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Comparative Evaluation of antimicrobial efficacy of MTA mixed with different concentrations of chlorhexidine and TAP against E Faecalis.

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Abstract: *MTA, a hydraulic cement that sets in the presence of water, is used as a root-end filling material but its antibacterial property is not ideal against E. faecalis which are mainly found in retreatment cases. The aimof the study is to determine whether substitution of different concentrations of Chlorhexidine or TAP (triple antibiotic paste) solution in place of sterile water as a mixing agent will enhance the antimicrobial activity of MTA. Samples of Pro root MTA were prepared by mixing with different concentrations of triple antibiotic solutions and chlorhexidine respectively. The antibacterial efficacy was studied by placing MTA samples in BHI agar infused with bacteria E faecalis (ATCC 29212)and zone of inhibition was measured.All samples exhibited antibacterial activity. The antibacterial activity between the groups differed statistically(p=0.001). TAP solution has better results amongst all groups.*
The antimicrobial activity of MTA was enhanced significantly when mixed with TAP than CHX and water.

Keywords:*MTA, Chlorhexidine, Triple antibiotic solution, E faecalis.*

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I. Introduction

Mineral trioxide aggregate(MTA) was developed as a root-end filling material.It is a hydraulic cement that sets in the presence of water.MTA consists of 50–75% (wt) calcium oxide and 15–25% silicon dioxide. These two components together comprise 70–95% of the cement. When these raw materials are blended they produce tricalcium silicate, dicalcium silicate, tricalcium aluminate and tetracalciumaluminoferrite. On addition of water the cement hydrates to form silicate hydrate gel.Chlorhexidine (CHX) is used as anendodontic disinfectant because of its broad antibacterial action.Two percent chlorhexidine gluconate has been proven to be the most effective method to remove or completely eliminate E. faecalisfrom dentinal tubules up to 100 μm.[1,2]

Sreegowri et al [3]studied sealing ability of white and gray MTA mixed with distilled water and 0.12% chlorhexidine gluconate as a root end filling material. They concluded that Chlorhexidine appears to be a good alternative to replace distilled water, as a solution to be mixed with MTA.Triple antibiotic paste (TAP), is a mixture of metronidazole, ciprofloxacin and minocycline. It has been demonstrated that this material is effective against the majority of endodontic pathogens.Enterococcusfaecalisa normal commensal of oral cavity possess a concern for endodontist as it is associated with persistent, asymptomatic endodontic infections. The occurrence in such infections ranges from 22% to 74%.[4]Thepurposeof this study is to determine whether substitution of different concentrations of CHX OR TAP (triple antibiotic paste) solution in place of sterile water as a mixing agent will enhance the antimicrobial activity of MTA.

II. Materials And Methods

Materials used wereProRoot MTA (DENTSPLY Tulsa Dental Specialties, DENTSPLY, USA), chlorhexidine gluconate (Neelkanth - Safe Plus, Jodhpur, India) Custom stock solution of TAP.Bacterial strain E faecalis ATCC 29212 & media BHI agar plates.Pro root MTA samples were prepared.MTA was mixed with water, with 0.1 mg/ml TAP, with100 mg/ml of TAP, with 0.12% CHX,& with 2% CHX respectively to form five groups.E. faecalis (ATCC 29212) were inoculated in 5 ml of brain heart infusion (BHI) and incubated at 37°C for 48 hours.Thirteen Petri plates with 20 ml BHI agar were inoculated with 0.1 ml of the experimental suspensions, using sterile swabs that were brushed across the medium, obtaining growth in junction.Freshly prepared specimens of five mm diameter from each test material were placed on agar plates. After incubation at

37^0 C for 24 hours and 7 days, zone of inhibition was measured. Six replicates were tested for each sample.The observations were tabulated & subjected to statistical analysis.

Statistical analysis

Mean and standard deviation for zone of inhibition in millimeters for groups were calculated.Kolmogorov Smirnov test showed that the data was normally distributed hence parametric tests were applied.One way ANOVA followed by Post hoc (Tukey) was applied for intergroup & intragroup comparison respectively. Level of significance was set at 0.05.

III. Results

The mean value for zone of inhibition for CHX at high concentration(13.5mm)is significantly different (p=0.001) than CHX at low concentration (9.33mm). Similar results were found for TAP at low conc (16mm) and high conc (27.67). Amongst CHX, TAP and control group, at low& high concentration; all were found to be significantly different (p=0.001) than each other. TAP exhibited betterresults amongst all.

