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Effect of Addition of an Anti-Oxidant in Calcium Hydroxide and Chlorhexidine, When Used in Combination as an Intra-Canal Medicament on Fracture Resistance of Root Dentin, at Two Different Time Intervals: An In-Vitro Study

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Abstract: ***During endodontic treatment, if intracanal medicaments- calcium hydroxide (CH) & chlorhexidine (CHX) were used for longer periods of time, they reduce the fracture resistance of dentin. Aim-The aim was to analyse the effect of an antioxidant in improving the fracture resistance of root dentin when mixed with CH&CHXat two different time intervals. Methodology- Forty single-rooted extracted human premolars were collected, cleaned and instrumentation was done till ProTaper F2. The canals were irrigated with 3% NaOCl and samples were divided into two groups. Group 1 - mixture of 1.5 g of CH & 1 ml of 2% CHX (CH-CHX), group 2 - mixture of 1.5 g of CH, 1 ml of CHX & 1 ml of 5% LP solution (CH-CHX-LP). After storage period of 1-week and 1-month, middle 8 mm root cylinder was sectioned & tested for fracture resistance.Statistical Analysis was carried out using Student t tests& ANOVA. Results- The mean fracture resistance of Group 1 after 1 week was 489.72 & after 1 month was 336.88 while the fracture resistance of Group 2 was 469.02 after 1 week and 409.00 after 1 month (p+<0.001). Conclusion- Addition of an anti-oxidant in CH-CHX combination maintain the strength and increase the longevity of the endodontically treated teeth.***

Keywords: Fracture resistance, Reactive oxygen species (ROS), Calcium Hydroxide, Chlorhexidine, Lycopene

1. Introduction

The primary cause of endodontic diseases is the presence of bacteria and their toxins in the root canal systems. Hence, reduction of the bacterial populations and their by-products to levels compatible with peri-radicular tissue healing and prevention of apical periodontics are the primary goal of endodontic treatment. This is achieved with instrumentation and use of appropriate irrigation regimen. Instrumentation and irrigation procedures have improved noticeably over the years; however, none of the existing techniques can completely clean the root canal system owing to the complexity of the root canal systems. Studies suggest that about 35% or more of the canal surface area remain unchanged, reducing the success rate to 68% of root canal treatment.[1] An approach for supplementing the disinfecting effects of conventional chemo-mechanical procedures with intra-canal medication has been recommended.[2]Calcium hydroxide (CH) has been widely used as an intracanal medicament in endodontic therapies.[3] It is a universally accepted inter-appointment intracanal medicament because of its biocompatibility, antimicrobial properties, and tissue dissolution ability.[4] Although calcium hydroxide is proven to be good intra-canal medicament in reducing the microbial growth,[5] literature have shown that there is decrease in mechanical properties of radicular dentin when calcium hydroxide is used as intra-canal medicament for more than 5 weeks.[4, 6,7]Chlorhexidine (CHX) is another intra-canal medicament that is routinely used in endodontics for reducing microbial growth.[8] It has properties such as substantivity and broad antibacterial spectrum. They are also used as an intra-canal medicament alone or combined with other medicaments (i.e. calcium hydroxide - CH).[9]In the ex vivo experiments, it was found that CHX may induce reactive oxygen species (ROS) production in the alkaline environment (saturated $Ca(OH)_2$solution).[10]So, although this combination has proven to be synergistic in decreasing the microbial growth, it has negative effect on fracture resistance of radicular dentin.[11]Addition of anti-oxidants may reduce reactive oxygen species. Very few studies have been undertaken to use antioxidants with intra-canal medicaments to improve fracture resistance of radicular dentin against the reactive oxygen species.The aim of this in-vitro study was to analyse the effect of an antioxidant in improving the fracture resistance of radicular dentin when mixed with calcium hydroxide and chlorhexidine as an intra-canal medicament, at two different time intervals (1 week and 1 month).

2. Methodology

2.1 Data Collection

Forty mandibular single-canaled premolars extracted for orthodontic reasons were selected for thisstudy. Teeth with cracks, caries, restorations and previous endodontic treatments were excluded. After extraction, the soft tissues, dental calculus, and stains were immediately removed from teeth and stored in normal saline at 4°C for further use.

2.2 Sample Preparation

An endodontic access cavity was prepared in each tooth and the working length was determined by visualizing the tip of a size 15 k-file at the apical foramen and subtracting 1 mm from the length of the file. Later, cleaning and shaping of root canals was done using ProTaper instruments till F2. Canals were irrigated with 1 ml of 3% NaOCl during use of each succeeding file. Further the canals were finally rinsed with sterile saline to remove any dentin debris remained in canal after instrumentation.
Samples thus obtained were randomly divided into two groups, (n=20).

Group 1 (CH-CHX): combination of 1.5 g of CH powder and 1 ml of 2% CHX as an intra canal medicament.

