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Effect of N-Acetylcysteine and calcium hydroxide as intracanal medicament on the microhardness of root dentin.

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Abstract

Background & Objectives: To evaluate and compare the effect of N acetylcysteine Calcium hydroxide on the microhardness of root dentin as intracanal medicament.

Methods: A total of 30 extracted single- rooted premolar teeth with single canals were selected and decoronated at the CEJ to obtain uniform root lengths of 12mm. The root canals were prepared to an apical size of 30 - 6% with Neo Endo file system. During instrumentation irrigation using 2.5 %, NaOCL was used as an irrigant between each instrument. Samples were divided into 3 groups depending on the medicaments used.

Group 1: Control (no medicament) (n=10)

Group 2: Calcium hydroxide (n=10)

Group 3:N acetylcysteine paste (n=10).

Medicaments in pastes forms were placed in the canal space up to the CEJ and the canal orifice was sealed with a temporary dressing. Depending upon the microhardness tests being conducted at 2 weeks (A) and 4 weeks (B) intervals, the groups were further subdivided (n=5) into Group 1A, Group 2A, Group 3A, Group1B, Group 2B, and Group 3B. The specimens were then longitudinally sectioned in a buccolingual direction to split the root. The root segments were horizontally embedded in auto polymerizing acrylic

resin leaving their dentin surface exposed. The dentin surface of the mounted specimens were ground flat and smooth and polished to obtain a smooth surface. The microhardness was determined at three different points foreach sample: in the coronal, middle, and apical thirds. Microhardness measurements were recorded using Vickers Microhardness Tester with 25 g load for 10 s.

Results: One-way ANOVA Test followed by Tukey's Post hoc Test and Student Paired t Test was used to compare the mean Micro hardness values at 2- & 4-weeks' period at different regions between 3 groups and in each group. The difference in the mean micro hardness between 3 groups in coronal, middle and apical region was statistically significant at $p<0.001$. Multiple comparisons of mean difference of microhardness showed significant results, highest microhardness with the control group followed by the N-Acetyl Cysteine group and the lowest with the Ca(OH)2 group. The test results showed that the mean micro hardness at 4 weeks' period in

Control group, N-Acetyl Cysteine group and Ca(OH)2 group at Coronal, Middle & Apical region showed relatively lesser values as compared to 2 weeks' period.

Interpretation & Conclusion: The result of the current study substantiated that NAC caused a significantly lesser reduction in the microhardness of the root dentin in comparison with the commonly used CaOH. Hence, with further studies, NAC can be explored to serve the purpose of a potent intracanal medicament in root canal therapy with its well-known benefits that favour bacterial eradication.

Keywords: N acetylcysteine, Calcium hydroxide, Microhardness, Intracanal medicament.

Introduction

Despite the significant progress made in "cleaning and shaping" techniques, the resistance of microorganisms is frequently associated with the ecological organization of bacteria in a three-dimensional structure known as biofilm [1]. Therefore, the use of intracanal medication to disrupt biofilms and thereby eradicate residual microbiological infections within root canals has been recommended to achieve a successful endodontic treatment [2,3]However failure of endodontic treatment may occur due to the complexity of root canal anatomy or antibiotic resistance of some bacteria such as Enterococcus faecalis , which isa gram-positive facultative anaerobic bacterium frequently isolated from root canals of teeth with pulpal necrosis and apical periodontitis[4,5].One of the most commonly used intracanal medications during endodontic treatment is Ca(OH)2 calcium hydroxide due its capacity to neutralize bacterial endotoxins and the stimulation of periapical repair [6].However, according to Andreasen's theory, calcium hydroxide has a proteolytic action that could weaken the tooth up to 50% in 1 year. He believed that the disruption in links between the collagen fibers and hydroxyapatite crystals could be the cause of dentin microhardness reduction [7] In a search for novel intracanal medicament, N-acetylcysteine (NAC) caught attention. N-acetylcysteine (NAC) is derivative of amino acid L-cysteine, with antioxidant properties and widely used as mucolytic agent in medical treatments [8,9. In addition, NAC has been actively studied by clinical microbiologists due to its high antibacterial activity, including against biofilm phenotypes, such as E. faecalis[10]. It has been reported that NAC reduces a self-produced polymeric matrix production, preventing bacterial adherence to surfaces, inhibiting biofilm formation and disrupting mature biofilms [11–13].When new products are first released onto the market, laboratory tests are essential to study their effect on dental tissues [14]. The evidence of mineral loss or gain

