An in Vitro Study Comparing the Effect of Nanocalcium Hydroxide Powder with Various Vehicles as Intracanal Medicaments on the Microhardness of Root Dentin

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Abstract:- An intra-canal medicament (ICM) is a drug commonly used in endodontics to disinfect root canals between appointments. It helps eliminate residual bacteria after root canal preparation, reduces inflammation in the pulp and periapical tissues, neutralizes tissue debris, and makes canal contents inert. Additionally, it acts as a barrier alongside the temporary filling to prevent leakage and facilitates the drying of persistently wet canals. Therefore, the use of an ICM is essential for creating a microbe-free environment before root canal obturation, promoting optimal healing of the periapical tissues. Recently, nano-calcium hydroxide (NCH) has been introduced in endodontics, offering superior antibacterial properties compared to traditional calcium hydroxide powder. This is due to NCH's larger surface area-to-volume ratio and increased charge density, which enhance its interaction with the environment, resulting in greater antibacterial activity. To improve the effectiveness of ICMs, various vehicles such as aqueous, viscous, and oil-based formulations are used. Polyethylene glycol, an oil-based vehicle, has antibacterial properties due to its hydrophilic nature, which helps reduce bacterial counts. Chitosan, a viscous vehicle, possesses multiple biological properties, including antibacterial, hypercholesterolemic, wound healing, mucoadhesive, and sustained-release characteristics. Chlorhexidine, an aqueous vehicle, is known for its strong antibacterial properties. These vehicles may influence the physical and chemical properties of root dentin. Microhardness testing can be used to assess potential mineral loss or gain in dental hard tissues. The aim of this study is to investigate the effect of nano-calcium hydroxide combined with various vehicles on the microhardness of root dentin.

Keywords:- *Nanocalcium Hydroxide, Vehicles, Intracanal Medicament, Microhardness.*

I. INTRODUCTION

An intra-canal medicament (ICM) is a drug commonly used in endodontics to disinfect root canals between treatment appointments[1].

It helps to eliminate any remaining bacteria after root canal preparation, also reduces inflammation in the pulp and surrounding tissues, neutralizes tissue debris, and makes the canal contents inert. It also acts as a barrier, preventing leakage when used alongside a temporary filling, and aids in drying persistently wet canals[2]. Thus, the use of an ICM is crucial for ensuring a microbe-free environment before the root canal obturation, which promotes optimal healing of the periapical tissues[3,4].

ICMs are typically delivered using various vehicles, which can be water-soluble, viscous, or oil-based. These vehicles directly influence the release, action, dissociation, and penetration of the medicament into the dentinal tubules.

Importantly, an ICM should not adversely affect the physical or mechanical properties of root dentin, such as microhardness, modulus of elasticity, and flexural strength, nor should it be cytotoxic to periapical tissues. However, some intracanal medicaments can negatively impact root dentin's physical properties, particularly its microhardness.

Microhardness is a measurement of mineral loss or gain in dental hard tissues, assessed by the penetration of an indenter into small areas[5].

Root dentin microhardness is typically correlated with mineral content: a decrease in microhardness indicates demineralization, while an increase signifies mineralization. Lower microhardness reflects softer dentin, which can affect the sealing ability of obturation materials and compromise the long-term prognosis of endodontically treated teeth[6,7].

Nano-sized calcium hydroxide particles have recently been introduced in endodontics. These particles, which are smaller than 100 µm, have a larger surface area-to-volume ratio and higher charge density, making them more reactive and enhancing their antibacterial activity. Nano-calcium hydroxide also offers better penetration into deeper layers of dentin, higher surface alterations, and superior antimicrobial properties, particularly against *Enterococcus faecalis*, a bacteria resistant to high pH[8]. As a result, they are highly suitable for use as intracanal medicaments in root canal therapy.