Group 2 (CH-CHX-LP):combination of 1.5 g of CH powder and 1 ml of 2% CHX as an intra canal medicament and1 ml of 5% LP solution was added to this mixture. About 5% LP solution was prepared by mixing 5 g of LP, which is in the form of powder to 100 ml of distilled water.

The experimental intra-canal medicament paste was applied into the root canals with a sterile lentulo-spiral in a slow speed hand piece and tamped on the canal space up to the cemento-enamel junction level. Later, the access cavities of all the samples were sealed with glass ionomer cement. The samples were sealed apically with flowable composite. Each group was further divided into two sub-groups (n=10), depending on storage period for 1 week and 1 month. After storage period, roots were sectioned with a diamond disc and straight hand piece at low speed, from coronal and apical side to obtain middle 8 mm of root cylinder. Each root cylinder was irrigated with distilled water to remove the medicaments.

2.3 Fracture Testing

Each root cylinder was subjected to the Universal Testing Machine (UTM; Instron) to evaluate the fracture resistance. The root cylinder was positioned vertically on lower fixed platform of UTM using a double-sided adhesive tape.[17] A loading fixture was lowered until the tip rested on root cylinder. Then, a vertical loading force was applied at a crosshead speed of 1 mm/min until the root cylinder fractured. The load at fracture was recorded and expressed in Newton units.

The values obtained were subjected to statistical analysis using Student t tests (two tailed, paired), Analysis of variance (ANOVA) test. The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data.

3. Results

The results of our study revealed that at one-week time interval themean values of fracture resistance of Group 1 (CH-CHX) was 489.72 and for Group 2 (CH-CHX-LP) 336.88 whereas after 1 month, 469.02 for group 1 and 409.00 group 2 respectively. The difference in mean values was highly significant at both the intervals, but at one-week interval, CH-CHX combination had better fracture resistance while after one-month interval, CH-CHX-LP combination showed higher resistance.

Table 1: Represents comparison of fracture resistance in terms of {Mean (SD)} at different time intervals using paired t test

Group	*Time interval*	*N*	*Mean*	*Std. Deviation*	*t value*	*P value*
Group 1 (CH-CHX)	*1 week*	10	489.720	11.5016	26.001	<0.001**
	1 month	10	336.880	14.9944		
Group 2 (CH-CHX-LP)	*1 week*	10	469.020	16.3162	9.604	<0.001**
	1 month	10	409.000	10.4810		

(p<0.05 - Significant*, p < 0.001 - Highly significant**)

Table 2: Represents comparison of the mean difference (1 week – 1 month) of fracture resistance in terms of {Mean (SD)} using ANOVA test

Group	*N*	*Mean*	*Std. Deviation*	F value	P value
Group 1 (CH-CHX)	10	152.8400	18.58866		
Group 2 (CH-CHX-LP)	10	60.0200	19.76325		
Total	20	106.4300	48.69595		

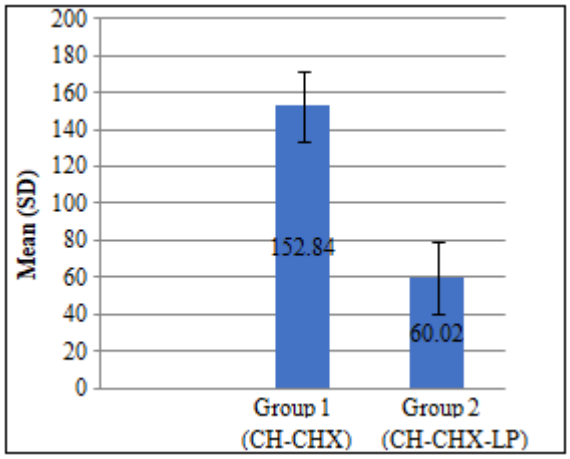

Figure 1: Represents comparison of the mean difference (1 week – 1 month) of fracture resistance of radicular dentin after using different Intracanal medicaments in terms of {Mean (SD)} using ANOVA test

4. Discussion

This in-vitro study was carried out to investigate whether the addition of natural antioxidant to intracanal medicament mixture has any influence on fracture resistance of radicular dentin. Mature permanent teeth were chosen in the study as an increase in frequency of fracture with immature teeth has been reported because of incomplete root development and

subsequent thinner dentinal walls.[12] Instrumentation of samples was done uptoproTaper file of size F2 for better penetration of irrigant and intra-canal medicament. Various studies have proven that larger sized apical preparations have a positive impact on mechanical efficacy of root canal irrigation especially in apical third.[13] To simulate the clinical conditions 3% sodium hypochlorite was used as an irrigant between the succeeding files during cleaning and shaping procedure of all the samples.[11]

The experimental design of sectioning the middle 8 mm root cylinder of the specimens was chosen to establish a baseline for measurement. The experimental set up of this study is similar to that described by Koppolu et al(2015).[11]The advantage of the sectioning is firstly, the standardization of size and secondly confirming the canals to be free of debris before the required to fracture the sample. The Fracture resistance of radicular dentin of each sample was thus recorded and data was subjected for statistical analysis. intra-canal medicament is removed. These 8 mm root cylinders were subjected to Universal Testing Machine to obtain the values of load.

