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COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF CALCIUM HYDROXIDE AND SILVER NANOPARTICLES AS INTRACANAL MEDICAMENT: AN IN-VIVO STUDY

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ABSTRACT The aim of this study was to evaluate and compare the antimicrobial activity of calcium hydroxide and silver nanoparticles as an intracanal medicament in patients with necrotic/infected pulp.

Materials And Methods– 30 single rooted permanent teeth diagnosed clinically and radiographically with necrotic/infected pulp were included. After complete disinfection, access opening was done and working length was determined. The first microbiological pre-treatment sample (S1) was collected by paper points. After completion of instrumentation, a post-instrumentation sample (S2) was taken and the teeth were divided into two groups: Group 1: calcium hydroxide, Group 2: Silver nanoparticles. The intracanal medicaments were left in place for 7 days. Post 1 week, S3 was taken. Samples collected were cultured on BHI agar and colony forming units were counted after 24 hours.

Results- Intergroup comparison was done using Mann Whitney U test & intragroup comparison was done using Friedman test & Wilcoxon test. It was observed that the percentage reduction was better with Group 2 (Silver nanoparticles), however, it was not statistically significant when compared to Group 1 (Calcium hydroxide).

Conclusion – It was concluded that silver nanoparticles showed significant antimicrobial activity and can be used as an effective root canal medicament as an alternative to calcium hydroxide dressing.

KEYWORDS : Antimicrobial; Intracanal medicament; Calcium hydroxide; Silver nanoparticles

INTRODUCTION

Complete debridement and adequate elimination of microbial irritants, including microorganisms and their toxins, is a fundamental prerequisite for successful endodontic therapy.[1] With current instrumentation techniques, an average of 40-50% of the root canal wall is untouched, leaving ample tissue in which microorganisms can survive and proliferate.[2] To ensure complete elimination of root canal bacteria, effective antimicrobial agents are required for a predetermined time period for predictable eradication of remaining bacteria.[3] Augmentation of the antibacterial protocol can be achieved by utilizing intracanal interappointment medicaments, which are capable of further reducing the bacterial load.[4]

Calcium hydroxide is most commonly used intracanal medicament due to its wide antimicrobial spectrum, alkalizing effect and inflammatory control.[5] Application of calcium hydroxide for a week has been shown to reduce the microorganisms which survived biomechanical instrumentation of the canal.[6] Its high pH alters the biological properties of bacterial lipopolysaccharides in the cell walls of gram negative species, thereby inactivating the membrane transport mechanisms.[7,8] The efficacy of this material depends on the penetration of hydroxyl ions into the dentinal tubules and accessory canals, where bacteria and their byproducts accumulate. Calcium hydroxide, as a physical barrier, can prevent canal reinfection and disrupt the nutritional supply of the residual microorganisms in the root canal system.[9] *E. faecalis* and *C.albicans* have been shown to be resistant to the antimicrobial effect of Calcium hydroxide because of its ability to penetrate the dentinal tubules and adapt to the changing environment.[7] Thus, the search for a better alternative is an ongoing process.

Nanomaterials may provide solutions to technological and environmental challenges in the areas of medicine including dentistry.[10] The antibacterial activity of silver nanoparticles against several species of endodontic bacteria has been reported in several investigations.[11] They provide greater contact surface in comparison with silver particles in an aggregate form; thus, a small volume of silver nanoparticles has an antimicrobial property comparable to that of much larger amount of aggregated silver.[9]

Several possible mechanisms have been proposed that involve the interaction of silver with biological macromolecules such as enzymes and deoxyribonucleic acid (DNA) through an electron-release mechanism. Silver nanoparticles have bactericidal effects resulting not only in inhibition of bacterial growth but also in killing bacteria.[10] Besides, antibacterial nanoparticles do not seem to provide the bacteria with the capacity to gain resistance. Therefore, they may serve as a promising irrigant/intracanal medicament in endodontics.[12]

The aim of this investigation was to comparatively evaluate the antimicrobial activity of calcium hydroxide and silver nanoparticles as intracanal medicaments *in vivo*.

