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Research Article

COMPARATIVE ANALYSIS OF THE EFFICACY OF CALCIUM HYDROXIDE AND CURCUMA LONGA AS ANINTRACANAL MEDICAMENT AGAINST ENTEROCOCCUSFAECALIS

Arun Kumar, S and Rama Brahmam Lanke

Department of Conservative Dentistry & Endodontics, Mgs Dental College & Research Centre, Sriganganagar, Masters In Environmental And Occupational Health Sciences, Western Kentucky University

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ABSTRACT

Back ground: The aim of this study is to evaluate and compare the efficacy of calcium hydroxide and turmeric powder [Curcuma Longa] as an intracanal medicament against Enterococcus faecalis using real-time quantitative polymerase chain reaction (qPCR).

Material and method: A total of 20 patients were selected for the study. The study is limited to the mandibular molar teeth with adequate crown structure. Patient selection is based on the prior diagnosis of the mandibular molar teeth with pulpal necrosis and periapical lesion that are suggested as ideal candidates for the root canal treatment. On the basis of the intracanal medicament used in the root canal space i.e., calcium hydroxide or Curcuma longa, the patients were then randomlycategorized into two groups (n=10). During the course of the study three different samples were collected (S1, S2, S3) per tooth at three different procedural intervals. The first sample is collected after the access opening of the tooth and is namedas S1, the second sample is collected after the chemo-mechanical preparation and irrigation with sodium hypochlorite and is named as S2, the third and the final sample is collected after 7 days of intracanal medicament placement and is named as S3. The three collected samples (S1, S2, S3) were then transferred to the microbiology lab for evaluating Enterococcus faecalis using real time PCR (Polymerase Chain Reaction); The statistical analysis was done using Student's T-Test and Paired Sample T-Test.

Results- Results of the present study showed that curcuma longa (Turmeric powder) is more effective in reducing the concentration of E.faecalis than calcium hydroxide. The group medicated with the Turmeric powder showed more significant differences between the samples S1 and S3 when compared to the group medicated with calcium hydroxide.

Conclusion- In conclusion, it can be stated that Turmeric powder is more effective against Enterococcus faecalis as compared to Calcium hydroxide.

INTRODUCTION

The most important objective of the root canal treatment is to provide complete debridement of the microorganisms from the root canal space[1]. One of the main causes of failure of root canal therapy is the persistence of microorganisms in the root canal space sundquest *et al*.[2]Primary endodontic infections are poly microbial, of which gram negative anaerobic bacteria are dominated in the canal space, on the other hand secondary endodontic infections are composed of few bacterial species.[3-6]Enterococcus fecal is was found to be the most prevalent bacteria in the root canal of teeth with chronic apical periodontitis and periapical infections. Enterococci is less dependent on the virulent factors and emergence as pathogen may be resistant to anti-microbial agents.[7]The procedural steps biomechanical and chemo-mechanicalpreparation of the root canal space may reduce the bacterial population but complete elimination is not possible. The possible reason for persistence of infection may be due to retention of micro organisms in dentinal tubules.[8-13] Usage of intra canal medicament helps in the elimination of intracanal bacteria and provides a conductive environment for periapical tissue repair.[9,10]

Calcium Hydroxide as an intracanal medicament was introduced in the year 1920. The widespread use of calcium hydroxide as an intracanal medicament is because of its properties such ashigh alkalinity (ph-12.5) and high antibacterial activity. Calcium Hydroxide has destructive effect over the bacteria's cellular membrane and protein structure (elka *et al*., 2005)[14]. Even though there are a number of

**Corresponding author: Arun Kumar, S*
Department of Conservative Dentistry & Endodontics, Mgs Dental College & Research Centre, Sriganganagar, Masters In Environmental And Occupational Health Sciences, Western Kentucky University

advantages associated with its usage as an intracanal medicament there are some disadvantages too such as developing resistance to Enterococci and Yeast. Curcuma longa (**Turmeric powder**) a member of "*Zingiberaeceae*" family has been used for thousands of years as a medicinal herb in Ayurvedic, Unani & Chinese medicines. It has antimicrobial, antioxidant, astringent, and other beneficial properties. Because of the above mentioned properties, It's usage in dentistry can be effectively as well.[15]According to a study conducted by Niamsa *et al*, curcuma longais effective against the Enterococcus Faecalis, Escherichia Coli, Klebsiella pneumonia, Candida Albicans, and Candida Kruseii. [niamsa *et al*, j of pharmacology and toxicology 2009].[16]Turmeric powder can be used as an Intracanal medicament because of its antibacterial properties, ease of availability, low cost and lack of adverse effects. The material has been already evaluated in vitro, so there is a need for in vivo evaluation.