Polyethylene glycol, a colorless, water-soluble, hygroscopic polymer, is fully miscible with water[9]. Its antibacterial effects stem from its hydrophilic properties, which help reduce bacterial populations[10].

Chlorhexidine is a cationic bisbiguanide compound containing two chlorophenyl rings and two biguanide groups linked by a hexamethylene chain. It is most stable in salt form and is basic in nature[11].

Chitosan is a polysaccharide derived from chitin, a component found in arthropod exoskeletons, through partial deacetylation. Composed of glucosamine and N-acetyl glucosamine copolymers, chitosan exhibits various biological properties, including antibacterial, hypercholesterolemic, wound-healing, mucoadhesive, and sustained-release characteristics[12,13].

II. SUBJECTS AND METHODS

➢ *Specimen Selection and Preparation*

This in-vitro study was reviewed by the institutional ethical committee for its ethical integrity and applications (859–2019). The power of the study was 95%, the level of significance was $P < 0.05$, and a sample size of 72 was estimated. Freshly extracted mandibular premolars with comparable root diameter, length, and taper were obtained, cleaned mechanically using an ultrasonic scaler and kept at room temperature in normal saline. The access cavities will be prepared using Endo access bur. Patency of the canal was achieved using a 10-k file and the working length was calculated by reducing one mm from the exit of the file at the apex.

➢ *Root Canal Preparation*

Standardized cleaning and shaping procedures was done using ProTaper rotary instruments up to size F3. After each instrumentation, 2 mL of 2.5% NaOCl for 1 min was used for passive irrigation, using disposible needle positioned one mm short of the working length. After 1 min of final irrigation with 2 mL of 17% ethylene diamine tetra acetic acid solution, the canal was washed with distilled water (5 ml) to eliminate any precipitate. Sterile paper points were used to dry the canals.

➢ *The specimens Were Randomly Divided into Four Groups:*

- Nanocalcium hydroxide mixed with Polyethylene glycol (Group 1; n = 18),
- Nanocalcium hydroxide mixed with Chitosan (Group 2; n = 18),
- Nanocalcium hydroxide mixed with Chlorhexidine (Group 3; n = 18),
- Nanocalcium hydroxide mixed with Distilled water (Group 4; n = 18).

➢ *Nano Calcium Hydroxide Paste Preparation*

Nano calcium hydroxide powder was mixed with different vehicles for all the groups to form a paste which was placed inside the root canal using lentulo spirals in each sample. Paste was compacted into the root canal using hand pluggers. While placing the medication, sufficient care was taken to ensure that no traces of the drug adhered to the external surface. Teeth were suspended in glass vials containing distilled water, such that the apical third only submerged in distilled water for 14 days. After 14 days, the ICM was removed by rinsing with saline from the root canal. Teeth sample were decoronated at cementoenamel junction using Dimond discs under continuous spray of water. Root samples were again sectioned horizontally into three sections such as coronal, middle and apical thirds. Coronal and apical root portions were discarded and only the middle third of the root were selected for the testing. Now, these middle root portions were mounted using autopolymerised acrylic resin. These mounted samples were subjected to microhardness test using Vickers hardness test.

Fig 1: Apical Third of Tooth Suspended in Glas Vial

Fig 2: Prepared Samples

➢ *Evaluation of Dentin Microhardness*

Mean microhardness value was measured under the magnification of 100X using Vickers microhardness tester. 25 gram of load was applied for time of 10 dwell seconds. The indentation were made in the mid root area 1mm away from the canal.

