Section A-Research paper

ISSN 2063-5346

In-vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine, ozonated water and double antibiotic paste used as an intracanal medicament

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Abstract

Background and Objective: The aim and objective of this study are to evaluate and compare the antimicrobial efficacy of ozonized calcium hydroxide, calcium hydroxide with chlorhexidine and double antibiotic paste when used as intracanal medicament.

Materials and methods: 30 extracted single-rooted, single canal human teeth were selected for the study. Teeth were decoronated and the length was standardized to 13mm. After cleaning and shaping of the canals, teeth were inoculated with E. fecalis and samples were collected before placement of medicament. Teeth were then divided into 3 groups. Group A: Calcium Hydroxide + Chlorhexidine; Group B: Calcium Hydroxide + Ozonized water & Group C: Double antibiotic paste. Microbial samples were collected after 48 hours and were evaluated.

Results: Kruskal Wallis test followed by Dunns Post hoc test were used for analysis, with level of significance P<0.5. Group A demonstrated significantly lesser CFUs/ml values as compared to Group B & Group C & the difference was statistically significant at p=0.04 & p=0.001 respectively. This was followed by Group B showing relatively lesser mean CFUs/ml as compared to Group C and however the mean difference was not statistically significant [p=0.17]. This infers that the mean CFUs/ml value was significantly lesser in Group A, followed by Group B and higher in Group C

ISSN 2063-5346

Conclusion: Calcium hydroxide mixed with chlorhexidine followed by calcium hydroxide mixed with ozonised water is an effective intracanal medicament against E.fecalis when compared to double antibiotic paste.

Clinical Significance: ozonated water can be considered a vehicle to carry calcium hydroxide as an intracanal medicament

Keywords: Calcium Hydroxide, Chlorhexidine, Double antibiotic paste, Intracanal medicament, Ozonized water.

Introduction: Microorganisms play an important role in the initiation and perpetuation of root canal infection. Even though there are a variety of microorganisms present in the root canal system, the predominant species that are found are obligate and facultative anaerobes. This is because of bacterial interactions and varying levels of oxygen in the canals. Hence the elimination of microorganisms becomes the priority for the successful outcome of endodontic treatment.[1] It is reported that even after chemomechanical preparation, microorganisms survive and thrive in the root canal. These bacteria multiply in the root canal during inter-appointment periods. Hence, intracanal medicaments play an important role in reducing this bacterial load, favoring the healing. [2,3]

Since its introduction in 1920 by Herman, Calcium hydroxide has been widely used in endodontics and is acknowledged as one of the most effective antimicrobial dressings. [4]
However, calcium hydroxide is not effective against all the bacteria. Several studies have reported the failure of calcium hydroxide to eliminate enterococci effectively, as they tolerate high pH values. Combined medicaments are used as intracanal medicaments to have additive or synergistic effects. Most commonly, calcium hydroxide mixed with 2% chlorhexidine gel has been used.[5-7] The cationic molecule of chlorhexidine binds to negatively charged bacterial cell wall leading to alteration of cell osmotic equilibration.[8]

Ozonated water is a known antimicrobial agent that oxidates cell walls and cytoplasmic membranes of bacteria, fungi, protozoa and viruses. The efficacy of ozonated water as a root canal irrigant has been studied and has been found to be efficacious against E. fecalis and Streptococcus mutans.[9] Double antibiotic paste, a combination of metronidazole and ciprofloxacin has been used as an intracanal medicament. Most commonly used in regenerative endodontics, it has been seen to be effective against various root canal microorganisms, including E.fecalis.[10,11]

Materials:

Selection and standardization of teeth: Thirty freshly extracted single-rooted human teeth with a single canal, verified by digital radiographic image, were used for the study. The study was submitted and approved by the Ethical committee. Root surfaces were cleaned using Gracey curette and all teeth were stored in distilled water until use.

Inclusion criteria for teeth selection were: no gross caries or fractures, patent canal, complete root formation, no external or internal root resorption, and no previous endodontic treatment done.

