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Microbiological Evaluation of the Level of Disinfection Attained by Mtad, 2% Chlorhexidine and 2.5% Sodium Hypochlorite – A Comparative *in vivo* Study

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A B S T R A C T

The aim of the study is to evaluate and compare the level of disinfection achieved by MTAD, 2% chlorhexidine and 2.5% sodium hypochlorite by counting the number of colony forming units. 30 patients satisfying the inclusion criteria were selected and randomly divided into three groups of 10 each. After access opening and working length determination, sterilized K files were used to collect preoperative samples and were transported to the Microbiology Laboratory. Following which biomechanical preparation of the canal was done for all cases. In Group I, Group II and Group III patients, MTAD, 2.5% Sodium Hypochlorite and 2% chlorhexidine were used for irrigation and disinfection, respectively. A sterilized no 15 file was reintroduced in the canal and post- operative samples were collected and transported to microbiology laboratory. Number of colony forming units was counted. The data obtained were analyzed using the one way ANOVA followed by Multiple Duncan's test as the post hoc test with a p value <0.05 as the level for statistical significance. Suggested that MTAD show statistically significant superior mean bacterial reduction of 81.73 and 91.45 for aerobes and anaerobes respectively when compared to 2.5% sodium hypochlorite (65.45, 71.64) and 2% chlorhexidine (68.82, 72.27). Mixture of tetracycline isomer, citric acid and a detergent (Tween 80) MTAD reduces the bacterial count significantly more when compared to 2.5% sodium hypochlorite and 2% chlorhexidine and seems to be an alternative to currently used irrigating solutions.

Introduction

Endodontic infections are poly microbial and mixed infections in nature. Apical periodontitis results from invasion of bacteria into the pulp and periapical tissues (Kakehashi *et al.,* 1965). The successful outcome of an endodontic treatment depends on the complete eradication of the microorganisms from the root canal system prior to obturation. Root canal disinfection is accomplished by mechanical and chemical means (Bystrom and Sundqvist, 1981). Mechanical procedures can considerably reduce the bacteria but does not predictably eradicate them. Hence, it is necessary to use chemical disinfecting agents during root canal therapy in order to obtain a successful outcome.

Of all the currently used substances in dentistry, sodium hypochlorite (NaOCl) appears to be the most commonly used irrigant in a concentration ranging from 0.5 to 6% (Walker, 1936). The factors contributing to its popularity are low cost, ease of availability and good shelf life. Potential bactericidal activity and excellent tissue dissolving action make it a good irrigant (Siqueira *et al.,* 1998; Beltz *et al.,* 2003). Its caustic nature (Hauman and Love, 2003) and inability to remove smear layer when used alone are the major disadvantages (Goldman *et al.,* 1981). Accidental injection of NaOCl into periradicular tissues can cause cytotoxicity.

Chlorhexidine gluconate is another irrigant widely used in dentistry which is having antiseptic and bactericidal properties. One of the most important properties of chlorhexidine is substantively contributing to its prolonged antimicrobial activity (Zehnder, 2006). When used as disinfectant, 2% chlorhexidine demonstrated significant inhibition against *E. faecalis* (Wang *et al.,* 2007). The major drawback of chlorhexidine is its inability to dissolve necrotic tissue remnants (Naenni *et al.,* 2004).

MTAD is one of the newer irrigating solutions containing a mixture of a tetracycline isomer, an acid and a detergent and has proved its ability in smear layer and debris removal and root canal disinfection and *E. faecalis* eradication (Shabahang *et al.,* 2003). It appears to be superior to chlorhexidine in antimicrobial activity.

The purpose of the present *in vivo* study is to evaluate and compare the level of disinfection achieved by MTAD, 2% chlorhexidine and Sodium hypochlorite by counting the number of colony forming units.

Materials and Methods

Patient selection

Thirty patients 15 to 45 years old presenting with intact single rooted non vital tooth with periapical pathosis, requiring endodontic treatment were selected for this study. Institutional ethics committee, Government Dental College, Kottayam gave approval for this research study. In addition, written informed consent was obtained from all the subjects taking part in this study. The patients were divided into three groups of 10 each. It was decided to irrigate the Group I patients with MTAD, Group II with 2.5% Sodium hypochlorite and Group III with 2% chlorhexidine.

