 5% NaOCl when compared to 5% $Ca(OCl)_2$. Calcium hypochlorite in aqueous solution liberates $Ca(OH)_2$. Wang and Hume postulated that $Ca(OH)_2$ does not penetrate dentin well because of buffering capacity of hydroxyapatite. According to Leonardo *et al*., 5% NaOCl showed lower surface tension compared to 5% $Ca(OCl)_2$ and so wettability and penetration of 5% NaOCl might be more than 5% $CaOCl_2$. The effect on microhardness of root canal dentin of 5% NaOCl is more than 5% $CaOCl_2$ at 300 µm from pulp dentin interface.[4,16]

In the present study, 10% $CaOCl_2$ showed more reduction of microhardness when compared with 5% $CaOCl_2$ and 5% NaOCl. Dutta and Saunders found that 5% and 10% (w/v) solutions of $Ca(OCl)_2$ had very similar available chlorine levels (4.15% and 4.1%, respectively). The hyperosmotic effect of 10% $Ca(OCl)_2$ solution caused tissue dehydration and explains more weight <5% $Ca(OCl)_2$ after 5 min and also hypothesized that an accidental periradicular introduction of $Ca(OCl)_2$ may cause less tissue irritation. Oliveira *et al*. showed that high concentrations of $Ca(OCl)_2$ and NaOCl significantly increase dentin permeability and may lead to greater sequestration of calcium ions, increasing, therefore, the surface demineralization. Their results support that the 5% $Ca(OCl)_2$ could act as a chelating solution causing inadvertent erosion of the canal walls. On that account, the use of $Ca(OCl)_2$ and NaOCl at concentrations >5% should be reevaluated as an irrigating solution because it significantly altered dentin roughness and could hinder the adhesion of endodontic sealers to dentin.[15,17]

A limitation of this present study is that the experimental conditions of the immersion tests differed substantially from clinical situations. In clinical situations, the root canal is a closed-end channel, and this may produce a vapor lock effect during irrigation. As a result, different parts of the root canal wall are affected differently by irrigation. In the tests, however, it is possible to evenly apply a relatively large amount of the irrigant so that it remains in close contact with the dentine surface. This is not the case in clinical situations. Further studies should evaluate the effect of these irrigants in a closed-canal system in conjunction with agitation devices such as sonic and ultrasonic agitation systems.[18]

When using root canal irrigants, there is no consensus on the ideal amount of reduction in root dentine microhardness that both facilitate mechanical instrumentation and avoid mineral loss and weakening of the dental hard tissues. The problem of mineral loss and dental softening after use of chelating agents may be addressed by future studies on dentine remineralization. Chemical solution softening effect on the dentinal walls could be beneficial in the clinic as it permits rapid preparation and negotiation of tight root canals. However, the degree of softening and demineralization action may have an influence of the physical and chemical properties of this heterogenic structure. These chemicals may also affect the adhesion of sealers and cement to the dentin.[19,20]

In the present study, 5% NaOCl and 10% $CaOCl_2$ showed maximum reduction in microhardness of root canal dentin. Hence, it is advisable not to use higher concentrations of NaOCl and $CaOCl_2$ which would alter the physical properties of dentin and jeopardize the tooth.

CONCLUSION

Within the limitations of this *in vitro* study, it can be concluded that:

1. Post-treatment microhardness of root canal dentin within the groups treated with 2.5% NaOCl, 5% NaOCl, 5% $CaOCl_2$, and 10% $CaOCl_2$ for 5 min showed a significant decrease in microhardness values ($P < 0.05$).
2. The decrease in root canal dentin microhardness was least with 2.5% NaOCl, followed by 5% $CaOCl_2$, 5% NaOCl, and 10% $CaOCl_2$.
3. There was a significant decrease in post-treatment microhardness between all the test groups except 5% NaOCl and 10% $CaOCl_2$. The microhardness values between 5% NaOCl and 10% $CaOCl_2$ were not statistically significant ($P < 0.05$).

Further studies should be conducted to evaluate the optimal concentrations of the irrigating solutions which help in efficient disinfection and smear layer removal without modifying the dentin microstructure.

