International Journal of Research and Reviews in Pharmacy and Applied science

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COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF ROUTINE ENDODONTIC IRRIGANTS WITH SURFACTANTS AGAINST MTAD ON *ENTEROCOCCUS FAECALIS* - AN IN VITRO MICROBIOLOGICAL STUDY.

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ABSTRACT

The purpose of this study was to evaluate the antimicrobial efficacy of 2.5% Sodium hypochlorite (NaOCl), 2% Chlorhexidine (CHX), 2% Iodine potassium iodide (IKI) with surfactants Triton X-100 (TX-100) and Cetrimide (CTR) against MTAD on *Enterococcus faecalis* (ATCC 29212) .The antimicrobial efficacy of irrigants was tested by Modified Kirby-Bauer method. Known volume of test irrigants and surfactants was introduced into the sterile paper discs and placed on the Mueller Hinton agar plates. After overnight incubation at 37^{o}C, zone of bacterial inhibition was measured.

The data obtained was statistically analyzed by ANOVA and Bonferroni Multiple Comparison test. There was no significant difference between 2.5% Sodium hypochlorite, 2% Chlorhexidine, 2% Iodine potassium iodide. Triton X-100 showed no zone of inhibition. Cetrimide showed zones of inhibition for all test concentrations. 0.5% CTR was found to be the effective concentration. MTAD was statistically significant over the routine endodontic irrigants and combination regimens.

The results of this study showed 0.5% cetrimide had strong antibacterial efficacy as routine endodontic irrigants (2.5% NaOCl, 2%CHX, 2% IKI) even at a lower concentration. MTAD had stronger antibacterial efficacy on *E.faecalis* when compared to routine irrigants and combination regimens.

KEY WORDS: Routine endodontic irrigants, MTAD, surfactants, combination regimens, Triton X-100 and Cetrimide.

INTRODUCTION

Successful endodontic treatment involves effective eradiation of the causative microorganisms during root canal treatment procedures. The chances of favorable outcome with root canal treatment are significantly higher if infection is eradicated effectively before the root canal system is obturated.[1] In failed root canal treatments *Enterococcus.faecalis* is commonly found in a high percentage and is able to survive in the root canal as a single organism or as a major component of the flora. [2]

The most commonly used endodontic irrigants includes 2.5% sodium hypochlorite (NaOCl), 2% chlorhexidene (CHX) and 2% iodine potassium iodide (IKI). A new product Biopure MTAD (Tulsa, Dentsply) is also available as an endodontic irrigant. Antimicrobial activity of Biopure MTAD is said to be superior against *E.faecalis* compared to other irrigants. [3,4] Its efficacy is proven to be promising against eight different strains of *E.*faecalis.[5] It is less cytotoxic irrigant than 5.25% NaOCl and 0.12%CHX .[6]

Sodium hypochlorite is proven to be broad spectrum antimicrobial agent.[7] A study showed 5.25% NaOCl is better in disaggregation of *E. faecalis* cells than MTAD.[8] 2.5% NaOCl was effective on *E.faecalis* biofilm generated on membrane filter discs.[9] The antimicrobial property of NaOCl is due to its tissue dissolving capacity.[10]

Chlorhexidene is also a potent broad spectrum antimicrobial agent and has the advantage of substantivity.[11] It is effective against *E.faecalis* even at lower concentration like 0.5% and 1% .[12] 2% chlorhexidine suggested to be resistant against colonization of *E. faecalis* and effective than $Ca(OH)_2$ paste. [13, 14]

Iodine potassium iodide was able to eliminate *Streptococcus faecium*, a closely related species to *E.faecalis*, from infected tubules within 10 mintues than $Ca(OH)_2$ which took 24 hrs.[15] IKI was suggested to be more potent than sodium hypochlorite or chlorhexidine in eliminating *E.faecalis*.[16] 2% IKI was reported to be less toxic than 5.25% NaOCl.[17]

Surfactants are surface active agents. In this study triton X-100 (TX-100) and cetrimide (CTR) were used as surfactants. 1% triton X-100 has been proved to improve the effectiveness of 5% NaOCl in removing smear layer and tubular debries when used during and after root

canal preparation[18] and caused better penetration of NaOCl into the dentinal tubules.[19] A study showed that triton X- 100 had better ability to lyse the exponentially growing *S.faecalis* than sodium dodecyl sulfate (SDS).[20]

Cetrimide (cetiltrimethyl ammonium bromide) is a quaternary ammonium compound and a cationic detergent that is effective against many Gram positive and Gram negative bacteria. Cetrexidin (Vebas, San Giuliano, Italy) contains 0.2% CHX & 0.2% cetrimide, and is more effective against *E.faecalis* and has lower cytotoxicity than 5.25% NaOCl.[21]

Thus the purpose of this study was to evaluate the antimicrobial efficacy of routine endodontic irrigants (2.5%NaOCl, 2%CHX, 2%IKI) with surfactants (triton X-100 & cetrimide) against MTAD on *Enterococcus faecalis*

MATERIALS AND METHODS

2.1 Bacterial Growth and Culture:

The present study was done on *Enterococcus faecalis* (ATCC 29212). From frozen stock culture, the bacteria was subcultured in Brain Heart Infusion broth. The optical density of the planktonic culture was adjusted to the 0.5 Mc Farland constant and later used for streaking on the Muller Hinton agar plates.

