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Comparative Evaluation of Effect of Three Different Calcium Hydroxide Combinations and Triple Antibiotic Paste on Root Dentine Microhardness - An in-Vitro Study

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Abstract:

Background: *Use of intracanal medicaments (ICM) during root canal treatment procedures have been considered as one of the necessary steps to further decrease the microbial population before obturation. Calcium hydroxide and triple antibiotic paste are the most commonly used intracanal medicaments. Their placement in the root canals exposes the radicular dentine to their action that may cause various surface alterations on it. These surface alterations have reported to decrease the microhardness of the dentine indicating dissolution and degradation. Hence, the goal of this study was to compare the effect of three different calcium hydroxide combinations and triple antibiotic paste on root dentine microhardness.*

Methodology: *Forty extracted human mandibular anterior teeth were taken for the study. After decoronation of the samples, pulp tissue extirpation and biomechanical preparation was done. The prepared teeth were then cut transversely from the middle third of each root such that two uniform root sections were obtained from a single tooth. The obtained root sections were randomly divided into four groups (n=20). Base line microhardness testing was then done. The sections were then placed into petridishes containing calcium hydroxide with glycerine (Group-I), distilled water (Group-II), chlorhexidine gluconate (Group-III) and triple antibiotic paste (Group-IV) respectively. Microhardness testing was repeated in the same manner after 24 hours, 3 days and 7 days.*

Statistical Analysis: *The values obtained after microhardness tests, were statistically analysed using One-way ANOVA test and Post-hoc bonferroni test .*

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Results: *Overall, the dentin micro-hardness values decreased after treatment with all the tested materials at different interval of time. Triple antibiotic paste group showed maximum decrease in micro-hardness.*

Keywords: *Intracanal Medicaments, Calcium Hydroxide, Triple Antibiotic Paste, Dentine Microhardness*

I. INTRODUCTION

The aim of root canal treatment is to prevent or cure the periapical pathosis. Hoshino E et al[1] in1996 stated bacterial infections to be the main cause of periapical pathosis. Hence, the success of root canal treatment depends not only on the removal of infected pulp tissue and dentine but also on the disinfection of the root canal system. Considering the limitations of mechanical preparation along with the complexity of the root canal systems, chemical preparation of the root canals has become a necessity. Termed as the chemo mechanical debridement; it is described as the cleaning and shaping of the root canal using instrumentation along with irrigants and intracanal medicaments that leads to reduction in the bacterial load in the root canal system[2].

Use of intracanal medicaments during root canal procedures have been considered as an indispensable step to decrease the microbial population before final root canal filling. Although biomechanical preparation of the root canal system does reduce the microbial content to a great extent but the use of intracanal medicaments have been anticipated to decrease the interappointment pain, the bacterial count and to prevent regrowth and render the canal contents inert[3].

According to Grossman[3,4], the ideal requirements of root canal medicament are as follow:

- It should be an effective antimicrobial agent.
- It should be nonirritating to periradicular tissues.
- It should remain stable in solution.
- It should have a prolonged antimicrobial effect.
- It should be active in presence of blood, serum, and protein derivatives of tissue.
- It should have low surface tension.
- It should not interfere with the repair of periradicular tissues.
- It should not stain tooth structure.
- It should not induce a cell-mediated immune response.

Triple antibiotic paste (TAP) is one of the most commonly used intracanal medicament in the endodontic regeneration.[1] TAP consists of three medicaments – metronidazole, ciprofloxacin and minocyclin (Lovelace et al 2011, Miller et al 2012)[5,6]. To maintain the chemical stability and ensure physiological compatibility, acids are further added in to the paste. This exposure of the acidic antibiotics to the dental hard tissues has a negative impact on their mechanical properties. Calcium chelation and hard tissue demineralisation has been seen to occur due to minocycline.

Calcium hydroxide is an intracanal medicament that is frequently used in endodontics. It was introduced by Hermann in the year 1920[3]. It is available in two forms, powder and paste. It has a pH of 12.5, has antibacterial properties and stimulates hard tissue formation. When available in powdered form, calcium hydroxide is mixed with different vehicles for ease of use and application. These vehicles used to mix and dispense the calcium hydroxide have a significant effect on its antibacterial property. The hydroxyl ions released from calcium hydroxide are highly oxidizing free radicals[5]. They destroy the bacteria by damaging the cytoplasmic membrane, bacterial DNA and protein denaturation.