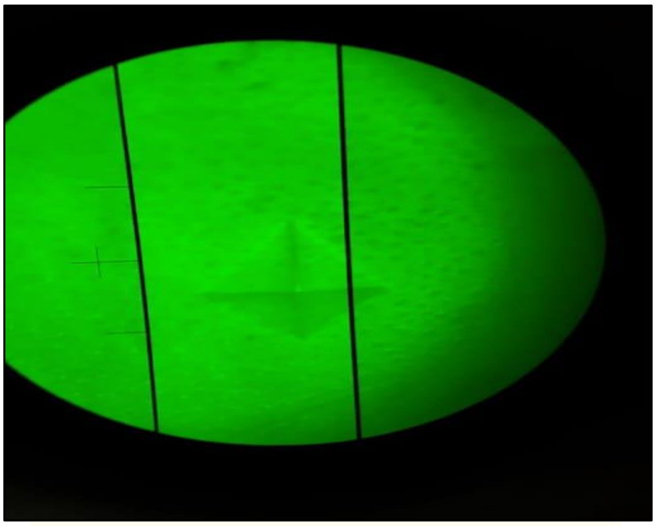

Fig 3: Indentation Marked in the Middle Third of Root.

➢ *Statistical Analysis*

Statistical analysis was performed with IBM SPSS 20 software. Analysis of Variance (ANOVA) test was used for comparing the significance of difference between the groups at 5% level of significance, followed by Post Hoc Test for multiple comparison between the groups that have significant difference .

III. RESULTS

Table 1: Descriptive Statistics for Microhardness of Different Groups

Group	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Polyethyelene Glycol	18	58	2.03	0.48	55	61
Chitosan	18	66.61	1.97	0.47	63	69
Chlorohexidine	18	66.89	2.19	0.52	63	70
Distilled Water	18	76.56	2.43	0.57	73	80

Table 2: Comparison of Microhardness between the Groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3104.49	3	1034.83	220.94	0.000
Within Groups	318.5	68	4.68		
Total	3422.99	71			

Table 3: Multiple Comparisons (Post Hoc Test)

Group (I)	Group (J)	Mean Difference (I - J)	Std. Error	Sig.	Inference
Polyethyelene Glycol	Chitosan	-8.61	0.72	0.000	Significant
	Chlorohexidine	-8.89	0.72	0.000	Significant
	Distilled Water	-18.56	0.72	0.000	Significant
Chitosan	Polyethyelene Glycol	8.61	0.72	0.000	Significant
	Chlorohexidine	-0.28	0.72	0.980	Not Significant
	Distilled Water	-9.94	0.72	0.000	Significant
Chlorohexidine	Polyethyelene Glycol	8.89	0.72	0.000	Significant
	Chitosan	0.28	0.72	0.980	Not Significant
	Distilled Water	-9.67	0.72	0.000	Significant
Distilled Water	Polyethyelene Glycol	18.56	0.72	0.000	Significant
	Chitosan	9.94	0.72	0.000	Significant
	Chlorohexidine	9.67	0.72	0.000	Significant

The difference between the mean hardness between the groups is significant (P Value < 0.05) Polyethylene Glycol has least value of microhardness and distilled water has the highest.

Multiple pairwise comparison shows that the difference in the mean microhardness between Polyethylene Gylcol , Chitosan, Cholrohexidine and Distilled water is statistically significant only difference between Chitosan and Cholrohexidine is not significant.

