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Effect of triple antibiotic paste and calcium hydroxide intracanal medicaments on dentin microhardness

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Abstract:
Intracanal medicaments may alter dentin microhardness, potentially compromising the structural integrity of endodontically treated teeth. Therefore, it is of interest to compare the effects of triple antibiotic paste (TAP), calcium hydroxide ($Ca(OH)_2$) and a control on root dentin microhardness using extracted human teeth and a Vickers hardness tester. Both medicaments significantly reduced dentin microhardness compared with the control at the coronal, middle and apical thirds ($p < 0.05$). TAP produced the greatest reduction in microhardness, whereas calcium hydroxide better preserved dentin hardness, indicating its superior suitability as an intracanal medicament. Thus, data show the preferential use of calcium hydroxide we encourage further research on natural intracanal medicaments and advanced methods for assessing dentin integrity.

Keywords: Calcium hydroxide ($Ca(OH)_2$), triple antibiotic paste (TAP), microhardness, intra-canal medicament, root canal treatment, vickers hardness.

Background:
Root canal infections are polymicrobial in nature, involving both aerobic and anaerobic species. The ideal root canal treatment requires through microbial elimination for treatment success **[1, 2]**. Due to the complex internal anatomy of the root canals, the chemo-mechanical preparation alone is not sufficient and there is necessity of the use of intracanal medicaments as adjuncts. The intracanal medicaments are antimicrobial agents which act as interappointment dressings that eradicate residual microorganisms and dissolve necrotic tissue, thus complementing the synergistic action of instrumentation, irrigation and obturation **[3, 4]**. The most widely used intracanal medicament is calcium hydroxide. Clinically it is applied to attain bacteria under control, break down the organic residues, give therapy for periapical inflammatory action, stop root resorption by inflammation, restore organization of inorganic component of the tooth and can be used as a temporary obturating material between appointments **[5]**. It is proved to be highly effective in treatment of teeth with chronic periapical lesions. $Ca(OH)_2$ is known by its capacity to neutralize bacterial endotoxins and the stimulation of apical and periapical repair **[6].** A newer formulation called triple antibiotic paste (TAP) - Ciprofloxacin, metronidazole and minocycline together form TAP. The abovementioned antibiotics exhibit effective antimicrobial and antibacterial properties. The American Association of Endodontics (AAE) protocol advocates the use of TAP in regenerative endodontic procedures. Its use is also advocated in the treatment of radicular resorptive defects, root fracture and also in the non-surgical management of periapical pathosis **[7, 8].** Therefore, it is of interest to report the effects of intracanal medicaments such as $Ca(OH)_2$ and TAP on affecting the microhardness of dentin.

Materials and methods:
This is an *in vitro* study that used human teeth to measure the variation in dentin microhardness when intracanal medication was used. The intracanal medicaments used in the study were triple antibiotic paste composed of ciprofloxacin, metronidazole and minocycline (TAP) and calcium hydroxide paste. They were compared with control group for better evaluation. Thirty extracted single-rooted mandibular premolars with single canals were selected for this study. They were collected from adult patients who require extraction for orthodontic purpose. Teeth with caries, fractures, or calcified/obliterated canals were excluded and all samples were confirmed to be free of calculus and debris. Teeth were thoroughly cleaned from any blood streaks or calculus deposits. They were stored in saline solution at room temperature till time of use. Teeth were decoronated transversally at the cemento-enamel junction with a double-faced diamond disc (Microdont LDA, Brazil) at low speed with water coolant to ensure a uniform working length of 13 to 15 mm. The Working lengths of the sample teeth were established by inserting a size 15 K-file (Mani, Inc, Japan) to the root canal terminus until it became visible through the apical foramen and then subtracting 1 mm. Root canal preparation was done by ProTaper rotary files (Dentsply, sirona) till size F5. The