Table no 1. Comparison between the different groups

Group	Low conc	High conc
CHX	9.33±1.03	13.5±0.83
TAP	16±0.63	27.67±0.51
Control	7±0.63	
F value	240	1674.459
P value	0.001*	0.001*

*p≤0.001 (highly significant)

Table no 2. Post hoc Tukey's test

Concentration	Combinations	Mean difference	p-value
Low	CHX vs TAP	6.67	0.001*
	CHX vs Control	2.67	0.001*
	TAP vs Control	9.33	0.001*
High	CHX vs TAP	14.17	0.001*
	CHX vs Control	6.83	0.001*
	TAP vs Control	21.0	0.001*

*p≤0.001 (highly significant)

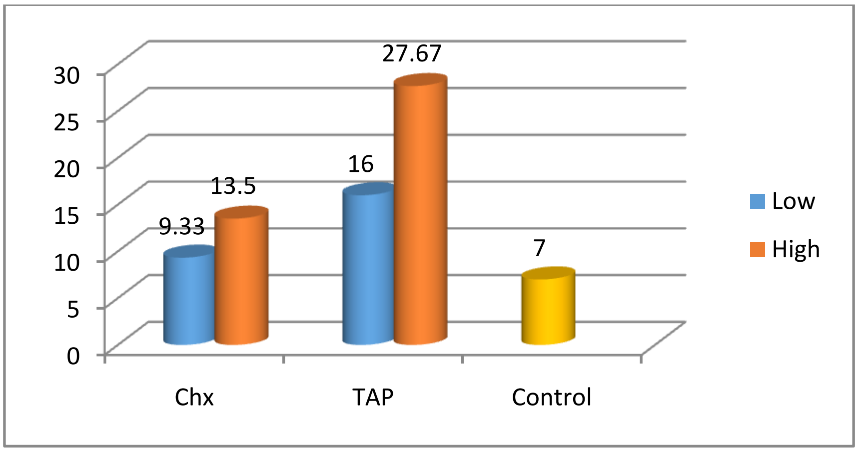

Chart showing zone of inhibition amongst diff groups.

IV. Discussion

The treatment outcome depends on successful elimination of the associated microorganisms and infected tissues as well as effective sealing of the root-end or perforation site to prevent future recontamination.

MTA was developed to fulfill a need of an ideal root-end filling material for surgical endodontic treatment.It can induce bone,PDL& cementum formation thus leading to regeneration rather than repair.[5]It produces high pH environment hence, has antibacterial property. It has decreased solubility so maintain a hard and excellent marginal seal.MTAexhibitedzoneof inhibition which shows a self-inherent antimicrobial property. This property is associated with the high pH it possesses. Initial pH is 10.2 which rise to 12.5 in 3 h.[6] The high pH of 12 is known to halt the growth of *E. faecalis*.[7]

In the present study, the antimicrobial activity of pro root MTA when mixed with different concentrations of triple antibiotic solutions and chlorhexidine respectively was investigated.

Zone of inhibition around MTA with higher concentration TAP were found to be larger than other groups and also statistically significant. This finding is similar to the finding of a study conducted byNaveenS et al[8] which concluded that zone of inhibition for MTA/ antibiotic mixture was more than MTA/ chlorhexidine and MTA/ water. The advantage of using triple antibiotic solution is that it will decrease the likelihood of resistant bacterial strains.TAP contains both bactericidal (metronidazole, ciprofloxacin) and bacteriostatic (minocycline) agents to allow for successful revascularization. Metronidazole is a nitromidazole compound that exhibits a broad spectrum of activity against protozoa and anaerobic bacteria. Minocycline is a semisynthetic derivative of tetracycline with a similar spectrum of activity.TAP was proved to be biocompatible. Tetracycline inhibits collagenase and matrix metalloproteinases, is not cytotoxic and increases the level of interleukin-10, an inflammatory cytokine. In addition, metronidazole and ciprofloxacin can generate fibroblasts.

Hernandez E.P et al[9]studied the effect of ProRoot MTA mixed with chlorhexidine on apoptosis and cell cycle of fibroblasts and macrophages.They concluded that the fresh mix of ProRoot MTA with 0.12% CHX gluconate induced apoptosis of macrophages and fibroblasts.Their findings suggested that the potentially beneficial antimicrobial effect of CHX may be accompanied by an increase in the cytotoxicity of the resulting MTA-based material.Maryam Bidar et al[10]concluded that the addition of 0.12, 0.2, or 2% CHX to MTA and CEM significantly enhanced the antimicrobial activity of these two cements.As the antimicrobial effect of 0.12% CHX did not differ from that of 2% CHX, with a lower toxicity, adding CHX at concentration of 0.12% to MTA and CEM was suggested.Stowe et al[11]concluded that the substitution of 0.12% chlorhexidine gluconate for sterile water in tooth-colored ProRoot MTA enhanced the antimicrobial effect of the MTA in vitro.

Mittag et al[12] concluded that when mixed with distilled water pure MTA –G has shown to produce a bactericidal effect against E. faecalis, while no antibacterial effect was seen with MTA-W. They also concluded that MTA-W when mixed with chlorhexidine, leads to bactericidal effects against E. fecalis for at least 48 hours.

V. Conclusion

Within the limitations of this study,it is concluded that using chlorhexidine and triple antibiotic solution in place of sterile water increased the antibacterial efficacy of pro root MTA. Triple antibiotic solution exhibitedgreater antibacterial efficacy than chlorhexidine gluconate.

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