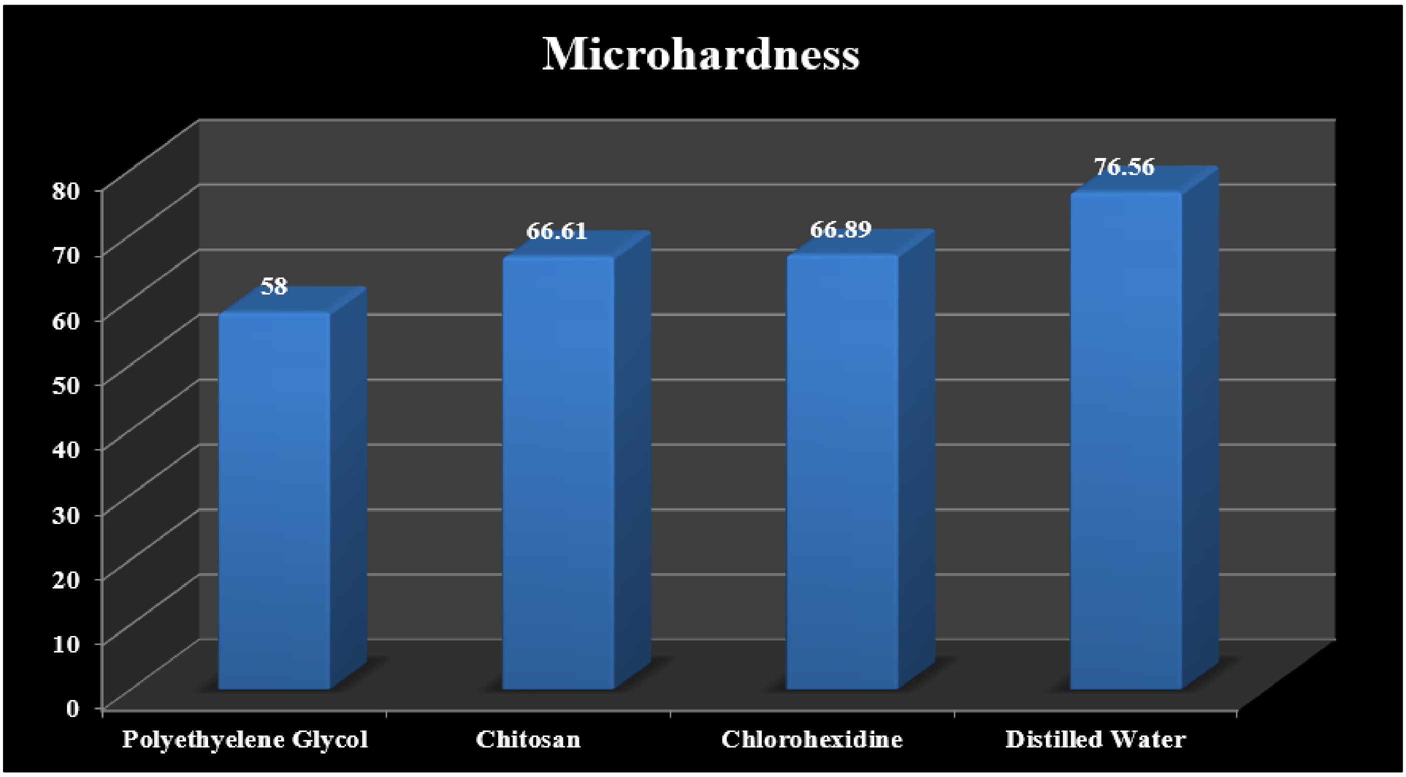

Fig 4: Graphical Representation of Comparison between Different Groups

IV. DISCUSSION

In this study, Nano-calcium hydroxide (NCH) pastes, combined with different vehicles, were placed inside the root canal , and their effects were assessed by measuring the microhardness.

Due to its smaller size and larger surface area, NCH can penetrate deeper into the dentin and effectively eliminate endodontic bacteria within the dentinal tubules. Nanoparticles exhibit superior antibacterial properties against endodontic pathogens.

Previous Studies have also shown that NCH causes fewer changes in dentin microhardness than conventional calcium hydroxide (CH) when used as an intracanal medicament for 4 weeks[14].

There are two main methods for testing microhardness: Knoop and Vickers. In this study, Vickers microhardness testing was used, as it is more sensitive to measurement errors, less influenced by surface conditions, and capable of testing small specimens with high accuracy. It also allows for the evaluation of surface changes in deeper dental hard tissues.

Significant changes in dentin microhardness after the application of ICMs suggest that these medicaments directly impact the dentin structure and its components. A reduction in microhardness indicates changes in the mineral content of dental hard tissues, which in turn affects the modulus of elasticity and flexural strength of dentin[15].