Operative procedures

Under strict aseptic conditions, the involved tooth was isolated and endodontically

accessed. Electronic apex locator was used to determine the working length which was confirmed by radiographic method. Sterile 15no.ISO K files were used to negotiate up to working length of the canal and to obtain the pre-operative samples. Then these contents were transferred into preheated Stuart's Transport Medium taken in a sterile vial for anaerobic culture and into sterile vial for aerobic culture and tightly sealed. These samples were to Microbiology Laboratory at Tropical Institute of Ecological Sciences within two hours of collection. This was the first sample.

Cleaning and shaping had done for all cases. Group I patients were irrigated with MTAD, GROUP II patients with 2.5% Sodium hypochlorite & for GROUP III patients 2% chlorhexidine was used. Sterilized15no. ISOK files were reintroduced in the canal and fresh samples taken. These contents were also transferred into preheated Stuart's Transport medium taken in a sterile vial for anaerobic culture and into sterile vial for aerobic culture and tightly sealed and sent to microbiology laboratory at TIES within two hours of collection. This was the second sample.

Laboratory procedures

For anaerobic culture, samples were inoculated on Brain Heart Infusion broth and incubated under anaerobic condition for 24 hrs. It was then transferred into thioglycollate medium and incubated for 24–72 hours under McIntosh and Fildes Anaerobic Jar at 37^0C. The jar was opened after 48–72 hours. For aerobic culture, samples were inoculated on blood agar and Nutrient agar and were placed in an incubator at 37^0C for 18–24 hours. Both aerobic and anaerobic plates were examined with the help of hand lens and after that colony forming units were counted by the microbiologist at TIES.

Results and Discussion

Data were analysed using computer software, Statistical Package for Social Sciences (SPSS) version 16. Table 1 and graph 1 shows Analysis of variance (One Way ANOVA) was performed to compare three different groups.

The mean reduction in the aerobic colony counts obtained for Group I (MTAD), Group II (NaOCl) and Group III (CHX) treatment modality along with Standard deviation values were 81.73±8.475, 65.45 ± 6.699 and 68.82 ± 7.026. From the table it is clear that 'F' value after doing one way ANOVA test is 14.665 and P value is <0.05, which indicate that test is highly significant.

The mean reduction in anaerobic CFU obtained for Group I (MTAD), Group II (NaOCl) and Group III (CHX) treatment modality along with Standard deviation values were 91.45 ± 12.871, 71.64 ± 7.527 and 72.27 ± 6.798. From the table it is clear that 'F' value after doing one way ANOVA test is 15.588 and P value is < 0.05, which indicate that test is highly significant. To elucidate multiple comparisons between groups, Duncan's Multiple Range Test was also performed along with ANOVA as post hoc test. Group I (MTAD) showed superior bacterial reduction in comparison with and Group II (NaOCl) and Group III (CHX). This result was statistically significant. Group III (CHX) showed statistically insignificant superior CFU reduction when compared to Group II (NaOCl) for both aerobic and anaerobic colony counts.

The eradication of the microbes from the infected root canals is a complicated task. The recontamination of the obturated canals with microorganisms may lead to treatment failure. As mechanical instrumentation of the canals cannot completely serve the purpose of debridement and microbial

elimination the use of endodontic irrigants is central to a successful disinfection. An ideal irrigant should be able to destroy microorganisms and their by-products without producing any harm to the host tissue (Yesilsoy *et al.,* 1995).

NaOCl has been consistently used as an endodontic irrigant since its introduction for the last 70 years thanks to its antimicrobial activity and tissue dissolving capacity. Antimicrobial activity of sodium hypochlorite is based on its high pH which interferes with the cytoplasmic membrane integrity and also biosynthetic alterations in cellular metabolism. The ability to dissolve tissue and the rate of dissolution is directly proportional to concentration of NaOCl (Baumgartner and Cuenin, 1992). Not only the actions but also the toxicity and caustic potential of NaOCl also increase with the concentration. Also, its disagreeable taste and smell may force us to search for an alternative irrigant with comparatively less side effects (Pashley *et al.,* 1985).