2.2Preparation of Irrigating solutions, Surfactants and combination regimens:

2.5% NaOCl was prepared by diluting 5% NaOCl (Prevest Denpro limited). 2% CHX was prepared from chlorhexidine digluconate (Sigma, 20% aqueous solution). 2% IKI was prepared by dissolving 2g of iodine (Merck) and 4g of potassium iodide (Himedia) in 94mL of physiological saline.[22] MTAD (Tulsa, Dentsply) was mixed as per manufacturer's instructions.

Surfactants Triton X-100 (Himedia) and Cetrimide (Himedia) were used in the study. They were used in the concentrations of 0.25%, 0.5%, 1% and 2%. These concentrations were freshly prepared.

Combination regimens of sodium hypochlorite, chlorhexidine and iodine potassium iodide with 0.5% CTR was prepared.

2.3 Antimicrobial Susceptibility Test:

The modified Kirby – Bauer method was followed to check the antimicrobial efficacy. Using a Sterile swab (Himedia) *E. faecalis* culture was evenly spread on the solidified 20ml of Mueller Hinton Agar plates. This was followed by placing Sterile disc (Himedia) with known

volume of solutions (irrigants, surfactants as well as combination regimens). In this study 20µl of the solution was used on each disc. Six replicates for each solution were kept. Then petriplates were placed in an incubator at 37^0C. After the overnight incubation the zone of inhibition was measured.

RESULTS & DISCUSSION

The results of the antimicrobial efficacy of routine endodontic irrigants, surfactants and combination regimen are summarised in table 1-3. The data obtained were statistically analyzed by ANOVA and Bonferroni Multiple Comparison test.

This study was aimed to compare the efficacy of routine endodontic irrigants with surfactants in comparison to MTAD in eliminating *E.faecalis. E.faecalis* was chosen as the test organism because it has been found to be associated with failed root canal treatments .[23]

The results of our study implicated that MTAD showed significantly greater zone of inhibition than 2.5% sodium hypochlorite, 2% chlorhexidine and 2% Iodine potassium iodide. There was no significant difference in the antibacterial efficacy between 2.5% NaOCl, 2% CHX and 2% IKI. The results obtained are in accordance with the results of Shabahang S et al. [24], Giardino L et al.[25]

In the surfactant group (Triton X-100 & Cetrimide), Triton X-100 did not show any zone of inhibition at any concentrations (0.25%, 0.5%, 1%, 2%) tested. However cetrimide showed antimicrobial efficacy at all concentrations (0.25%, 0.5%, 1%, 2%). Since there was no statistical significance between 0.5% and any of the other test concentrations 0.5% was taken as the effective concentration for the combination regimen with the routine endodontic irrigants.

The combination regimen of 2.5% NaOCl, 2% CHX and 2% IKI with 0.5% cetrimide was not statistically significant over the routine endodontic irrigants. Although there was a increase in the antibacterial actitivity with the addition of 0.5% cetrimide to 2.5% NaOCl and

2% CHX; it was found to be less than 2% IKI. MTAD was found to be statistically significant than any of the combination irrigant regimen tested.

MTAD contains 3% doxycycline (tetracycline isomer) 150mg/5ml that potentiates its antibacterial activity. In addition it has 4.25% Citric acid, 0.5% polysorbate80 (detergent), which could enhance its antibacterial activity[26].

CONCLUSION

Within the limitations of this study it can be concluded that 0.5% cetrimide has a strong antibacterial efficacy as routine endodontic irrigants (2.5% NaOCl, 2%CHX, 2% IKI) even at a lower concentration. MTAD was found to have a stronger antibacterial efficacy when compared to the routine irrigants and combination regimens.

TABLES:

Sl no	Irrigant	Mean of zone of inhibition (cm)	Mean difference (control-experimental)	P value
1	2.5% NaOCl	1.700	1.1667*	<0.001
2	2% CHX	1.733	1.1333*	<0.001
3	2%IKI	1.950	0.9167*	<0.001
4	MTAD(Control)	2.867	-	-

Table 1: Showing mean values of zone of inhibition by routine irrigants and MTAD, * The mean difference is significant at the .05 level.