Many studies have shown that the placement of intracanal medicaments in the root canal exposes the radicular dentine to their action which certainly affects its composition.[7,8] Hence, this study was conducted to compare the effect of three different calcium hydroxide combinations and triple antibiotic paste on root dentine microhardness.

II. MATERIAL AND METHODOLOGY

Clinically intact 40 extracted human mandibular anterior teeth were used in the study. Debris and soft tissue remnants on the root surfaces were removed. The coronal portions were sectioned from the radicular portions which were followed by the biomechanical preparation of their root canals.

After completion of the root canal preparation, the roots were cut transversally from the middle third in 2 mm sections using a diamond disk such that two sections will be obtained from middle third of each root [Fig.1] The samples were divided randomly into four groups (20 each).

- Group I - Calcium hydroxide with Glycerine
- Group II - Calcium hydroxide with Distilled water
- Group III - Calcium hydroxide with Chlorhexidine gluconate
- Group IV - Triple Antibiotic Paste

Baseline microhardness testing was done using a microhardness tester. [Fig.2] The sections were then placed in petridishes, containing a 2 mm depth of intracanal medicaments and vehicle combination for 7 days.

Microhardness testing was repeated in the same manner after 3 and 7 days. The obtained data was statistically analyzed.

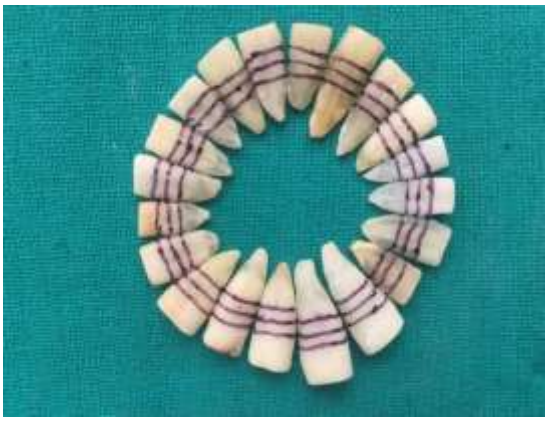


Fig.1

Fig. 2

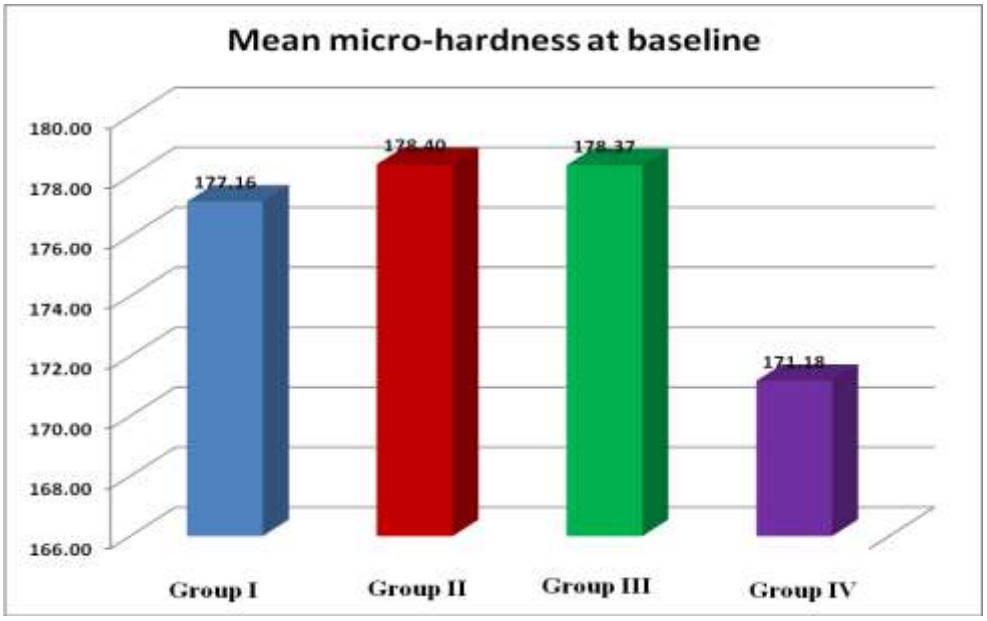
III. RESULTS

The mean micro-hardness at baseline was compared using the One-way ANOVA test and the post-hoc bonferroni test. There was no significant difference in mean micro-hardness at baseline between all Groups as shown in Table 1, Table 2 and Graph 1.