Crowns of all selected teeth were sectioned using a diamond disc (Strauss diamond disc, 0.17). The length of all specimen were standardized to 13mm. Size #10 K file (Mani, Inc, Japan) was inserted into the root canal till the tip of the file was visible at the apical

ISSN 2063-5346

foramen under the operating microscope(4X). The file was then withdrawn 1mm and working length was estimated. The root canal orifice was enlarged using orifice shaper rotary file (SX, Protaper Gold, Dentsply). Root canals were manually enlarged to size 20, K file (Mani, Inc, Japan) followed by the enlargement of the canals with Protaper Gold rotary files. The last file used was finishing file F3 (Protaper Gold, Dentsply), to maintain the standardization of the root canal. Irrigation was done using 2.5% sodium hypochlorite solution for each file used. Finally, the root canals were irrigated with 17% EDTA and physiologic saline solution as final rinse. Each specimen was stored in saline solution before the experiment. The apical foramen and external surfaces were sealed. The specimens were sterilized in an autoclave at 121^{o} C for 15 minutes.

The root canals were contaminated with strains of E. fecalis (ATCC 29212) using an insulin syringe. The samples were incubated at 37oC for 21 days in an incubator. Every once in 3 days, brain heart infusion broth containing the microbial sample was added. After 21 days, the first sample was collected. Sterile paper points were placed in the root canal for 1 minute and transferred to Eppendorf tubes containing sterile saline. The specimens were randomly divided into 3 groups (n=15).

Group 1: Calcium hydroxide powder (Prevest DenPro, India) combined with chlorhexidine 2% (Consepsis, Ultradent) (n=15)

Group 2: Calcium hydroxide powder combined with ozonized water. (n=15)

Group 3: Double antibiotic paste (n=15)

The double antibiotic paste was freshly prepared by mixing antibiotic powders of metronidazole (Metrogyl 400mg tablet, JB Chemicals and Pharmaceuticals) and ciprofloxacin (Ciplox 500mg tablet, Cipla India) with propylene glycol. Lentulospirals were used in slow speed handpiece to place the intracanal medicament into the canals. The root canal orifices were sealed with temporary cement (Cavit G, 3M, ESPE). The samples were then incubated at 37oC for 14 days. After this period, a second sampling was performed. The first and second samples were transferred to blood agar plates, within 1 hour of collecting the sample. These were then incubated at 37^{o}C for 48hrs. The number of viable E. fecalis cells was obtained, and colony -forming units per ml were measured. Results were statistically analyzed.

Statistical analysis: The results obtained were statistically analyzed using Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp. Descriptive statistics include expression of CFUs levels of E. faecalis in terms of mean and standard deviation (SD).

Inferential Statistics: Kruskal Wallis test followed by Dunns Post hoc test were used to compare the mean CFUs between 3 study groups. The level of significance [P-Value] was set at $P<0.05$.

Results: Mean comparison of bacterial counts obtained after application of intracanal medications showed significant reduction (Table 1). Group A demonstrated significantly lesser CFUs/ml values as compared to Group B & Group C & the difference was statistically significant at $p=0.04$ & $p=0.001$ respectively. This was followed by Group B showing relatively lesser mean CFUs/ml as compared to Group C and however the mean difference was not statistically significant [$p=0.17$]. This infers that the mean CFUs/ml value was

ISSN 2063-5346

significantly lesser in Group A, followed by Group B and higher in Group C. When Group B and C were compared, Group B had greater reduction in bacterial count than GroupC (Table 2).

Comparison of mean CFUs/ml [in log10 values] b/w 3 groups after application of Intra canal Medicaments using Kruskal Wallis Test

Groups	N	Mean	SD	Min	Max	p-value
Group A	15	3.108	2.653	0.00	8.71	0.005*
Group B	15	5.847	2.727	1.43	10.48	
Group C	15	8.088	3.102	3.34	13.98	

The test results demonstrate that the mean CFUs/ml [in log10 values] for Group A was 3.108 ± 2.653, Group B was 5.847 ± 2.727 and Group C was 8.088 ± 3.102. This mean difference in the CFUs/ml [in log10 values] between 3 groups after application of Intra canal Medicament was statistically significant at p=0.005