in dental hard tissues could be indirectly estimated by microhardness measurement [15,16]. Since there is nonsufficient information about the effect of N-acetylcysteine (NAC) on fracture resistance of root dentin over time period, this study was conducted. Therefore, the aim of this study was to evaluate and compare the effect of N acetylcysteine and Calcium hydroxide on the microhardness at the coronal, middle, and apical thirds of root dentin at 2 weeks and 4 weeks period when used as an intracanal medicament.

Materials And Methods

Preparation of tooth specimens

A total of 30 extracted single-rooted premolar teeth with single canals were selected for this study. They were collected from adult patients requiring extractions for orthodontic purpose. Teeth with any signs of previous root caries, cracks, curved or blocked canals were excluded. Teeth were thoroughly cleaned from any soft tissue or calculus deposits then they were stored in saline solution at room temperature till the time of use. Teeth were decoronated transversally at the cemento-enamel junction(CEJ) with a double-faced diamond at low speed with water coolant to ensure a uniform sample length of 12 mm.

Canal Preparation

Working lengths were established by inserting a size 15 K-fle (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 using Neo Endorotary flesh (Dentsply, sirona) till size #30 6%.Canals in all groups were irrigated with a standardized volume of 2 mL of 2.5% sodium hypochlorite between each file. The canals were finally rinsed with saline to remove any dentine debris remaining in the canal after instrumentation. Root canals were then dried with paper points.

Placement of intracanal medicament

The teeth were then randomly assigned to 3 groups (n=10), based on the treatment Group 1: Control group: no medicament; Group 2: Calcium hydroxide intracanal medicament and Group 3: N-acetylcysteine. Both the Calcium hydroxide powder and the N-acetylcysteine were mixed with sterile saline solution 1 gm: 1mL proportion respectively over a sterile glass plate until smooth and homogenous. The paste was inserted into the root canal using a K-file#30 in samples of subgroup (A) for 2 weeks and in samples of subgroup (B) for 4 weeks. The canal orifices were sealed using a temporary dressing.

Determination of microhardness

Specimens were longitudinally sectioned in a bucco-lingual direction after complete mechanical preparation. Sectioning was done using a double-faced diamond disk at low speed, by making indentations on either side, without passing through the canal space. This was followed by using a chisel and mallet to split the root. The root segments were then horizontally embedded in auto polymerizing acrylic resin leaving their dentin surface and canal exposed. The dentin surface of the mounted specimens was ground flat and smooth with a series of ascending grades of abrasive papers under distilled water.

Figure 1: Dentin microhardness determination in each specimen with a Vickers hardness tester

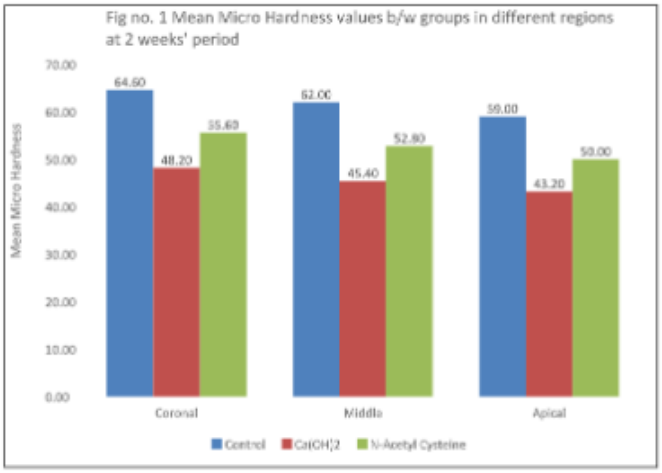
Dentin microhardness was determined in each specimen with a Vickers hardness tester [(Figure 1). The indentations were made with a Vicker's diamond indenter using 200 gm load and a dwell time of 15 seconds. The indentations were done at 0.5 mm level from the root canal spaces at coronal, middle, and apical thirds, and their means for each sample were calculated at two weeks and four weeks for the respective subgroups. The diamond-shaped indentations were carefully observed & average length of 2diagonals was used to calculate the microhardness.