REFERENCES

1. Qing Y, Akita Y, Kawano S, Kawazu S, Yoshida T, Sekine I, *et al.* Cleaning efficacy and dentin micro-hardness after root canal irrigation with a strong acid electrolytic water. J Endod 2006;32:1102-6.
2. Garcia AJ, Kuga MC, Palma-Dibb RG, Só MV, Matsumoto MA, Faria G, *et al.* Effect of sodium hypochlorite under several formulations on root canal dentin microhardness. J Investig Clin Dent 2013;4:229-32.
3. Guivarc'h M, Ordioni U, Ahmed HM, Cohen S, Catherine JH, Bukiet F, *et al.* Sodium hypochlorite accident: A Systematic review. J Endod 2017;43:16-24.
4. Leonardo NG, Carlotto IB, Luisi SB, Kopper PM, Grecca FS, Montagner F, *et al.* Calcium hypochlorite solutions: Evaluation of surface tension and effect of different storage conditions and time periods over pH and available chlorine content. J Endod 2016;42:641-5.
5. Aslantas EE, Buzoglu HD, Altundasar E, Serper A. Effect of EDTA, sodium hypochlorite, and chlorhexidine gluconate with or without surface modifiers on dentin microhardness. J Endod 2014;40:876-9.
6. Eskandarinezhad M, Asghari V, Janani M, Frough M, Reihani, Rahimi S, *et al*. Evaluation of the effects of *Triphala* on dentin micro hardness as irrigation solutions. J Ayurveda Holistic Med 2015;3:58-67.
7. Ghisi AC, Kopper PM, Baldasso FE, Stürmer CP, Rossi-Fedele G, Steier L, *et al.* Effect of super-oxidized water, sodium hypochlorite and EDTA on dentin microhardness. Braz Dent J 2014;25:420-4.
8. Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. J Dent 1999;27:43-6.
9. Kandil HE, Labib AH, Alhadainy HA. Effect of different irrigant solutions on microhardness and smear layer removal of root canal dentin. Tanta Dent J 2014;11:1-11.
10. Aranda-Garcia AJ, Kuga MC, Chavéz-Andrade GM, Kalatzis-Sousa NG, Hungaro Duarte MA, Faria G, *et al.* Effect of final irrigation protocols on microhardness and erosion of root canal dentin. Microsc Res Tech 2013;76:1079-83.
11. Sayin TC, Serper A, Cehreli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent naOCl treatment on the microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:418-24.
12. Massoud SF, Moussa SM, Hanafy SA, El Backly RM. Evaluation of the microhardness of root canal dentin after different irrigation protocols (*in vitro* study). Alex Dent J 2017;42:73-9.
13. Slutzky-Goldberg I, Maree M, Liberman R, Heling I. Effect of sodium hypochlorite on dentin microhardness. J Endod 2004;30:880-2.
14. Oliveira LD, Carvalho CA, Nunes W, Valera MC, Camargo CH, Jorge AO. Effects of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:125-8.
15. Oliveira JS, Raucci Neto W, Faria NS, Fernandes FS, Miranda CE, Abi Rached FJ Jr., *et al.* Quantitative assessment of root canal roughness with calcium-based hypochlorite irrigants by 3D CLSM. Braz Dent J 2014;25:409-15.
16. Reddy P, Balla S, Raghu SS, Velmurugan N, Gupta GT, Sahoo HS. Effect of 5% calcium hypochlorite on mechanical properties of root dentin: An *in vitro* study. J Operative Dent Endod 2016;1:56-9.
17. Dutta A, Saunders WP. Comparative evaluation of calcium hypochlorite and sodium hypochlorite on soft-tissue dissolution. J Endod 2012;38:1395-8.
18. Kara Tuncer A, Tuncer S, Siso SH. Effect of QMix irrigant on the microhardness of root canal dentine. Aust Dent J 2015;60:163-8.
19. Patil CR, Uppin V. Effect of endodontic irrigating solutions on the microhardness and roughness of root canal dentin: An *in vitro* study. Indian J Dent Res 2011;22:22-7.
20. Ulusoy Öİ, Görgül G. Effects of different irrigation solutions on root dentine microhardness, smear layer removal and erosion. Aust Endod J 2013;39:66-72.