METHODOLOGY

SELECTION OF TEETH

Thirty single rooted teeth with necrotic or infected pulp as diagnosed clinically and radiographically were selected for the study. Prior clearance of the protocol from the Ethical committee taken and the study protocol was registered with clinical trials registry of India. Written informed consent from each patient was taken.

Inclusion Criteria:

Patients with non-contributory medical history, intact permanent teeth without any previous restoration, with a necrotic or infected pulp as diagnosed clinically and radiographically, with adequate coronal structure for proper isolation, temporization, and restoration were included.

Exclusion Criteria:

Patients with systemic conditions, acute periapical abscess, retreatment cases, patients on antibiotic therapy within three months, teeth with calcified canals, sinus opening, immature apex, internal or external resorption or periodontal pockets >5 mm and pregnant women were excluded.

First Treatment Session

Oral cavity was disinfected with chlorhexidine solution. Each tooth was anaesthetized and isolated with rubber dam followed by caries removal. Access cavity was prepared and the working length was determined with an electronic apex locator. Pretreatment Sample (S1) was obtained by injecting normal saline (5ml) into the root canal and circumferentially pumping #10 K-file (Mani, Inc. MDCI Ltd, Japan) (1 mm short of working length).

A sterile paper point (Metabiomed, India) was immersed into the transport media and was placed into the canal for 60 seconds (Figure 1). It was immediately transported to the test tube containing transport media (Peptone water, HiMedia Laboratories Pvt. Ltd, India) (Figure 2). Three paper point samples were taken for each tooth. Working length was confirmed radiographically. Biomechanical preparation was done using step back technique upto master apical size #40. Saline as an irrigant was used followed by collection of post instrumentation sample (S2) in the same manner as S1.

It was sent to laboratory for processing within 2 hours. After second sampling, the canals were dried and medicaments were dispensed:

Group I: Calcium Hydroxide Paste

Calcium hydroxide powder (ProDent Ratnagiri, India) was mixed with propylene glycol (AVARICE Laboratories Pvt. Ltd, India) to obtain paste like consistency. This was carried into the canal by using lentulospiral (Dentsply Maillefer, Switzerland).

Group II: Silver Nanoparticle Dispersion

Silver nanoparticle dispersion (Nano Labs, India) was applied and kept in the root canals using a paper point. Access cavity was sealed with Cavit (Orafil–G™, PREVEST DenPro, India). The medicaments were left in place for 7 days.

After 7 days, post medication sample (S3) was taken after removing the medicament. Teeth were obturated by lateral condensation technique using gutta percha (Metabiomed, India) and restored with composite (Spectrum®, Dentsply India Pvt Ltd., india). Microbiological samples (S1, S2, S3) were preincubated for 30 minutes and shaken vigorously in a vortex mixture for 60 seconds and then were plated on Brain heart infusion agar (HiMedia Laboratories Pvt. Ltd, India) and colonies were counted after 24 hours using classic bacterial counting method (Figure 3 and 4). Observations were statistically analyzed.