The results of our study revealed that the Group 1 (CH-CHX) had maximum value of fracture resistance than Group 2(CH-CHX) after one-week time interval, but after one-month time interval, Group 2 had maximum value of fracture resistance. Statistical difference between the mean values was highly significant at both the intervals.

The reasons for reduction in fracture resistance of root dentin after I month of placement of CH-CHX intra-canal medicament may be explained on the basis of properties of $Ca(OH)_2$. Long-term exposure to $Ca(OH)_2$ will change the organic matrix of tooth structure, and therefore alters the mechanical properties of dentin. It has been shown that $Ca(OH)_2$ tends to dissolve soft tissue, and this action takes place by denaturation and hydrolysis of the pulp tissue.[4, 7] The interaction of collagen fibres and hydroxyapatite crystals is disrupted by the exposure to $Ca(OH)_2$ and so alters physical properties of dentin.[14]

Another reason for decrease in fracture resistance after I month time-interval could be attributed to CHX. Although CH-CHX combination has proven to be synergistic in decreasing the microbial growth, it has negative effect on fracture resistance of radicular dentin. This may be mainly because of the release of reactive oxygen species (ROS) by CHX in an alkaline environment.[11]This is probably due to the formation of hydroxyl ion which is the dissociation product of $Ca(OH)_2$ at an alkaline pH.[10]

Literature supports that there was decrease in fracture resistance of radicular dentin when CH was used as intracanal medicament. In the systematic review by Yassen and Platt many authors quoted that strength of radicular dentin was significantly decreased at a period of >1-month. So, time would be required for CH to penetrate into dentin and denature the collagen fibrils making dentin susceptible to fracture.[6]

The results of our study are in accordance with various studies [4,6,7,15] as far as the time interval is concerned, though there were some differences in the study designs like, Andreasen et al used immature mandibular sheep incisors, whereas in our study, we used mature human premolar teeth. Another difference was that in all these studies only $Ca(OH)_2$ was tested whereas we used the combination of CH-CHX.

The higher values of fracture resistance in Group 2 are attributed to addition Lycopene (LP) in the medicament paste with combination of CH and CHX.

Lycopene, a carotenoid compound, is a natural pigment synthesized by plants. It is a tetraterpene assembled from eight isoprene units composed entirely of carbon and hydrogen, containing 11 conjugated and 2 non-conjugated carbon-carbon double bonds (c = c). Lycopene exerts potent anti-inflammatory effects through its action as an antioxidant and free radical scavenger. Lycopene is naturally accumulated in ripe tomatoes, watermelons, red chillies, and guavas, giving them their characteristic red color. Tomato and tomato-based foods account for more than 85% of lycopene. Due to its potent antioxidant potential, lycopene obtained from tomato extract was taken up for the study. Since it was used on the external surface of the hard tissues, a minimum concentration of 5% lycopene extract was chosen for this study.[16]

In the present study when Lycopene (LP) was added to the mixture of CH and CHX, fracture resistance of radicular dentin was higher at 1-month interval than that of without addition of Lycopene (LP) to the mixture of CH and CHX. This can be explained by the free-radical scavenging property of Lycopene. The quenching of ROS by LP may be due to its structure containing high number of conjugated double bonds, which has the maximum oxygen-quenching ability. This is a physical quenching process that is, the carotenoid remains intact and can undergo further cycles of single oxygen quenching. Further, LP effectively deactivates the electronically excited sensitizer molecules that are involved in the generation of radicals and singlet oxygen.[17]

The results of our study are in alignment with this study, but interestingly, in our study after 1-week time interval the values of fracture resistance of Group 2 (CH-CHX-LP) were lower than Group 1 with CH-CHX combination. Though there is no obvious reason for this observation, it may be possible the production of ROS may be less when CH-CHX is kept in the canal for shorter duration and it increases with longer time-interval. This may result in less oxidative stress and less destruction of dentin in short time-interval. Hence addition of Lycopene may not have been so beneficial at 1-week time-interval. Another possibility is that the concentration of Lycopene penetration at site is less initially and increases with time. Hence the fracture resistance of CH-CHX-LP may have been decreased at 1-week than at 1-month.

5. Conclusion

Within the limitations of this in vitro study it can be concluded that exposure to CH-CHX for an extended time weakens root structure and increases the fragility of teeth. Addition of antioxidants like Lycopene can help in

maintaining the strength of root dentin. However, the results are more encouraging and need to bevalidated with a larger sample size, long-term evaluationwith *in vitro* models and randomized control trials beforeextrapolating the results into the clinical scenario.

6. Conflict of Interest

Authors have no conflict of interest

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