Original Article

Comparison of the Antimicrobial Efficacy of MTAD and EDTA against Enterococcus Faecalis in Vitro

Uzair Aftab[1], Madiha Zaighum[2], Darshana[1], Talha Asad Khan[1], Rafia Khan[2] and Sheba Ramzan[3]

ABSTRACT

Objective: To compare antibacterial efficacy between 5.25% NaOCL/17% EDTA and 1.3% NaOCL/MTAD irrigation protocols.

Study Design: Comparative and in vitro study

Place and Duration of Study: This study was conducted at the Operative Dentistry Department at the Isra Dental College Welfare OPD, ISRA University Hospital from December, 2013 to December, 2014.

Materials and Methods: E. Faecalis bacteria was grown for 24hr on Brain Heart Infusion agar. E.faecalis was inoculated in root canals of single canaled decoronated teeth (14mm). Step back preparation was done with MAF of 40 # K File. Between every file change root canals were irrigated in group I with 5.25% NaOCl and the finishing rinse was done with 17% EDTA, in group II between each file change root canals were irrigated with 1.3% NaOCl and the finishing rinse was done with MTAD and in Group III between every file change irrigation of canal was done with 5.25%NaOCl. Bacterial sample was taken with a # 40 paper point. Bacterial Culture was done on sheep blood agar and then colonies were counted on a colony counter.

Results: There was difference in the effectiveness of all the three irrigation protocols. It was revealed that mean bacterial count was significantly lower with irrigation protocol 5.25%NaOCl/17%EDTA (i.e. Group I) and irrigation protocol 1.3%NaOCl/MTAD (i.e. Group II) as compared to control group (i.e. Group III=irrigation protocol with 5.25%NaOCl).

Conclusion: 5.25%NaOCl/17%EDTA showed similar antibacterial efficacy to that of 1.3%NaOCl/MTAD against E. faecalis.

Key Words: Enterococcus faecalis, Irrigation. Sodium Hypochlorite, MTAD, Dye penetration

Citation of article: Aftab U, Zaighum M, Darshana, Khan TA, Khan R, Ramzan S. Comparison of the Antimicrobial Efficacy of MTAD and EDTA against Enterococcus Faecalis in Vitro. Med Forum 2023;34(3):67-70.

INTRODUCTION

Endodontic infections have been well documented to be of microbiotic origin.[1] The primary purposes of non-surgical root canal therapy, is to eliminate the microorganisms and the associated infected and necrotic pulpal remnants in order to prevent or cure apical periodontitis.

[1.] Department of Operative Dentistry, Isra University, Karachi.
[2.] Department of Operative Dentistry, Isra Dental College, Hyderabad.
[1.] Department of Operative Dentistry, Liaquat University Hospital Hyderabad..

Correspondence: Dr. Madiha Zaighum, Assistant Professor of Operative Dentistry, Isra Dental College Hyderabad.
Contact No: 03003025920
Email: madihaz_pk@hotmail.com

Received: October, 2022
Accepted: January, 2023
Printed: March, 2023

It has been shown that failure of endodontic treatment is due to the presence of microorganisms either having remained in or re-colonized the obturated root canal.[3] number of studies have mentioned that Enterococcus faecalis is the one of the predominant bacteria in teeth with persistent periapical periodontitis.[2,3,4] Numerous measures have been described to reduce the number of microorganisms in the root canal system, including the use of various instrumentation techniques, irrigation regimens, and intracanal medicaments.[5]

Sodium Hypochlorite (NaOCl) is, to date the most widely used endodontic irrigant because of its good tissue solvent action[6], has a broad spectrum of antimicrobial activity [7]but the major disadvantages of NaOCl are its cytotoxic effect (pain, swelling, bruising and numbness) if injected into the periapical tissues[8], the chelating agent ethylene diamine tetra-acetic acid (EDTA) in its different physical forms and formulations is most commonly used for smear layer removal. It is highly biocompatible, shows high efficiency in removing the smear layer and may detach biofilms adhering to root canal walls.[9]

Recently, a new irrigating solution containing a mixture of tetracycline isomer, citric acid, and detergent Tween

80 (MTAD) has been introduced as a final rinse solution for removing the smear layer from the surface of the root canal[9], and is also capable of eradicating bacteria from infected root canals. MTAD is less cytotoxic than a range of endodontic irrigants, including, 3% hydrogen peroxide, EDTA and 5.25% NaOCl.[10] Torabinejad et al[11] showed that the effectiveness of the MTAD was enhanced when low concentration of NaOCl is used as an intracanal irrigant before the use of MTAD as a final rinse. Shabahang and Torabinejad found the combination of 1.3% NaOCl as a root canal irrigant with MTAD as a final rinse was more effective against E. faecalis than 5.25% NaOCl with 17% EDTA.[12] Davis et al[13] used in vitro experiments to show that 5.25% NaOCl exhibited less antimicrobial efficacy against E. faecalis than BioPure MTAD, demonstrating that BioPure MTAD is a viable medicament against E. faecalis.