	Baseline			
	Mean	Std. Deviation	F-value	p-value
Group I	177.16	10.91	0.164	0.920
Group II	178.40	9.80		
Group III	178.37	17.86		

Group IV	171.18	50.76		

Table 1: Mean microhardness of radicular dentine at baseline.



Graph.1 Graphical representation of the mean microhardness of radicular dentine at baseline.

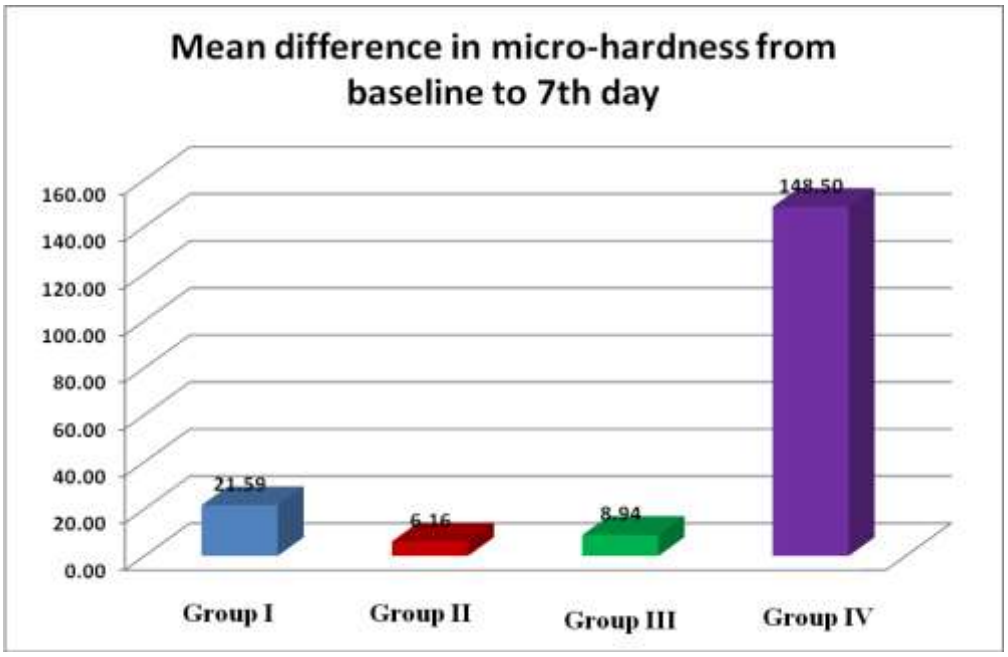
		Mean Difference	p-value
Group I	Group II	-1.24	1.000
Group I	Group III	-1.21	1.000
Group I	Group IV	5.98	1.000
Group II	Group III	0.03	1.000
Group II	Group IV	7.22	1.000
Group III	Group I	7.19	1.000

Table 2: Mean difference in radicular dentine microhardness at baseline using Post-hoc bonferroni test.

The mean difference in micro-hardness from baseline to 7th day was compared between all Groups using the One-way ANOVA test. There was a significant difference in mean difference in micro-hardness from baseline to 7th day. (Table 3 and Graph 2)

	Mean	Std. Deviation	F-value	p-value
Group I	21.59	8.06	86.977	0.000
Group II	6.16	6.52		
Group III	8.94	2.55		
Group IV	148.50	46.66		

Table 3: Mean difference in microhardness from baseline to 7th day



Graph 2: Graphical representation of mean difference in microhardness from baseline to 7th day.

The inter-group comparison of mean difference in micro-hardness from baseline to 7th day was done using the post-hoc bonferroni test. The mean difference in micro-hardness from baseline to 7th day was significantly more among Group IV compared to other Groups. (Table 4)

		Mean Difference	p-value
Group I	Group II	15.43	0.861
Group I	Group III	12.65	1.000
Group I	Group IV	-126.91	0.000*
Group II	Group III	-2.78	1.000
Group II	Group IV	-142.34	0.000*
Group III	Group IV	-139.56	0.000*

Table 4: Intergroup comparison of mean difference in microhardness from baseline to 7th day.

- Overall, the dentin micro-hardness values decreased after treatment with all the tested materials at different interval of time.
- Inter-group comparison showed significant difference between triple antibiotic paste group and calcium hydroxide groups.
- Group IV exhibited maximum decrease in micro-hardness followed group I whereas group III and group II presented similar results.