Statistical Analysis

One-way ANOVA Test followed by Tukey's Post hoc Test was used to compare the mean Micro hardness values at 2 & 4 weeks' period at different regions between 3 groups. Student Paired t Test was used to compare the mean Micro Hardnes.

Results

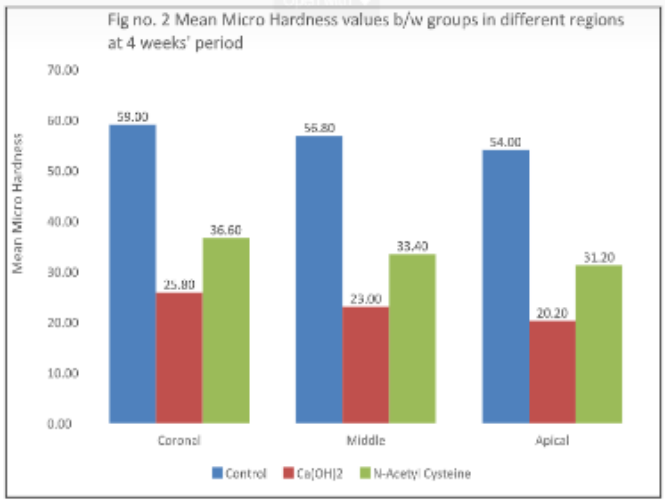
Both intracanal medicaments reduced the root dentin microhardness when compared to the control over periods of 2 and 4 weeks. The differences in the mean micro hardness between 3 groups in coronal, middle and apical region were statistically significant. Multiple comparison of mean difference between groups revealed that Control group showed significantly highest mean micro hardness as compared to Ca(OH)2 group and N-Acetyl Cysteine group. This was then followed by N-Acetyl Cysteine group showing significantly higher mean micro hardness as compared to Ca(OH)2 group at 2 weeks period. (Graph 1).



Graph 1: Mean Micro Hardness values between groups in different regions at 2 weeks period

The difference in the mean micro hardness between 3 groups in coronal region was statistically significant. Multiple comparison of mean difference between groups revealed that Control group showed significantly highest.

mean micro hardness as compared to Ca(OH)2group and N-Acetyl Cysteine group. This was then followed by N-Acetyl Cysteine group showing significantly higher mean micro hardness as compared to Ca(OH)2 group at 4 weeks. (Graph 2)



Graph 2: Mean Micro Hardness values between groups in different regions at 4 weeks period

The test results showed that the mean micro hardness at 4 weeks' period in Control group at Coronal, Middle &

Apical region showed relatively lesser values [59.00 ± 6.60, 56.80 ± 7.01 & 54.00 ± 6.63] as compared to 2 weeks' period [64.60 ± 2.07, 62.00 ± 2.45 & 59.00 ± 2.55]. However, the mean difference in the micro hardness between 2 & 4 weeks in all the regions in control group was not statistically significant.

Discussion

Intracanal medicaments may negatively affect important physical properties of root dentine, such as microhardness [17]. Microhardness is a measurement indicating mineral gain or loss in dental hard tissues and is measured by an indenter that penetrates microscopic areas [15,16]. Root dentine microhardness is often correlated with mineral concentration.