Kho[14] and Baumgartner[13] compared the antimicrobial efficacy of irrigation between 1.3% NaOCl / Bio pure MTAD and 5.25% NaOCl/15% EDTA in the apical 5mm of roots infected with E. faecalis, and found no difference between antimicrobial efficacies of both protocols. These differences ensure that the efficacy of Bio Pure MTAD remains somewhat controversial. So further research is required.

MATERIALS AND METHODS

Experimental study in vitro was conducted in the department of operative dentistry and ISRA clinical laboratory in Hyderabad Pakistan.

A total of 116 single rooted teeth with single canal were collected out of which 90 were selected; those that were fractured, grossly carious and showing signs of resorption or calcification were excluded. All teeth were stored in normal saline. Each tooth was cleaned from stains and calculus via scaling. Sterilization of all teeth was done in an autoclave at 121°C / 15 lbs for 20 minutes Then the presence for single canal in teeth was confirmed by radiographs. Decoronation of all teeth was done by a low speed hand piece using a diamond disc, to approximately 14mm K-file #15 (Dentsply) was used to determine the working length. E. Faecalis was the bacteria used in the present study. The growth of bacteria was done in Brain Heart Infusion culture for 24hrs at 37°C. Colonies of bacteria were added to 9ml sterile saline. Turbidity was checked with 0.5 Mc. Farland Turbidity Standard. With the help of tuberculin syringe a 10 µl suspension of E.Faecalis was inoculated in the root canals under aseptic conditions inside a laminar air flow hood. After the incubation period, the teeth were rinsed thoroughly with normal saline after removal from the test tubes. Now the cleaning and shaping was done with step back technique and the master apical file was #40 K-file distributed into three (3) groups; Group I (5.25%NaOCl/17%EDTA) Group II (91.3%NaOCl/MTAD) and Group III- Irrigation with 5.25%NaOCl (control group).

Group-I: When the preparation root canal is completed 17% EDTA (Smear Clear, Sybron Endo) was used (Fig.III-11). 1ml of EDTA was left in the root canal for 1 min followed irrigation with 5ml of 5.25%NaOCl to get rid of the smear layer. Excess moisture was removed by #35 absorbent paper point (Dentsply International), followed by the placement for 10 seconds of # 40 absorbent paper point to the whole extent of the working length of the root canal. This paper point was transferred to 2ml sterile normal saline in a test tube which was then vortexed for 1min.

Group-II: The root canals of teeth in this group were cleaned, shaped, and irrigated with 1.3%NaOCl One milliliter of 1.3% NaOCl was employed between each file size. At the end of the preparation, 1ml of MTAD was kept in the canal for 4 min to get rid of the smear layer and then was irrigated with 4ml of MTAD. The exposure period for MTAD was approximately 5 minutes. Excess moisture was removed by #35 absorbent paper point (Dentsply International), followed by the placement for 10 seconds of # 40 absorbent paper point to the whole extent of the working length of the root canal. This paper point was transferred to 2ml sterile normal saline in a test tube which was then vortexed for 1min.

Group-III: In this group we used only 5.25% NaOCl for irrigation. Along the usual process of preparation one milliliter of 5.25% NaOCl was employed between each file size. Excess moisture was removed by #35 absorbent paper point (Dentsply International), followed by the placement for 10 seconds of # 40 absorbent paper point to the whole extent of the working length of the root canal. This paper point was transferred to 2ml sterile normal saline in a test tube which was then vortexed for 1min.

Bacterial culture was done from each test tube, using a standard Loop technique to achieve a semi quantitative culture on 5% sheep blood agar. The inoculated agar plates were stored in an incubator under aerobic condition at 37°C for 48hrs. The colony count for the samples from each group was performed digitally.

RESULTS

Anti-bacterial efficacy of each group is shown in Table I, there was no significant difference in antibacterial efficacy using three irrigation protocol, but there was significant difference in mean antibacterial activities among Group I and III (p=0.001) and Group II and III (p=0.001). So in overall comparison, it can be concluded that Group I and Group II Have significantly better antibacterial activity than Group III Table 2.