Comparison of mean CFUs/ml [in log10 values] before & after application of Intra canal Medicament in each group using Wilcoxon Signed Rank Test

Groups	Time	N	Mean	SD	Mean Diff	p-value
Group A	Before Medicament	15	20.787	4.056	17.679	<0.001
	After Medicament	15	3.108	2.653		
Group B	Before Medicament	15	21.515	6.268	15.668	<0.001
	After Medicament	15	5.847	2.727		
Group C	Before Medicament	15	22.955	5.411	14.866	<0.001
	After Medicament	15	8.088	3.102		

The mean CFUs/ml [in log10 values] in all the 3 study groups showed significantly lesser values after application of Intra canal medication as compared to before application time and the mean differences observed in all the groups were statistically significant at p<0.001.

Discussion: The results of this in vitro study showed that calcium hydroxide mixed with chlorhexidine showed a significant reduction in E. fecalis. However, calcium hydroxide mixed with ozonated water showed a reduction in E. fecalis counts when compared to double antibiotic paste as intracanal medicament.

ISSN 2063-5346

Calcium hydroxide has been widely used in the field of endodontics due to its various biologic properties. It has antimicrobial activity, tissue dissolving ability, inhibition of tooth resorption and induction of repair by hard tissue formation. [2,12-15]

The antimicrobial activity of calcium hydroxide is related to the release of hydroxyl ions in an aqueous environment.[16] For the effective action of calcium hydroxide, the hydroxyl ions must diffuse through dentine and pulpal remnants. An aqueous suspension of calcium hydroxide has a high cytotoxic potential.[17 18]

Various vehicles have been used to carry calcium hydroxide inside the root canal. Since endodontic infections are polymicrobial, combining two medicaments can produce additive or synergistic effects. Chlorhexidine has been shown to reduce bacterial count because of its ability to adsorb dentin, and its action on bacterial cell walls and cytoplasmic membrane, leading to loss of osmotic balance, leakage of intracellular material and cell death. Various studies have shown the efficacy of 2% chlorhexidine gel when used alone or in combination with calcium hydroxide against many root canal microorganisms.[19-21]

Studies have also proven the synergistic effect of calcium hydroxide and chlorhexidine having a higher antibacterial effect than when calcium hydroxide was mixed with saline.[22-23] The results of this study is in agreement with the above mentioned studies, wherein there is a significant reduction of E. fecalis.

The application of ozone has gained popularity in endodontics because of its antibacterial effect. Ozone is a triatomic highly unstable compound that has a very short half life. Ozone gas produced in a clinical setting has high oxidation potential and is 1.5 times more effective than chloride. It has been found that ozone has antibacterial effect on E.fecalis. [24] The efficacy of antibacterial effect of ozone as root canal irrigant has been evaluated, and found to be effective against various root canal microorganisms.[25]

In this study, ozone water was used as a vehicle to carry calcium hydroxide as an intracanal medicament. This combination had a significant reduction in the CFU/ml of E. fecalis. However, the efficacy was found to be lesser than the combination of calcium hydroxide mixed with chlorhexidine. This could be attributed to the short half life of ozone and the better substantivity of chlorhexidine. [26] In search for obtaining a sterile root canal system, various antibiotics have been used as intracanal medicaments. Triple antibiotic paste, double antibiotic paste, and amoxicillin-clavulanate are a few that have been evaluated for their efficacy. Due to the drawback of discolouration of minocycline in triple antibiotic paste, double antibiotic paste gained popularity. Double antibiotic paste has a combination of metronidazole and ciprofloxacin. Various studies have shown a reduction in the bacterial count as well as a significantly longer residual antibacterial effect with double antibiotic paste [27,28] In this study, a reduction in E. fecalis was seen after the application of double antibiotic paste. However, the efficacy was lesser when compared to the other two groups.

Conclusion: Within the limitations of this study, it can be concluded that ozonated water can be used as a vehicle to carry calcium hydroxide as an intracanal medicament. There was significant amount of reduction in number of CFU/ml of E. fecalis. However, 2% chlorhexidine gel remains the vehicle of choice according to the results of this study.

ISSN 2063-5346

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