In this study, aqueous, viscous, and oil-based vehicles were used to prepare Nano-calcium hydroxide as an intracanal medicament, and the microhardness of the dentin was evaluated 1 mm from the pulp cavity. Pashley et al. proposed an inverse relationship between dentin microhardness and tubular density, stating that as tubular density increases, dentin microhardness decreases[16].

The results indicated that all combinations of Nano-calcium hydroxide with vehicles, such as Polyethylene Glycol, Chlorhexidine, and Chitosan, reduced the microhardness of root dentin, except for distilled water, which was used as a control group. These significant changes in dentin microhardness suggest a direct effect of the medicaments on the dentin structure and its physical and chemical properties.

Mandana Naseri and Leila Eftekhar previously reported that when Nano-calcium hydroxide was mixed with sterile water, it caused limited changes in dentin microhardness. In this study, distilled water was mixed with calcium hydroxide because it does not contain free ions and is less likely to chemically react with root dentin. The results showed that Polyethylene Glycol and Chitosan, when mixed with Nano-calcium hydroxide, caused a greater reduction in dentin microhardness compared to Chlorhexidine.

Chitosan, a polysaccharide with chelating properties, promoted a greater reduction in microhardness[17]. Previous studies have suggested that the demineralizing action of chelating agents affects the chemical and physical properties of root dentin, leading to changes in microhardness and surface roughness[18,19]. Chitosan's hydrophilic nature facilitates close contact with root canal dentin, allowing it to be adsorbed easily onto the canal walls and penetrate deeper into the dentinal tubules[20].

Chlorhexidine had the least effect on dentin microhardness. Matrix Metalloproteinases (MMPs), such as MMP-2, MMP-8, and MMP-9, are found in the dentinal matrix. During dental caries, MMPs exhibit proteolytic activity, with MMP-1 and MMP-8 acting as collagenases that degrade type I collagen[21].

Chlorhexidine is a potent inhibitor of MMPs and can reduce their catalytic activity. By inhibiting proteinase activity, chlorhexidine prevents the release of MMPs, preserving the collagen fibrils in the dentinal surface and potentially contributing to the preservation of dentin microhardness[22].

Previous studies have also shown that Polyethylene Glycol, when combined with calcium hydroxide, increases antimicrobial activity against *Enterococcus faecalis*. Polyethylene Glycol can reduce the particle size of calcium hydroxide, improving its penetration into dentinal tubules and contributing to a reduction in dentin microhardness[23].

A potential limitation of this study is that it was conducted at room temperature, rather than at body temperature, suggesting that further in vivo studies are necessary to validate these findings under physiological conditions.

V. CONCLUSION

This study concludes that combination all vehicles with Nanocalcium hydroxide, except distilled water, significantly reduceded the microhardness of root dentin. Nanocalcium hydroxide combined with polyethylene glycol resulted in the greatest reduction in microhardness of root dentin, followed by Nanocalcium hydroxide with chitosan. Nanocalcium hydroxide with chlorhexidine showed the least effect on microhardness of root dentin.