Concn (%)	Mean of zone of inhibition (cm)	Var(i)	Var(j)	Mean difference(i-j)	P value
2	2.000	0.5% (control)	0.25%	0.1000	0.237
1	1.917		1.0%	-0.0500	1.000
0.5	1.867		2.0%	-0.1333	0.052
0.25	1.767				

Table 2: Showing mean value of zone of inhibition by Cetrimide(CTR).

Sl no	Combination regimen VS MTAD	Mean of zone of inhibition (cm)	Mean difference (control-experimental)	P value
1	2.5%NaOCl+0.5%CTR	1.900	0.9667*	<0.001
2	2.0% CHX+0.5%CTR	1.850	1.0167*	<0.001
3	2.0%IKIl+0.5%CTR	1.633	1.2333*	<0.001
4	MTAD (control)	2.867	-	-

Table 3: Showing mean value of zone of inhibition by combination regimens and MTAD.* The mean difference is significant at the .05 level

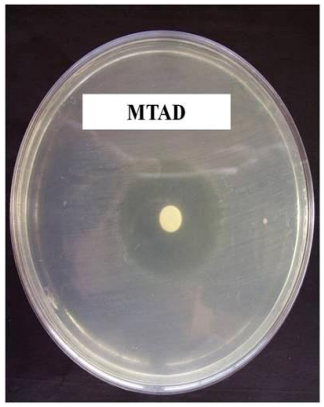
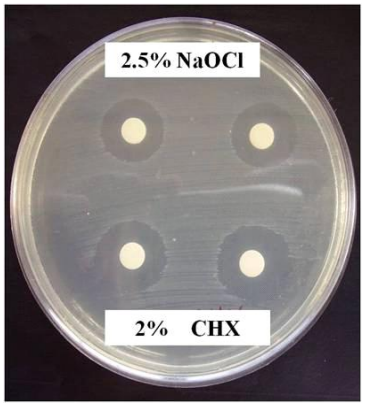


FIGURE.1



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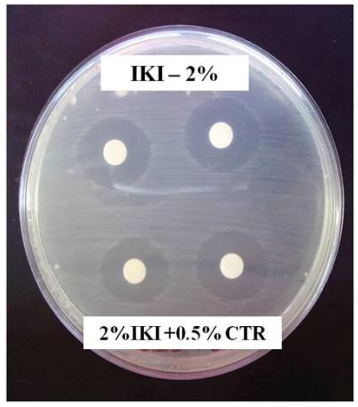

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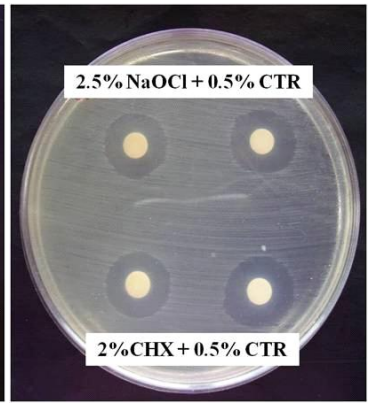

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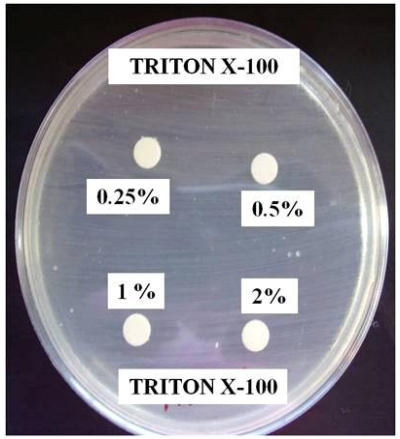

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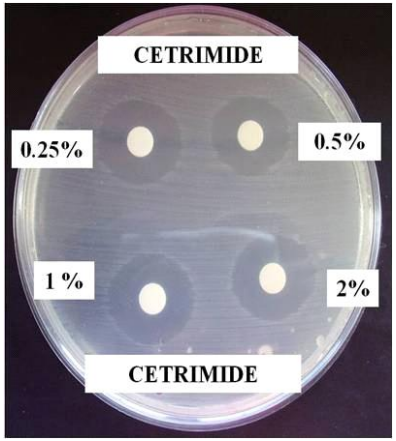

FIGURE.6

LEGENDS:

TABLE LEGENDS:

Table 1: Showing mean values of zone of inhibition by routine irrigants and MTAD.

Table 2: Showing mean value of zone of inhibition by Cetrimide (CTR).

Table 3: Showing mean value of zone of inhibition by combination regimens and MTAD.