Chlorhexidine gluconate is considered to be good antimicrobial agent with prolonged time of activity. When compared to NaOCl, it is relatively non-toxic and less foul taste (Jhonson and Remeikins, 1993). It can be used as an alternative to NaOCl in open apex cases and NaOCl allergic patients (Kaufman and Kella, 1989). Its inferior tissue dissolving action is a major drawback as a primary endodontic irrigant (Marley *et al.,* 2001).

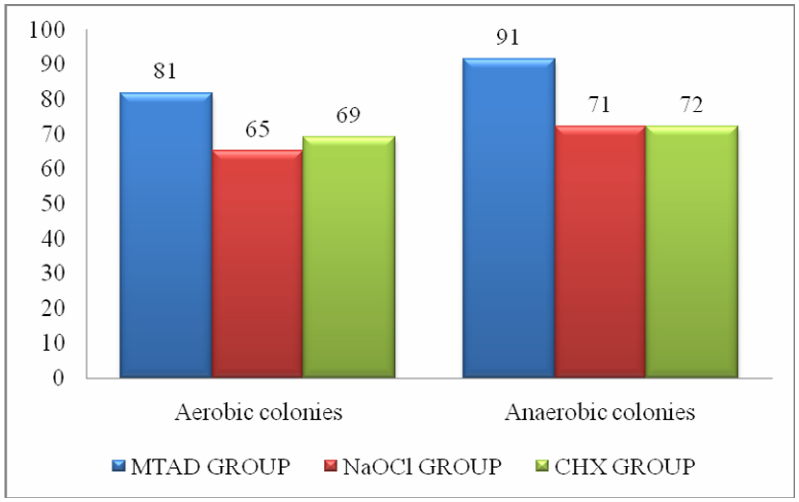
Bio Pure MTAD is a mixture of a tetracycline isomer, an acetic acid, and Tween 80 detergent and was introduced as a final root canal rinse after biomechanical preparation. MTAD shows excellent smear layer removal efficiency without significantly changing the structure of dentinal tubules (Torabinejad *et al.,* 2003a). In comparison with other endodontic irrigants and medicaments it is less cytotoxic. Torabinejad *et al.* (2003b) proposed that the efficiency of the MTAD can be increased when NaOCl is used as an irrigant before the application of MTAD as a final rinse for 5 minutes. Newberry *et al.* (2007) proposed that BioPure MTAD showed potent activity against *E. faecalis* as a final irrigant.

Table.1 Analysis of variance (One Way ANOVA) of bacterial colony count ($\times 10^3$) reduction comparing three treatments

Colony Type	Group	Mean Reduction	± SD	F value	P value
Aerobic	MTAD	81.73[a]	8.475		
	NaOCl	65.45[b]	6.699		
	CHX	68.82[b]	7.026	14.665	< 0.05
Anaerobic	MTAD	91.45[a]	12.871		
	NaOCl	71.64[b]	7.527		
	CHX	72.27[b]	6.798	15.588	< 0.05

a, b: Means with same superscript within each type do not differ each other (Duncan's Multiple Range test)