REFERENCES

[1]. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Dental Traumatology 1985 Oct;1(5):170-175.
[2]. Chong BS, Pitt Ford TR. The role of intracanal medication in root canal treatment. International Endodontic Journal 1992 March;25(2):97-106.
[3]. Fabricius L, Dahlén G, Sundqvist G, Happonen RP, Möller ÅJ. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth. European journal of oral sciences 2006 Aug;114(4):278-85.
[4]. Kawashima N, Wadachi R, Suda H, Yeng T, Parashos P. Root canal medicaments. International dental journal. 2009 Feb;59(1):5-11.
[5]. Prabhakar A., Taur S., Hadakar S., Sugandhan S. Comparison of antibacterial efficacy of calcium hydroxide paste, 2% chlorhexidine gel and turmeric extract as an intracanal medicament and their effect on microhardness of root dentin: An in vitro study. *Int. J. Clin. Pediatr. Dent.* 2013;6:171.
[6]. Hasheminia S.M., Norozynasab S., Feizianfard M. The effect of three different calcium hydroxide combinations on root dentine microhardness. *Res. J. Biol. Sci.* 2009;4:121–125.
[7]. Marickar R., Geetha R., Neelakantan P. Efficacy of contemporary and novel Intracanal medicaments against enterococcus faecalis. *J. Clin. Pediatr. Dent.* 2014;39:47–50.
[8]. Dianat O, Saedi S, Kazem M, Alam M. Antimicrobial activity of nanoparticle calcium hydroxide against Enterococcus faecalis: an in vitro study. Iranian Endodontic Journal 2015 Winter;10(1):39.
[9]. Chen J, Spear SK, Huddleston JG, Rogers RD. Polyethylene glycol and solutions of polyethylene glycol as green reaction media. Green Chemistry 2005 Jan;7(2):64-82.
[10]. Carreira Cde M, dos Santos SS, Jorge AO, Lage-Marques JL. Antimicrobial effect of intracanal substances. Journal of Applied Oral Science 2007 Oct;15(5):453-458
[11]. Nerwich A, Figdor D, Messer HH. pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide . Journal of Endodontics 1993 June 1;19(6):302–306.
[12]. Estrela C, Pesce HF. Chemical analysis of the liberation of calcium and hydroxyl ions from calcium hydroxide pastes in connective tissue in the dog. Part I. Brazilian Dental Journal. 1996;7(1):41–46.
[13]. Chen J, Spear SK, Huddleston JG, Rogers RD. Polyethylene glycol and solutions of polyethylene glycol as green reaction media. Green Chemistry 2005;7(2):64-82.

[14]. Naseri M, Eftekhar L, Gholami F, Atai M, Dianat O. The effect of calcium hydroxide and Nano-calcium hydroxide on microhardness and superficial chemical structure of root canal dentin: An ex vivo study. J Endod. 2019;45:1148–54.
[15]. Pashley D, Okabe A, Parham P. The relationship between dentin microhardness and tubule density. Dent Traumatol. 1985;1(5):176-79.
[16]. Pashley, David Henry, et al. "Collagen degradation by host-derived enzymes during aging." Journal of dental research 83.3 (2004): 216-221.
[17]. Kurita K. Chemistry and application of chitin and chitosan. Polym Degrad Stab 1998;59:117-120.
[18]. Hargreaves KM, Berman LH, Eds. Cohen's Pathways of the Pulp 11th ed. 2016.
[19]. Mohammadi Z, Shalavi S, Jafarzadeh H. Ethylenediaminetetraacetic acid in endodontics. Eur J Dent 2013; 7(Suppl. 1): S135-42.
[20]. Christian P, Von der Kammer F, Baalousha M, Hofmann T. Nanoparticles: structure, properties, preparation and behaviour in environmental media. Ecotoxicology 2008; 17(5): 326-43.
[21]. Elgezawi M, Haridy R, Almas K, Abdalla MA, Omar O, Abuohashish H, Elembaby A, Christine Wölfle U, Siddiqui Y, Kaisarly D. Matrix Metalloproteinases in Dental and Periodontal Tissues and Their Current Inhibitors: Developmental, Degradational and Pathological Aspects. Int J Mol Sci. 2022 Aug 11;23(16):8929.
[22]. Abdelazeem, Mohamed. "The Effect of chlorhexidine irrigation on root dentin microhardness in regenerative endodontics using double antibiotic paste." (2022).
[23]. Sidiqa AN, Zakaria MN, Cahyanto A, Joni IM, Maskoen AM. Carbonation inhibitor by polyethylene glycol encapsulation of calcium hydroxide fine particles to improve antimicrobial and root canal penetration properties. Heliyon. 2023 Jul 6;9(7):e18005.