FIGURE LEGENDS:

Fig1: Zone of inhibition of MTAD (Control)

Fig2: Zone of inhibition of 2.5% NaOCl & 2% CHX

Fig3: Zone of inhibition of 2 %IKI & 2% IKI +0.5% CTR

Fig4: Zone of inhibition of 2.5% NaOCl +0.5% CTR & 2% CHX+0.5% CTR

Fig5: Zone of inhibition of Surfactant - Triton X-100

Fig6: Zone of inhibition of Surfactant – Cetrimide (CTR

ACKNOWLEDGEMENTS

The authors are grateful to Miss Geethashri,(research assistant), Central Research Laboratory- A.B.S.M.I.D.S and Ms. Neevan D,Souza (statistician) for their continuous support throughout the work.

REFERENCES

1. Kavim IE, Kennedy J, Hussey D. The antimicrobial effects of root canal irrigation and medication. Oral surg oral Med oral Pathol 2007;103(4):560-569.
2. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to Calcium hydroxide. Int Endod J 2002;35:221-228.
3. Davis JM, Maki J, Bahcall JK. An in vitro comparison of the antimicrobial effects of various endodontic medicaments on *Enterococcus faecalis*. J Endod 2007;33(5):567-569.

4. Krause TA, Liewehr FR, Hahn CL. The antimicrobial effect of MTAD, Sodium hypochlorite, Doxycycline and Citric acid on *Enterococcus faecalis*. J Endod 2007;33(1):28-30.
5. Newberry BM, Shabahang S, Johnson N, Aprecio RM, Torabinejad M. The antimicrobial effect of Biopure MTAD on eight strains of *Enterococcus faecalis:* An in vitro investigation. J Endod 2007;33(11):1352-1354.
6. Yasuda Y et al. Effect of MTAD on differentiation of Osteoblast like cells. J Endod 2010;36(2):260-263.
7. Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5% sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol 1983;55:307-312.
8. Giardiana L et al. Comparative evaluation of antimicrobial efficacy of sodium hypochlorite, MTAD and tetraclean against *Enterococcus faecalis* biofilm. J Endod 2007;33(7):852-855.
9. Spratt DA, Pratten J, Wilson M, Gulabivala K . An in vitro evaluation of the antimicrobial efficacy of irritants on biofilms of root canal isolates. Int Endod J 2001;34:300-307.
10. Cobankara FK, Ozkan HB, Terlemez A . Comparison of organic tissue dissolution capacities of sodium hypochlorite and chlorine dioxide. J Endod 2010;36(2):272-274.
11. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% Chlorhexidine gluconate separately and combined as endodontic irritants. J Endod 1998;24:472-476.
12. Sassone LM et al. Antimicrobial activity of different concentrations of NaoCl and Chlorhexidine using a contact test. Braz Dent J 2003;14(2):853-866.
13. Williamson AE, Cardon JW, Drake DR. Antimicrobial susceptibility of monoculture biofilm of a clinical isolate of *Enterococcus faecalis*. J Endod 2009;35(1):95-97.
14. Ballal V et al. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. Australian dental Journal 2007;25(2):118-121.
15. Safavi K, Spangberg L, Langeland K. Root canal dential tubule disinfection. J Endod 1990;16:207-210.
16. Orstavik D, Haapasalo M. disinfection of endodontic irrigats and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990;6:142-149

17. Barnhart BD et al. An in vitro evaluation of the cytotoxicity of various endodontic irrigants on human gingival fibroblasts. J Endod 2005;31(8):613-615.
18. Gambarini G. Shaping and cleaning the root canal system: A scanning electron microscope of a new instrumentation and Irrigation technique. J Endod 1999;23(12):800-803.
19. Berutti E, Marini R, Angenetti A. Radiation ability of different irritants into dental tubules. J Endod 1997;23(12):725-727.
20. Cornett JB, Shockman GD. Cellular lysis of *Streptococcus faecalis* induced with Triton X-100. Journal of Bacteriology 1978;135(1):153-160
21. Oncag O et al. Comparison of antimicrobial and toxic effects of various root canal irrigants. Int Endo J 2003;36:423-432.
22. Spanberg L, Engstrom B, Langeland K. Biologic effects of dental materials – Part III: Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg Oral Med Oral pathol 1973;36:856-870.
23. Sandqvist G. Taxonomy, Ecology and Pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol 1994;78:522-530.
24. Shabahang S, Pouresmail M, Torabinyad M. In vitro antimicrobial efficacy of MTAD and sodium hypochlorite. J Endod 2003;29(7):450-452.
25. Giardino L et al. Antimicrobial effect of MTAD, Tetraclean, Cloreximid and sodium hypochlorite on three common endodontic pathogens. Indian J Dent Research 2009;20(3):12-16.
26. Torabinejad M et al. A new solution for the removal of the smear layer. J Endod 2003; 29:170-175.