canals were irrigated with a standardized volume of 2 mL of sodium hypochlorite between each file. The canals were finally rinsed with sterile saline to remove any dentine debris remaining in the canal after instrumentation. Root canals were then dried with sterile paper points. After drying the root canals, they were randomly assigned to three groups and the intracanal medicament were used as group 1 with calcium hydroxide paste, group 2 with Triple antibiotic paste and group 3 with saline which act as a control. The antibiotic pastes were fabricated by grounding one tablet of each antibiotic such as ciprofloxacin, metronidazole and minocycline to obtain a mixture 1:1:1 by weight, measured in a precision scale. The pastes were then produced by mixing the powder with saline solution. Specimens were stored at 37°C and 100% humidity for 90 days and the apices were sealed with heated glue to prevent leakage. The coronal, middle and apical thirds were sectioned and isolated. Each segment was further divided to yield two dentin discs of 2 mm thickness, verified using a Vernier caliper. Discs were polished with silicon carbide papers and finished with aluminum oxide paste. The discs were then stabilized in acrylic molds using wax.

Microhardness evaluation:

Root dentin microhardness was measured using a Vickers hardness tester (Future Tech Corp FM-700, Tokyo, Japan). A Vickers diamond indenter was applied under ×40 using a 200 g load for 15 s. Three indentations were placed 100 µm from the surface, spaced at least 500 µm apart and positioned approximately 1 mm from the canal wall. The mean of the three diagonal measurements was used to calculate the Vickers hardness number. A control group established baseline values.

Statistical analysis:

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests, data showed parametric (normal) distribution. One way ANOVA was used to compare between more than two groups in related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® statistics version 20 for windows.

Results:

Comparisons of three groups with micro-hardness at different position of sample teeth were done with One way ANOVA and respectively shown in **Table 1**. The ANOVA test revealed a significant difference in three groups with the Ca $(OH)_2$ and TAP affected the microhardness of root dentin at the coronal, middle and apical section with significant decrease in dentine microhardness when compared with control group. The TAP group showed least microhardness values than the other two groups and TAP caused significant decrease in dentin microhardness when compared with calcium hydroxide comparison of microhardness at the coronal, middle and apical third of all three groups were described in **Table 2.** There was statistically significant difference detected in between both the coronal and apical sites, while the middle site was not significantly different between both coronal and apical sites. The mean VHN of calcium hydroxide and TAP was significantly reduced when compared with control but TAP showed greater decrease in the mean VHN than Calcium hydroxide in the apical, middle third and coronal third of root dentin.

Table 1: Comparison of microhardness of Ca $(OH)_2$, TAP and control group

VHN	Ca(OH)2	TAP	Control	P
Coronal	62.1±2.3	53.3±2.2	70.3±3.1	0
Middle	64.4±3.1	57.42±.5	72.0±2.1	0.1
Apical	67.2±2.4	60.2±2.6	75.1±2.6	0

Table 2: Comparison of microhardness of apical, middle and coronal third of root dentin

VHN	Coronal	Middle	Apical	P
$Ca(OH)_2$	62.1±2.3	64.4±3.1	67.2±2.4	0.1
TAP	53.3±2.2	57.42±.5	60.2±2.6	0
Control	70.3±3.1	72.0±2.1	75.1±2.6	0

Discussion:

Successful endodontic treatment depends on effective eradication of microorganisms from the root canal system while preserving the structural integrity of radicular dentin. Intracanal medicaments are routinely used to eliminate residual bacteria that persist after biomechanical preparation, particularly in teeth with persistent infections or regenerative procedures **[9]**. However, prolonged exposure to these medicaments may alter the physical and mechanical properties of dentin, including microhardness, which reflects the mineral content and structural integrity of dental tissues **[10]**. Therefore, evaluating the influence of intracanal medicaments on dentin microhardness is essential for selecting agents that provide antimicrobial efficacy without compromising tooth strength. In the present study, Vickers microhardness testing was used because it is a reliable and reproducible method for assessing changes in dentin hardness with minimal influence from specimen size or surface characteristics **[11]**. Single-rooted teeth were selected to minimize anatomical variability and improve the consistency of measurements. The null hypothesis was rejected because both calcium hydroxide and triple antibiotic paste (TAP) significantly reduced dentin microhardness compared with the control group after 21 days of intracanal medication. Although both medicaments reduced dentin microhardness, calcium hydroxide demonstrated a significantly smaller reduction than TAP. Similar findings have been reported by Patri *et al.* **[12]**, Naseri *et al.* **[13]**, and Naeem *et al.* **[14]**, who attributed the reduction in dentin microhardness following calcium hydroxide application to its high alkalinity, which promotes degradation of the inorganic phase and denaturation of the organic collagen matrix. Nevertheless, the reduction produced by calcium hydroxide remained lower than that caused by TAP, suggesting better preservation of dentin structure. The present study also showed that TAP produced the lowest microhardness values among all groups. This observation agrees with the findings of Wang *et al.* **[15]**, who reported that prolonged exposure to TAP significantly decreases dentin microhardness. The acidic nature of ciprofloxacin, metronidazole, and minocycline promotes demineralization of dentin by chelating calcium ions from

hydroxyapatite, thereby reducing the mineral content and weakening the dentin matrix. These findings are consistent with the biological rationale that acidic intracanal medicaments adversely affect dentin mechanical properties. Region-wise analysis revealed significant differences between the coronal and apical thirds, whereas the middle third showed no significant difference compared with either region. These findings are consistent with those reported by Ballal *et al.* **[16]** and Sahebi *et al.* **[17]**, who suggested that regional variations in dentinal tubule density contribute to differences in dentin microhardness. The coronal dentin contains a greater density of dentinal tubules than the apical region, influencing mineral distribution and the penetration of intracanal medicaments. Recent investigations continue to support the influence of intracanal medicaments on dentin biomechanics. Thakur *et al.* **[18]** reported that prolonged intracanal dressing significantly alters dentin mechanical properties, emphasizing the need for careful selection of medicaments. Similarly, Nogueira *et al.* **[19]** highlighted that intracanal medicaments should achieve effective antimicrobial action while minimizing adverse effects on dentin structure. Palekar *et al.* **[20]** further emphasized balancing antimicrobial efficacy with preservation of dentin integrity, whereas Elgamal *et al.* **[21]** showed that newer intracanal formulations may provide favorable antimicrobial activity with reduced detrimental effects on dentin microhardness. These findings reinforce the importance of developing intracanal medicaments that maintain both biological effectiveness and structural preservation. Overall, the findings of the present study indicate that although both calcium hydroxide and TAP reduce dentin microhardness after prolonged application, calcium hydroxide preserves dentin integrity significantly better than TAP. Therefore, clinicians should consider not only the antimicrobial effectiveness but also the long-term effects of intracanal medicaments on dentin mechanical properties when selecting an intracanal dressing. Future studies should evaluate newer bioactive and natural intracanal medicaments using advanced techniques such as micro-computed tomography, nanoindentation, and mineral density analysis to better understand their effects on root dentin.

Conclusion:

Triple antibiotic paste significantly reduced dentin microhardness compared with calcium hydroxide across the coronal, middle, and apical thirds. Calcium hydroxide better preserved dentin microhardness, supporting its use as a safer intracanal medicament. Further studies should evaluate new natural intracanal medicaments using advanced analyses of mineral content and dentinal tubule penetration.

Limitations:

Main limitation of this study is that it was an *in vitro* study and certain clinical circumstances such as the oral temperature, presence of exudates and the surrounding periapical tissues can influence the clinical results which may differ from *in vitro* environment. Additional studies are needed to prove the impact of $Ca(OH)_2$ on the microhardness of root dentine through other specific types of tests as the mineral volume assessment and the penetration ability of $Ca(OH)_2$ within dentinal tubules.

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