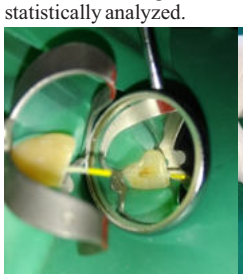

Figure 1 - Root Canal Sample Taken With A Paper Point

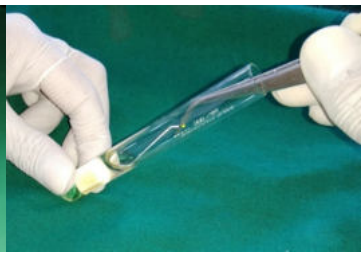

Figure 2 - Paper Point Sample Insertion Into Transport Media

Figure 3 - Digital Colony Counter

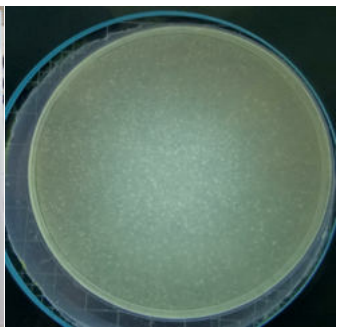

Figure 4 - Culture Plate Showing Bacterial Growth In Magnified View

RESULTS

Intergroup comparison was done using Mann Whitney U test & intragroup comparison was done using Friedman test & Wilcoxon test. The level of significance was set at 0.05. It was observed that there was significant reduction of bacterial count from stages S1 (after access opening) to S2 (after biomechanical preparation) and S3 (after medicament placement) in both the experimental groups (Table 1) The percentage reduction was better with Group 2 (Silver nanoparticles), however, it was not statistically significant when compared to Group 1 (Calcium hydroxide). (Table 2)

Table 1: Shows Mean Of Reduction Of Bacterial Count At S1, S2 And S3 For Calcium Hydroxide Paste And Silver Nanoparticle Dispersion

Reduction in colony counts X 10^5 at						
Group		S2 from S1 (1)	S3 from S1 (2)	S3 from S2 (3)	P[c] value	Post hoc pairwise comparison[c]
1	Mean	35.04	59.76	24.72	0.001, S	2>1>3
	N	15	15	15		
	Std. Deviation	42.26	111.79	68.24		
2	Mean	18.83	39.92	21.09	0.001, S	2>1,3
	N	15	15	15		
	Std. Deviation	22.63	27.39	10.28		
P[a] value		0.098, NS	0.744, NS	0.002, S		

[a]Mann Whitney U test, [b]Friedman test, [c]Wilcoxon test

Table 2: Shows Percentage Reduction Of Bacterial Count At Stages S1, S2 And S3 For Calcium Hydroxide Paste And Silver Nanoparticle Dispersion

Percentage reduction in colony counts at						
Group		S2 from S1 (1)	S3 from S1 (2)	S3 from S2 (3)	P[c] value	Post hoc pairwise comparison[c]
1	Mean	32.30	45.16	19.34	0.001, S	2>1,3
	N	15	15	15		
	Std. Deviation	19.12	20.68	18.27		
2	Mean	18.38	47.22	36.42	0.001, S	2>3>1
	N	15	15	15		
	Std. Deviation	24.72	22.05	15.62		
P[a] value		0.061, NS	0.806, NS	0.003, S		

[a]Mann Whitney U test, [b]Friedman test, [c]Wilcoxon test

DISCUSSION

Microorganisms are the main cause of pulpal and periapical diseases. Mechanical preparation of the root canal is the main mechanism to reduce the bacterial load in canals.[11] Although the mechanical instrumentation tries to debride infected canal walls, but mechanical instrumentation alone may result in incomplete cleaning of the root canal walls as it cannot eliminate microorganisms from untouched areas of the root canal system.[13] Therefore, chemical irrigants and intracanal medicaments seem necessary for eradication of infected tissues and microorganisms in addition to mechanical debridement.[14] Calcium hydroxide was introduced to dentistry by **Herman** in 1920. The antimicrobial activity of calcium hydroxide is related to the release of hydroxyl ions in an aqueous environment.[15] The hydroxyl ions alter the pH gradient of the cytoplasmic membrane damaging its protein.[16] It plays a major role as an inter-appointment dressing in the disinfection of the root canal system. It can also be used as an endodontic sealer, pulp capping agent, in apexification, pulpotomy cases and in weeping canals.[15] ***Bystrom et al***, in their in vivo study, reported that root canals treated with calcium hydroxide had fewer bacteria than those dressed with camphorated phenol or camphorated monochlorophenol (CMCP).[17] ***Shuping et al*** showed that placement of calcium hydroxide for atleast 1 week rendered 92.5% of canals bacteria free.[18] The major disadvantage of calcium hydroxide is its limited effectiveness in disinfecting dentinal tubules. Other disadvantage include difficulty in complete removal and its effect on setting reaction of zinc oxide eugenol based sealers.[19] ***Sathorn et al.*** evaluated eight clinical trials involving 257 cases and concluded that calcium hydroxide had limited effectiveness in eliminating bacteria from human root canals when assessed by culture techniques.[20]