Real time quantitative polymerase chain reaction (qPCR) was introduced as a novel method to quantify bacterial specimen in biological samples providing rapid detection and quantification. It is highly specific and sensitive. The technique has been proven to be more sensitive than the conventional bacterial culture in detection of targeted bacterial species[18-23].

In order to compare the efficacy of calcium hydroxide and Curcuma longa as intracanal medicament against Enterococcus Faecalisthe study has been undertaken.

MATERIAL AND METHODOLOGY

A total of 20 patients were selected for the study. The study is limited to the mandibular molar teeth with adequate crown structure. Patient selection is based on the prior diagnosis of the mandibular molar teeth with pulpal necrosis and periapical lesion that are suggested as ideal candidates for the root canal treatment. On the basis of the intracanal medicament used in the root canal space i.e., calcium hydroxide or Curcuma longa, the patients were then randomly categorized into two groups(n=10). During the course of the study three different samples were collected (S1, S2, S3) per tooth at three different procedural intervals. The first sample is collected after the access opening of the tooth and is named as S1, the second sample is collected after the chemo-mechanical preparation and irrigation with sodium hypochlorite and is named as S2, the third and the final sample is collected after 7 days of intracanal medicament placement and is named as S3.

After the completion of diagnosis, the selected tooth is isolated using rubber dam and was disinfected with 3% hydrogen peroxide solution. This is followed by the use of 3%sodium hypochlorite solution and subsequently neutralized by 5% sodium thiosulfate. The access preparation was then performed with a sterile endo access bur. Just before reaching the pulp chamber, the second stage of disinfection was done in the similar manner. After the completion of access preparation, three sterile paper points were placed in the distal canal consecutively to a level approximately 0.5 mm short of the root apex (on the basis of diagnostic radiographs), to soak up the fluid in the canal. Each paper point was left in the canal for at least 1 minute. The paper points were then transferred aseptically to tubes containing saline. In this way sample S1 was prepared. The working length of the root canals were determined using radiographs and apex locators. This is followed by the irrigation of the root canals with 17% EDTA (Ethylene Diamine Tetra Acetic Acid Solution) to remove the smear layer. During the instrumentation, the root canals were irrigated with 2ml of 3% sodium hypochlorite. After the completion of the instrumentation, 3ml of 5% Sodium thiosulphate was used to inactivate Sodium hypochlorite and is followed by the usage of saline. The canals were later dried with sterile paper points and second sample (S2) was collected in the similar manner.

Group A: In this category of patients, Calcium hydroxide isused as an intracanal medicament. After bio mechanical preparation of the canal space, calcium hydroxide was placed in the canal space using lentulo spirals and spreaders. After the placement of medicament, the access cavity was temporarily sealed using Cavit temporary restoration.

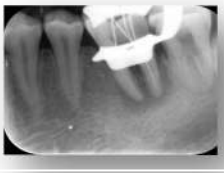
Group B: In this category of patients, Curcuma longais used as an intracanal medicament. The powder of Curcuma longais mixed with saline and made into apaste. After the completion of the biomechanical preparation of the root canal space,the paste was placed into the canal space using lentulo spirals and spreaders. After the placement of medicament, the access cavity was temporarily sealed using Cavit temporary restoration.

The patients were recalled after 7 days of medicament placement and following the same disinfection protocol the temporary dressing was removed. The medicament was flushed out using saline and the third sample S3 was taken. Teeth were later obturated using lateral condensation technique.

Table1 Comparison of Samples S1,S2, and S3 in 2 groups i.e., calcium hydroxide andCurcuma longa with respect to the with log cfu counts for E. faecalis

Groups	Time	Mean	St, deviation	Mean difference	Sd. Difference	%of change	Paired t	P-value
Calcium hydroxide	S1	4.87	0.83	0.45	0.66	7.36	1.6823	0.0129
	S2	4.42	0.34					
	S1	4.87	0.83	0.53	0.67	8.15	1.8264	0.1011
	S3	4.34	0.38					
	S2	4.42	0.34	0.08	0.16	0.85	0.7639	0.4645
	S3	4.34	0.38					
Curcuma longa	S1	5.19	0.60	0.40	0.47	9.07	3.1734	0.0134
	S2	4.79	0.53					
	S1	5.19	0.60	0.99	0.61	18.96	5.1297	0.0006
	S3	4.20	0.40					
	S2	4.79	0.53	0.59	0.54	10.88	3.0155	0.0146
	S3	4.20	0.40					
				P- <0.05				

All the three samples (S1, S2, S3) collected at three different procedural intervals were subjected to quantitative real time polymerase chain reaction to evaluate the levels of total bacteria (Enterococcus Faecalis). Statistical analysis was done using students' t test and paired t test with the significant level set at $p<0.05$.

