



COMPARISON OF THE EFFECT OF DIFFERENT IRRIGATING SOLUTIONS ON THE MICROHARDNESS OF ROOT CANAL DENTIN: AN IN-VITRO STUDY.

Dental Science

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ABSTRACT

AIM: The aim of present study is to compare and evaluate the changes in microhardness of root canal dentin following application of different irrigating solutions.

MATERIALS AND METHODS: A total of 50 freshly extracted single rooted teeth were selected and decoronated at 15 mm from root apex. Root canal instrumentation was done using step back technique and roots were sectioned longitudinally using diamond disc dividing into two halves; which were then polished and embedded into acrylic blocks separately. Half section of each specimen was sent for pretreatment microhardness testing whereas other half was immersed in different irrigating solution as per group in closed glass plate for 5 minutes. 50 specimens were divided into 5 groups of 10 teeth according to the irrigating solution used. Group A : 3% Sodium hypochlorite, Group B : 17% EDTA (Ethylene diamine Tetraacetic acid), Group C : 2% Chlorhexidine, Group D : Chloroquick solution, Group E : Saline (Control group). All the samples were placed in respective irrigant for 5 minutes at 37°C. All the tested specimens were then subjected to microhardness testing using Vickers hardness tester. Readings were recorded and statistical analysis was done using One-way ANOVA and Tukey's Post Hoc test.

RESULTS: 1) The result of present study showed that microhardness of root canal dentin decreases following application of different irrigating solutions. 2) The microhardness of root canal dentin was found to be comparatively least affected by using Chloroquick irrigating solution followed by 2% Chlorhexidine, 3% Sodium hypochlorite and 17% EDTA.

CONCLUSION: Chloroquick is an efficient root canal irrigant, as it was found to be least detrimental to root dentin microhardness. Besides, it has additional advantages like antimicrobial properties and efficient in smear layer removal.

KEYWORDS

Sodium Hypochlorite, Edta, Chloroquick Solution, Chlorhexidine, Microhardness, Vicker's Hardness Tester.

INTRODUCTION:

Endodontic treatment is aimed to eliminate microorganism and organic matter from pulp space with minimal harm to the dental hard tissue. Mechanical instrumentation produces a layer of organic and inorganic material called the smear layer that occludes the orifices of dentinal tubules.¹ Irrigation solution is essential to remove debris and smear layer created by instrumentation process. Irrigation plays an indispensable role in lubrication, gross debridement, destruction of microbes and dissolution of tissues.² An irrigants with the ability to remove smear layer without any erosion to the dentin and also to eradicate the microorganisms simultaneously would be considered standard.³

Dentin microhardness depends on the amount of calcified matrix per square millimetre. Thus, the determination of microhardness can provide appropriate evidence of mineral loss or gain in dental hard tissue.⁴ Irrigants react with the calcium ions of hydroxyapatite crystals of radicular dentine which leads to changes in microstructure of dentine and changes in calcium phosphorus ratio.⁵

Ballal NV et al stated that, changes in mineral content ratio reduces the microhardness⁶, increases the permeability and solubility of the root canal dentine. A decrease in microhardness can affect adhesion and sealing ability of sealers to the dentinal walls of root canals. So, it is necessary to use such a root canal irrigant which causes least reduction in dentin microhardness during root canal treatment.

Sena NT et al⁷ found that, sodium hypochlorite with a concentration ranging between 1 and 5.25% is the most commonly recommended endodontic irrigant because of its well-recognised antimicrobial activity and organic tissue dissolving ability. However, its capability to

remove the smear layer from root dentin appears to be limited.⁸

According to Zhang K et al⁹, demineralising agents such as ethylene diamine tetraacetic acid (EDTA) is used efficiently with sodium hypochlorite to remove the smear layer.

Chlorhexidine is broad spectrum antimicrobial agent in dentistry. Its properties such as antimicrobial action, substantivity and a relative absence of toxicity have led to the use as an endodontic irrigant¹⁰. Okino LA et al stated that it lacks the tissue dissolution capacities of sodium hypochlorite¹¹. Therefore, it has been suggested that chlorhexidine should not be used in place of sodium hypochlorite, but instead as a final irrigant followed by EDTA¹².

Chloroquick is a new formulation of 5.25% sodium hypochlorite with 18% hydroxyethylidene bisphosphonate (HEBP), also known as etidronic acid. It is available in 2 vial system which is then mixed with each other according to manufacturer's instructions. It has been reported to be efficient in smear layer removal and microbes eradication that are resistant to conventional endodontic irrigants¹.