Decreased microhardness reflects demineralization of root dentine, and vice versa. A decrease in root dentine microhardness reflects softer root dentine that negatively.

affects the sealing ability of obturation materials, leading to a compromised prognosis of the endodontically treated tooth [17]. Calcium hydroxide (CH) paste has been commonly used as an intracanal medicament due to its high alkalinity and proteolytic action which in turn eliminates bacterial toxins [6,18]. However, the use of intracanal medicaments may negatively affect the physical and mechanical properties of radicular dentin [19]. The use of triple antibiotic paste (TAP) and Ca (OH)2 was found to significantly reduce dentin flexure strength, microhardness, and root resistance to fracture [19-21]. Furthermore, these medicaments were also found to affect the chemical structure of dentin. [22]

According to a previous study by Hosoya et al, peak pH change using calcium hydroxide with an aqueous vehicle is found after 14 days, after which pH declines over time [23]. Therefore, it is suggested that the time required for optimum intracanal activity when using CH with an aqueous vehicle is at least 14 days. In another study, by Uluso et al. the CH and NAC powders were handled with saline and intracanal dressing for all groups and maintained for 14 days the study showed the effectiveness of NAC similar to calcium hydroxide. [24] Hence the minimum time period in the present study was kept at 2 weeks. The microhardness measurement was performed in three points at the coronal, middle, and apical third of the root canal dentin. The mean Vickers hardness number (VHN) was calculated for each specimen. The microhardness of the dentin depends on the tubular density, which varies from one area to another on the root dentin surface. Therefore, the current study design followed Pashley et al., [15,16] who stated that the tubular density affects microhardness - as the tubular density increases, the dentin microhardness decreases. The selection of the Vickers microhardness tester over the Knoop hardness tester was due to the suitability and practicality of the Vickers test for evaluating surface changes of deeper dental hard tissues. Yassen et al reported that the application of CH for 1 week may result in significant superficial collagen degradation or demineralization of radicular dentin, which leads to an increase of fragility and a reduction of toughness.[19] They also concluded that a 3-month application of CH paste medicaments significantly reduced the root fracture resistance of teeth when compared with a 1-week application .The results obtained were in agreement with the present study in which a significant reduction was observed overtime. Yilmaz et al assessed the effect of various endodontic regeneration agents on the microhardness of radicular dentin after time intervals and concluded that no difference was seen between the control and CH groups after 1week, but a significant reduction of microhardness was observed after 4 weeks of evaluation[25], which was

consistent with the results of the present study in which the dentin microhardness of the CH group was higher after2 week (42.85) but a significant reduction(33.0)was seen after the 4-week follow-up. Yoldas et al also assessed dentin microhardness at 1, 3, and 7 days after CH application and concluded that the use of CH combinations for intracanal dressing softens dentin,[21] which can also be concluded from the presentstudy. This could be attributed to CH (pH 12.5)molecules having a highly alkaline inorganic structure, and because of their small size, they can penetrate the intrafibrillar structure of the mineralized collagen fibrils and alter the 3-dimensional conformation of tropocollagen. As a result, it reduces the elastic modulus and microhardness of dentin[21].Rajakumaran et al, in their study used NAC as a root canal irrigant and evaluated its effect on the microhardness of dentine of which the results showed lowered reduction than the experimental group EDTA[26]. N-acetyl cysteine induced a lower reduction in root dentin microhardness than EDTA and this could beowing to its softer chelating nature and lower depth of demineralization. This reasoning might be the possible explanation for the lower reduction in microhardness than the CH group in the present study. Further studies should be carried out to evaluate the current results with the use of NAC as an intracanal medicament.

Conclusion

The results of the current study substantiated that NAC caused a significantly lesser reduction in the microhardness of the root dentin in comparison with the commonly used intracanal medicament CaOH. Hence, with further studies, NAC can be explored to serve the purpose of a potent intracanal medicament in root canal therapy with its well-known benefits that favor bacterial eradication.

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