Nanosilver is a newly manufactured product by the use of nanotechnology.[9] Nanoparticles (NPs) have a greater surface-to-volume ratio (per unit mass) than non-nanoscale particles of the same material, and therefore are more reactive.[21] Silver colloids have distinctive properties such as good conductivity, chemical stability, catalytic and antibacterial activity.[10] They possess bactericidal effects rather than bacteriostatic effect.[22] Silver nanoparticle solution has been recommended as an alternative to root canal irrigating solution not only for its strong bactericidal potential but also for its biocompatibility, especially in lower concentrations.[23] ***Wu D et al*** in their study concluded that silver nanoparticle as a medicament and not as an irrigant showed potential to eliminate residual bacterial biofilms during root canal disinfection.[23] ***Moghadas et al*** showed that nanosilver prevented the growth of common root canal bacteria.[13] ***Bo et al*** concluded that 0.1% and 0.2% nanosilver gel is more effective on *Enterococcus faecalis* biofilm compared to chlorhexidine and camphorated phenol.[24]

The results of this study demonstrated there was significant reduction of bacterial count from stages S1 (after access opening) to S2 (after biomechanical preparation) and S3 (after medicament placement) in both the experimental groups. There was no significant difference in the colony counts of both the experimental groups although the percentage reduction in colony counts was slightly better for silver nanoparticles than calcium hydroxide.

This could be explained by the smaller size and higher surface area per unit mass[5] of the silver nanoparticles because of which it penetrates the dentinal tubules deeper and at a greater concentration[25] than calcium hydroxide. Silver nanoparticles show multiple antibacterial mechanisms such as adherence and penetration into the bacterial cell wall, leading to the loss of integrity of bacterial cell membrane and cell wall permeability due to their extremely large surface area, which provides better contact with microorganisms.[23]

The bacterial membrane contains sulphur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorous containing compounds like DNA. As silver nanoparticles enter the bacterial cell, a low molecular weight region is formed in the centre of the bacteria to which the bacteria conglomerate, thus protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, inhibit cell division finally leading to cell death.[26]

Sadeghi et al. evaluated the effect of nanosilver solution on *Actinomyces viscosus* and *Streptococcus sanguinis* and showed that nanosilver solution had suitable antimicrobial properties against these species and this effect was achieved even in lower concentration.[27] ***Sotiriou and Pratsinis*** evaluated the antimicrobial effects of silver ions and nanosilver particles and concluded that the antimicrobial activity of both was similar.[28] ***Guangquan et al.*** also showed that synthesized silver nanoparticles could efficiently inhibit bacteria and fungi.[29] In contrast to our study result, ***Alabdulmohsen ZA et al*** investigated the bactericidal effect of silver nanoparticles in reducing bacterial infection in root canal when used as intracanal medicament alone or in addition to calcium hydroxide. Their results showed that the antibacterial effect of silver nanoparticles was lower than that of calcium hydroxide or combination of both the materials.[4] ***Mozayeni et al*** compared the antifungal activity of 2% chlorhexidine, calcium hydroxide and nanosilver gels against *Candida albicans*. Their results showed that calcium hydroxide and 2% chlorhexidine had better antifungal effect than nanosilver.[9] In contrast to their results, in our study silver nanoparticles showed better percentage reduction in the colony counts than calcium hydroxide though there was no statistically significant difference between them. This variation could be due to the differences in the mode of application, concentration, and particle diameter used. Further *in vivo* studies are required to better evaluate the effectiveness of silver nanoparticles as an intracanal medicament.

CONCLUSION

Therefore, within the limitations of this study, it can be concluded that silver nanoparticle has better antimicrobial activity in comparison to calcium hydroxide and thus can be considered a suitable alternative to calcium hydroxide as an intracanal medicament.

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