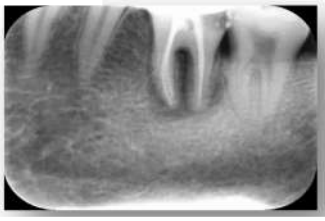


After access opening of diagnosed teeth

Collected samples S1,S2,S3.

Testing of the samples

Applied biosystem



Radiograph showing after

RESULTS

The results of the two medicaments used in this study showed antibacterial activity at 7-day intervals. The root canals of the teeth after access opening (S1 sample) were medicated with intracanal medicaments calcium hydroxide and turmeric powder. Initially both the medicaments showed significant bacterial reduction ($p<0.05$).However, inter group quantitative comparison disclosed significant differences between the medicaments used. Curcumin showed a remarkable reduction in bacteria i.e., 19% of bacterial difference from S1 to S3 sample; on contrary calcium hydroxide showed only 8% bacterial reduction from S1 to S3 sample. From the above results it can be said that Curcumin is most effective against the Enterococcus Faecalis Curcuma longa shows statistically highly significant difference in reduction of bacteria from S1(p-0.0006)and S2(p-0.01). Where as calcium hydroxide shows statistically significant difference in S1 and S3 (p-0.04) but no significant difference in S2 and S3 (p -0.46).

DISCUSSION

The presence of bacteria in the root canal space may lead to the failure of root canal treatment. Several studies have demonstrated the possible ways in which bacteria invade into dentinal tubules.[24,25] Present study is an vivo study and the bacterial count is done using real time quantitative polymerase chain reaction. Real time quantitative PCR assay also allows relative or absolute quantification of bacteria of intest including species that cannot be cultured or difficult to culture in vitro. The advantage of qPCR over conventional PCR is that this assay does not require post PCR manipulation of PCR products which results in reduced chance of carryover-contamination of the products[26,27]. Also, qPCR requires less time and laboratory work to obtain results from various types of samples.[28] Enterococcus Faecalis was chosen as a test organism in this study because it is one among the few facultative organisms associated with persistent apical periodontitis and is difficult to eliminate from the root canals. (basrani *et al* 2002, gomes *et al* 2003).[29] The reason for choosing Calcium hydroxide as a medicament in this study is because of its widespread clinical application as a medicament. For the calcium hydroxide medicament to be effective against the bacteria located within the dentinal tubules, the hydroxyl ions from calcium hydroxide should diffuse into dentin at sufficient concentrations and should exceed the dentin buffering capacity, reaching ph levels sufficient to destroy bacteria (gomes *et al* 2003, haapasalo 2000)[30]. In Endodontics, recent measures were being undertaken to use biological medication extracted from the nature, this is due to the cytotoxic reactions and inability to eliminate the bacteria completely by most of the commercial intracanalmedicaments. Besides their inability to eliminate the bacteria completely, many antibiotics were shown to develop resistance towards microorganisms. Curcuma longa is effective against the staphylococcus albusand staphylococcus aureus in concentrations up to 1:5000. In the current study, Curcuma longais shown to be more efficacious than calcium hydroxide in reducing the concentration of Enterococcus Faecalis. The group medicated with Curcuma longa showed 19% difference in samples S1(before medication) to S3(after medication), while the group medicated with calcium hydroxide showed only 8% difference. Calcium hydroxide mixed with saline was ineffective against E. Faecalis (siquera *et al*).[11]E. Faecalis'resistance to calcium hydroxide, probably is due to an effective proton pump mechanism which maintains optimal cytoplasmic ph levels. Vijay Singh *et al* conducted a similar in vitro study, and concluded that 1% chlorhexidine is more effective than Curcuma longaand calcium hydroxide is least effective against E. Feacalis. In this study, Curcuma longashows effective antibacterial affect against E. Faecalis than calcium hydroxide. The mechanism behind this behind the Curcumin against the E. Faecalis has been explained by many authors on the basis of a hypothesis which states that it is due to bacterial cell wall perturbation[lanicotti *et al* 2004].[31]

CONCLUSION

In sum, from the above discussions and documented results it can be stated that Curcuma longais comparatively more efficacious against Enterococcus faecalis than calcium hydroxide. Curcuma Longa can be used as an effective alternative for intracanal medication because of its outstanding properties which includes biocompatibility, antioxidant properties and potent inhibitor of different bacterial cultures. However, there is a need for further studies to appraise the efficacy of Curcuma longaas an intracanal medicament.

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