However, till date no study has been reported for a comparative evaluation of changes in root dentine microhardness caused by 3% sodium hypochlorite, 17% EDTA, 2% chlorhexidine and Chloroquick. Hence the aim of this invitro study is to compare and evaluate the changes in microhardness of dentin caused by different irrigating solutions i.e., 3% sodium hypochlorite, 17% EDTA, 2% chlorhexidine and Chloroquick using Vickers microhardness test.

MATERIAL AND METHODS:

50 freshly extracted human single rooted mandibular premolar teeth

extracted for orthodontic reasons were selected for this study (fig. 1). The teeth were stored in a sterile solution at room temperature. The study protocol was approved by institutional ethical board. The presence of single rooted canal was confirmed on radiograph for each tooth. All the teeth were decoronated to standardize the root length of 15 mm and to get a flat coronal reference point using a diamond disc and Vernier calliper respectively (fig. 2). Patency of the root canal was established by passing #10 K file (Mani, Japan) until its tip was visualized at the apex. Working length was considered 1 mm short of file length. Each canal was prepared using Protaper Universal rotary system (Dentsply Maillefer, Switzerland) with torque-controlled motor (X-smart, Dentsply Maillefer, Switzerland) upto an apical preparation of F3 file using crown down technique. Normal saline was used as a root canal irrigant between each subsequent file in all experimental groups during instrumentation.

A total of 50 specimens were randomly divided into 5 groups according to irrigants used, where Group A, B, C and D were test groups and Group E was control group. The groups were as follows:

Group A (n=10): 3% Sodium hypochlorite (Prime Dental, India)
Group B (n=10): 17% EDTA (Prevest DenPro™)
Group C (n=10): 2% Chlorhexidine (Prevest DenPro™)
Group D (n=10): Chloroquick (Neelkanth Dental and surgical Factory, Jodhpur, India).
Group E (n=10): 0.9% W/V Normal saline (Fresenius Kabi).

The roots were sectioned longitudinally using diamond disc with low speed micromotor under water cooling giving two halves (fig. 3). Each half root section was horizontally mounted in acrylic blocks exposing dentin surfaces and labelled accordingly (fig. 4). Microhardness testing was done at middle third of root of each sample at 500 micrometres from pulp dentin interface and measured as a pre-treatment Vickers hardness number.

Exposed dentin of other half of the root segments of each tooth were treated with 5 ml of respective irrigating solutions for 5 minutes (fig. 5). After 5 minutes, all specimens were rinsed with distilled water and dried with sterile paper points. (Meta Biomed, Korea). Specimens were then subjected for testing post-treatment microhardness number to evaluate the effect of the irrigant solutions on the dentin surface.

MICROHARDNESS TESTING:

Vickers diamond microhardness tester (Model: VM 50 PC) was used for measurement. Midroot region was used during the measurement. Indentations were made with Vickers diamond indenter at 500 micrometres from pulp dentin interface. 100 gm load per 15 seconds was applied during each indentation (fig. 6). The hardness value of each sample was recorded as a Vickers hardness number and results were sent for statistical analysis.

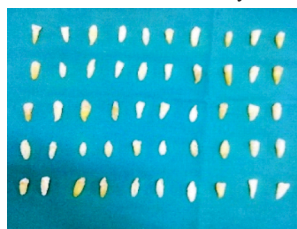


Fig.1: 50 extracted mandibular Premolars selected as specimens



Fig.3: Longitudinally sectioned root into two halves



Fig. 2: Decoronated mandibular premolar at 15 mm from the apex



Fig.4: Root sections horizontally mounted in acrylic blocks

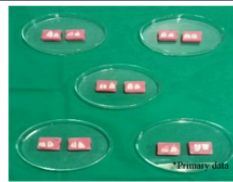


Fig.5: Specimens treated with respective irrigants in closed glass plates

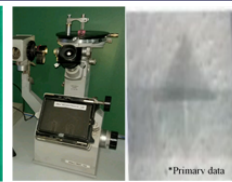


Fig.6: Vickers hardness test and indentation on sample glass plates

RESULTS:

One-way ANOVA showed statistically significant difference between different irrigants ($p < 0.05$). All irrigating solutions except normal saline (control group) showed reduction in the microhardness. The reduction in microhardness among the tested group were as follows 17% EDTA > 3% Sodium hypochlorite > 2% chlorhexidine > Chloroquick > Normal saline (Fig. 7).