IV. DISCUSSION

Intracanal medicament placement into the root canal system exposes the radicular dentine to its action, which may cause certain surface alterations. Changes in the mineral content ratio reduce the microhardness and increase the permeability and solubility of the root canal dentin[9]. A decrease in toughness of dentin indicates dissolution and degradation.[10] But for a successful root canal treatment outcome the use of intracanal medicaments is an indispensable must. Calcium hydroxide and triple antibiotic paste as intra canal medicaments have given promising results since a very long time.

Use of TAP as an endodontic regenerative material while treating the resistant infections have been reported[11,12]. While using TAP as an intracanal medicament the interval of its use that exert an antibacterial effect has been reported to have a range from 7 to 21 days[13,14]. Calcium hydroxide is one of the most common intracanal medicament, which is also used in regenerative treatments[15,16] with its duration of use ranging from 24 hours to one week to exert an antimicrobial effect[17,18]. Thus, this study was carried out to find their effect on radicular dentin microhardness within seven days interval of use.

Comparative evaluation of effect of three different calcium hydroxide combinations and triple antibiotic paste on root dentine have shown that when calcium hydroxide was mixed with distilled water (Group II) and chlorhexidine (Group III) the microhardness of dentine was relatively higher than the calcium hydroxide-glycerine combination (Group I). Yoldas et al[19] in 2004, Rivera EM et al[20] in1994, Oztan MD at el[21] in 2002 have shown similar results as our study, where calcium hydroxide with glycerine decreases the microhardness more when compared with calcium hydroxide with distilled water or normal saline combination. The greater reduction in dentine hardness found after the $Ca(OH)_2$–glycerine combination could be explained by the different penetration ability of the other combinations into dentinal tubules. Yassen et al[22] in 2013 stated that calcium hydroxide can penetrate the intrafibrillar milieu of mineralized collagen fibrils and may cause changes in the three-dimensional conformation of tropocollagen, leading to a decreased elastic modulus and microhardness of mineralized dentin. So, it would be reasonable to assume that the dissolution effect of calcium hydroxide would affect dentine. One explanation of this might be the humectant property of glycerine in keeping substances moist due to its hygroscopicity.

On the other hand, when we compared all three calcium hydroxide combinations with TAP, TAP showed maximum decrease in dentine microhardness. Triple antibiotic mixtures used are acidic materials (pH 2.9) that may have demineralizing effects. Significant decrease in the phosphate/amide ratio in triple antibiotic paste-treated dentin indicate that the paste has a demineralization effect on the surface of the radicular dentin.[10] Results are in accordance with Yassen GH et al[22] that showed significant decrease in microhardness with triple antibiotic paste and double antibiotic paste than calcium hydroxide.

Also, many studies have shown that when calcium hydroxide paste is combined with 2% of chlorhexidine, it had shown significantly higher antibacterial effect than other intracanal medicaments[23,24,25,26]. A study by Ghabraei S et al[27] in 2018 compared the efficacy of calcium hydroxide-chlorhexidine combination and triple antibiotic paste in eradicating the biofilm of E. faecalis. The results showed that triple antibiotic paste took seven days whereas calcium hydroxide and chlorhexidine combination was effective in three days. Also, a study by Evans MD et al[23], Gomes BPFA et al[24] and Schafer and Bossmann[25] concluded that calcium hydroxide and chlorhexidine combination was better as an antimicrobial agent while using as an intracanal medicament in comparison to calcium hydroxide-normal saline combination.

Considering the results of present study and the studies mentioned above, the use of calcium hydroxide and chlorhexidine combination can give promising results as an intracanal medication while minimally affecting the physical and mechanical properties of radicular dentine.

V. LIMITATIONS:

- In the present study, the dentin discs were fully immersed in the test solution. This condition does not simulate clinical application where the tested agents are placed into the root canal.
- Although microhardness determination does not provide specific information about the structure and mechanical properties of the dentin, determining microhardness provides indirect evidence of mineral loss or gain in dental hard tissues.

VI. CONCLUSION

- Within the limitations of this study, it was concluded that use of calcium hydroxide and triple antibiotic paste as an intracanal medicament has an effect on the microhardness of radicular dentin.
- *Triple antibiotic paste* showed a significant decrease in micro hardness when compared with calcium hydroxide groups.
- The effect on microhardness caused by calcium hydroxide depends upon the *type of vehicle* used.
- Changes in microhardness (amongst calcium hydroxide group) were observed as: *Calcium hydroxide with glycerine > Calcium hydroxide with chlorhexidine > Calcium hydroxide with distilled water*

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