Graph.1 Mean bacterial reduction of colonies (103) obtained by three irrigants



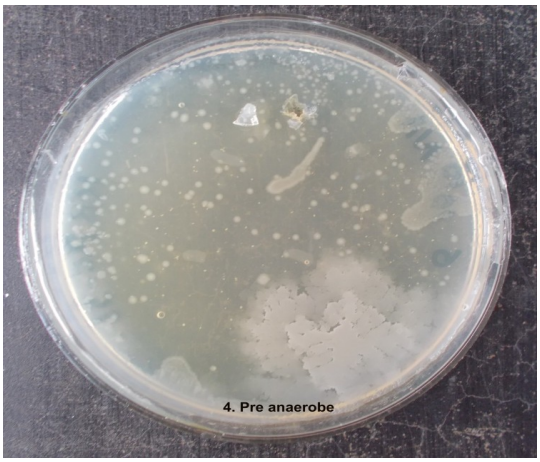


Figure.1 Pre-operative sample- anaerobe MTAD Case

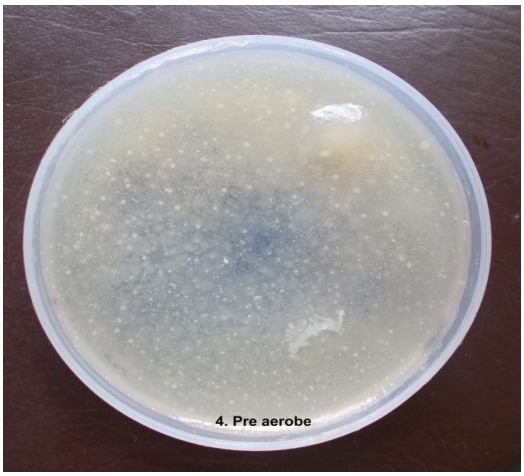


Figure.2 Pre-operative sample- aerobe MTAD Case

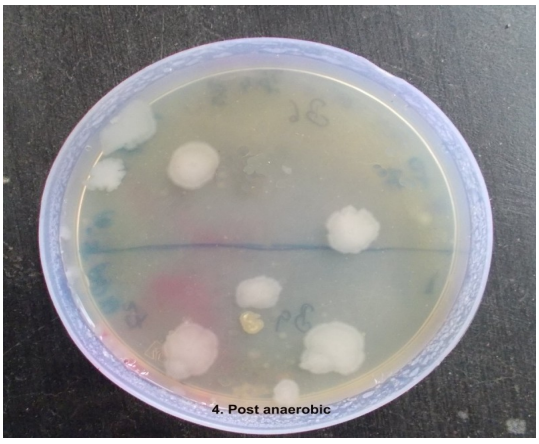


Figure.3 Post-operative sample- anaerobe MTAD Case

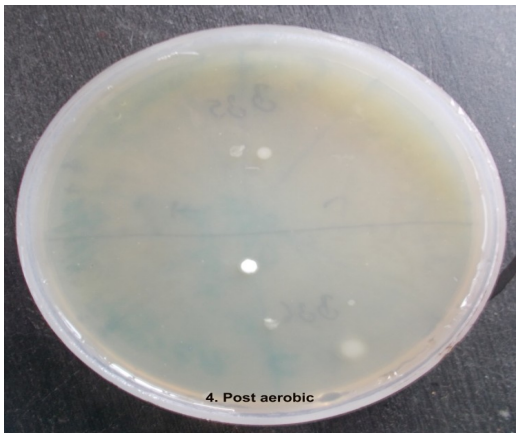


Figure.4 Post-operative sample- aerobe MTAD Case

The results from the present study show correlation with the findings of some other investigators. In the present study chlorhexidine shows marginally superior bacterial reduction than NaOCl. Jeansonne and White (1994) compared 5.25% sodium hypochlorite and 2% chlorhexidine gluconate *in vitro*, showed that bacterial colony count reduction obtained with 2% chlorhexidine was more when compared to 5.25% sodium hypochlorite, but these differences were not statistically significant. Ohara *et al.* (1993) reported that chlorhexidine gluconate was the most effective among six irrigants against anaerobic bacteria which he had tested.

Results of the present study demonstrated that MTAD attained a superior mean reduction of CFU for both aerobes and anaerobes in comparison with the other tested irrigants. The findings of our study show parallels with the results of Shabahang *et al.* (2003) who compared the ability of BioPure MTAD and 5.25% NaOCl to disinfect contaminated root canal with whole saliva. They evaluated that BioPure MTAD achieved a significantly superior bacterial reduction when compared to NaOCl. The reason behind these results might the reduction in surface tension of the MTAD by detergent which enhances the intimate contact of irrigant and there by its action in root canal dentin. Potent antibacterial effect can be attributed to the penetration of antibiotics up to 500µm into the dentinal tubules that are opened by citric acid (Giardino *et al.*, 2007).

From the present study, it can be evaluated that among the tested irrigants MTAD shows the maximum antimicrobial efficacy followed in descending order by 2% chlorhexidine and 2.5% Sodium hypochlorite.

Conclusion

Hence, it can be concluded that MTAD can be an effective intracanal irrigant for cleaning and disinfecting the root canal system and can be an alternative to traditionally used ones. The results obtained may be interpreted keeping in mind the limitations of this study, warranting further studies using larger sample number to investigate the clinical effectiveness of MTAD as a root canal disinfectant in comparison with other disinfecting agents.

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