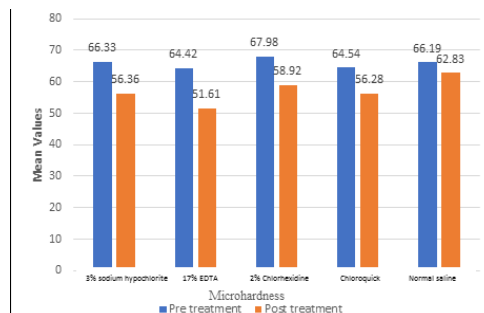


Fig. 7: Mean microhardness before and after treatment of specimens

STATISTICAL ANALYSIS:

The data was analysed with Statistical Package for Social Sciences (SPSS) for Windows 25.0 (SPSS, Inc. Chicago, Illinois). Confidence intervals were set at 95% and values of $p < 0.05$ were interpreted as statistically significant. One-way ANOVA was used to compare the four different irrigating solutions on the microhardness of the root canal dentin (Table 1 and 2).

Table 1: Pre-treatment one way ANOVA test

	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	86.45	4	21.61	2.7	0.04
Within Groups	356.7	45	7.9		
Total	443.1	49			

Table 2: Post-treatment one way ANOVA test

	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	674.47	4	168.61	13.74	0.0001
Within Groups	551.93	45	12.26		
Total	1226.40	49			

Tukey's Post-hoc test was computed to analyze in between group differences of five different irrigating solution (Table 3 and 4).

Table 3: Pre-treatment comparison of microhardness in different irrigating solutions across groups using Tukey's Post Hoc test.

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	Group B	1.91	1.25	0.55	-1.66	5.48
	Group C	-1.65	1.25	0.68	-5.22	1.92
	Group D	1.795	1.25	0.61	-1.78	5.37
	Group E	0.146	1.25	0.99	-3.43	3.72
Group B	Group A	-1.91	1.25	0.55	-5.48	1.66
	Group C	-3.56	1.25	0.06	-7.13	0.017
	Group D	-0.115	1.25	0.99	-3.69	3.46

	Group E	-1.764	1.25	0.63	-5.34	1.81
Group C	Group A	1.65	1.25	0.68	-1.92	5.22
	Group B	3.56	1.25	0.06	-0.017	7.13
	Group D	3.445	1.25	0.06	-0.13	7.022
Group D	Group E	1.796	1.25	0.61	-1.78	5.37
	Group A	-1.795	1.25	0.61	-5.37	1.78
	Group B	0.115	1.25	0.99	-3.46	3.69
	Group C	-3.445	1.25	0.06	-7.02	0.13
Group E	Group E	-1.649	1.25	0.68	-5.22	1.92
	Group A	-0.146	1.25	0.99	-3.72	3.43
	Group B	1.764	1.25	0.63	-1.81	5.34
	Group C	-1.796	1.25	0.61	-5.37	1.78
	Group D	1.649	1.25	0.68	-1.92	5.22

Table 4: Post-treatment comparison of microhardness in different irrigating solutions across groups using Tukey's Post Hoc test.

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	Group B	4.753	1.56	0.03*	0.30	9.20
	Group C	-2.555	1.56	0.48	-7.0	1.89
	Group D	0.079	1.56	0.99	-4.3	4.52
	Group E	-6.468	1.56	0.001*	-10.91	-2.01
Group B	Group A	-4.753	1.56	0.03*	-9.20	-0.30
	Group C	-7.308	1.56	0.0001*	-11.75	-2.85
	Group D	-4.674	1.56	0.03*	-9.12	-0.22
	Group E	-11.221	1.56	0.0001*	-15.67	-6.77
Group C	Group A	2.555	1.56	0.48	-1.89	7.00
	Group B	7.308	1.56	0.0001*	2.85	11.75
	Group D	2.634	1.56	0.455	-1.8	7.08
	Group E	-3.913	1.56	0.10	-8.36	0.53
Group D	Group A	-0.079	1.56	0.99	-4.52	4.37
	Group B	4.674	1.56	0.03*	0.22	9.12
	Group C	-2.634	1.56	0.45	-7.08	1.81
	Group E	-6.547	1.56	0.001*	-10.9	-2.09
Group E	Group A	6.468	1.56	0.001*	2.017	10.91
	Group B	11.221	1.56	0.0001*	6.77	15.67
	Group C	3.913	1.56	0.10	-0.53	8.36
	Group D	6.547	1.56	0.001*	2.09	10.99

DISCUSSION:

Microhardness of dentin may vary considerably between teeth. So, in the present study, the microhardness test was performed for each half sample of tooth at baseline and for other half sample of same tooth after treatment with irrigation solutions to evaluate the effect of the irrigant solution on the dentin surface.

The Vickers microhardness test was preferred in this study because of its appropriateness and practicality for evaluating surface changes of dental hard tissue treated with chemical agents. The microhardness measurement is one of the simplest non-destructive mechanical characterization methods¹³. Penetration of the indenter of Vickers hardness tester is twice as deep as Knoop. Furthermore, the hardness measurements obtained by the Knoop method are practically insensitive to the elastic recovery of the material, which made this test much more appropriate for the analysis of surface microhardness¹⁴.

The changes in the dentin microhardness can be attributed to the minimal resistance of increased number of widely opened dentinal tubules which were free of peritubular dentin near the pulp to the testing indenter¹⁵.

The number and diameter of dentinal tubules also play a key role in the effectiveness of irrigants¹⁶. Pashley et al¹⁷ stated that, an inverse correlation exists between dentin microhardness and tubular density. Degree of mineralization and amount of hydroxyapatite in the intertubular substances are cognitive factors in the determination of microhardness of dentin¹⁸.

The major factors that regulate the action of an irrigant are contact time and concentration. Ulusoy and Gorgul¹⁹ used the irrigants for 5 minutes as this duration is more realistic in terms of clinical practice. Accordingly, in this study samples were immersed in irrigating

solutions for 5 minutes to maintain uniformity between the five groups. In the present study, 3 % sodium hypochlorite was found to reduce the dentin microhardness. This reduction may be ascribed to its action of depletion of the organic phase of (type I collagen) of dentin²⁰.

Current results were similar with that of Saha SG et al²¹, who reported that 3% sodium hypochlorite does not significantly affect the dentin microhardness in contrast to the other two irrigants used i.e., 17% EDTA and 0.2% chitosan. Duvvi SA et al²² showed that, there was more reduction in dentin microhardness following application of 5% sodium hypochlorite compared to 2.5% sodium hypochlorite.

The present study revealed that, 17% EDTA decreased the microhardness of root canal dentin. The truth that 17% EDTA reduced the microhardness of dentin significantly could be due to its chelating property²³ or ability to dissolve the intertubular and peritubular dentin resulting in decreased modulus of elasticity and flexure strength.

Kalluru RS et al²⁴ found that, sodium hypochlorite and MTAD had not altered the microhardness of root canal dentin significantly whereas Chelating agents EDTA, EDTAC drastically reduced the microhardness of root canal dentin.

Kumar D et al²⁵ in his study demonstrated that, the microhardness of root dentin following application of MTAD as a final rinse was significantly less as compared to that of EDTA. Ballal NV et al⁶ concluded that, there was no significant difference between EDTA and maleic acid in the reduction of microhardness.

2% Chlorhexidine has antimicrobial properties similar to 5.25% sodium hypochlorite¹¹. Accordingly, in the present study, the use of 2% chlorhexidine solution decreased the microhardness of root canal dentin. Oliveira LD et al¹⁰ concluded that, 2% chlorhexidine and 1% sodium hypochlorite solutions reduced the microhardness of root canal dentin at 500 micrometres and 1000 micrometres from the pulp-dentin interface markedly.

Amin BK et al²⁶ demonstrated EDTA, sodium hypochlorite, and 2 % chlorhexidine significantly reduced the microhardness of root dentin in comparison with control group, while 0.2% chlorhexidine had no significant effect in the microhardness of dentin.

Ari et al² found the effect of 0.2% chlorhexidine solution on dentin microhardness and demonstrated that its use had harmless effect on root canal dentin.

De Deus G et al²⁷ stated that, HEBP is a weak chelating agent as compared to 17% EDTA and citric acid. These findings confirm that HEBP is a weak calcium-complexing agent that causes less change in dentin than other chelating agents.

Tartari T et al²⁸ suggested HEBP could be used as an effective alternative for other chelating agents as it has nominal effects on dentin structure and still shows better smear layer removal. The hydroxyethylidene bisphosphonate (HEBP), also known as etidronate is used systemically in patients suffering from osteoporosis or Paget's disease as it prevents bone resorption.

Lottanti S et al²⁹ concluded that, combination of HEBP and sodium hypochlorite can be used without interfering with the antimicrobial, biological and tissue dissolving properties of sodium hypochlorite.

According to Hegde V and Thakkar P³⁰, continuous soft chelating irrigation protocol performed with Chloroquick High shows better removal of smear layer at apical third when compared with conventional irrigation protocol.

A possible limitation of the present study is that, immersion tests were carried out at room temperature and not body temperature. Besides, in current study, efficacy of different irrigants was not evaluated in closed canal system in conjunction with agitation devices. So, further studies are required to be done to overcome limitations of this study.

CONCLUSION:

Within the limitations of this study, it can be concluded that, Chloroquick can be used as efficient root canal irrigant, as it was found to be least detrimental to root dentin microhardness when compared with 3% sodium hypochlorite, 17% EDTA and 2% chlorhexidine.

Besides, it has additional advantages like antimicrobial properties and efficient in smear layer removal.

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