Statistical Analysis: Research data was analyzed by using SPSS (version 22.0). Statistical tests were applied according to the data obtained.

1. Mean and standard deviation of antibacterial Efficacy (bacterial reduction) were calculated. (Table I)
2. Comparison of Mean and standard deviation of antibacterial efficacy among groups was done by one-way ANOVA. (Table 2)
3. A Tukey Post Hoc analysis was done for multiple comparisons of antibacterial efficacy among groups. (Table 3).

Table No.1: Mean Bacterial Reduction / Antibacterial Efficacy in Different Groups

Antibacterial Efficacy	Group I: (5.25%NaOCl/17% EDTA) n = 30	Group II: (1.3%NaOCl/MTAD) n = 30	Group III: (5.25%NaOCl) n = 30
Bacterial reduction CFU/ml	206.67 ± 125.762	146.67 ± 93.710	870.00 ± 269.290

Table No.2: Comparison of Bacterial Reduction / Antibacterial Efficacy with Different Protocols of Irrigation

	Group I (n=30)	Group II (n=30)	Group III (n=30)	F- Value	df	*p-value
Bacterial Reduction (in C.F.U)	206.67±125.76	146.67±93.71	870±269.29	149.33	2	0.001

Data is shown in mean followed by SD in parentheses;
*using One way ANOVA with p-value<0.05 as statistically significance
df=Degree of freedom; C.F.U=Colony forming unit;
Group I =Irrigation protocol 5.25% NaOCl/17% EDTA;
Group II=Irrigation protocol 1.3% NaOCl/MTAD;
Group III=Irrigation Protocol 5.25% NaOCl (control group).

Table No.3: Post-Hoc Analyses

	Bacterial Reduction (in C.F.U)		Mean differences	*p-value
	(n=30)	(n=30)		
Group I vs. Group II	206.67±125.76	146.67±93.71	60.00	0.404
Group I vs. Group III	206.67±125.76	870±269.29	-663.63	0.001
Group II vs. Group III	146.67±93.71	870±269.29	-723.23	0.001

Data is shown in mean followed by SD in parentheses;
*using Tukey post hoc analysis with p-value <0.05 as statistically significant
C.F.U=Colony forming unit;
Group I =Irrigation protocol 5.25% NaOCl/17% EDTA;
Group II=Irrigation protocol 1.3% NaOCl/MTAD;
Group III=Irrigation Protocol 5.25% NaOCl (control group).

DISCUSSION

The present in vitro study was conducted for the comparison of three irrigation protocols in antibacterial efficacy. The three protocols used were 5.25% NaOCL/17% EDTA (Group I), 1.3% NaOCL/MTAD (Group II) and 5.25% NaOCL (Group III).

In the present study antibacterial efficacy in terms of bacterial reduction (CFU/ml) was determined in all three groups in overall comparison, it can be concluded that Group I and Group II showed more bacterial reduction (CFU of E. faecalis) and have significantly better antibacterial activity than Group III.

The results of present study showed that when comparing 1.3% NaOCl/Biopure MTAD and 5.25% NaOCl/17% EDTA there was no significant difference in antibacterial efficacy of both protocols (p=0.404>0.05) which is in consistent with results of Kho and Baumgartner[14] compared the antimicrobial efficacy of irrigation between 1.3% NaOCl/Biopure MTAD and 5.25% NaOCl/15% EDTA in the apical 5mm of roots infected with E. faecalis .They reported 131 ± 291 mean CFU/mg in 5.25% NaOCl/15% EDTA group and 187 ± 237 CFU/mg in 1.3% NaOCl/ Biopure MTAD group and found no significant difference (p=0.495>0.05) in CFU of E. faecalis and antibacterial efficacy of both protocols.

Another study by Davis et al.[13] in which comparison of the antimicrobial effects of various endodontic medicaments (Biopure MTAD, 5.25% NaOCl and 2% CHX) on enterococcus faecalis conducted and reported that 5.25% NaOCl exhibited less antimicrobial efficacy against E. faecalis than BioPure MTAD (p<0.05), So in comparison the results of present study are consistent with this previous study as present study showed that NaOCl/Biopure MTAD irrigation protocol had significantly better antibacterial efficacy compared to 5.25% NaOCl (p=0.001<0.05).

However, study by Johal et al[15] on comparison of antimicrobial efficacy of 5.25% NaOCl /15 % EDTA and 1.3% NaOCl/Biopure MTAD for root canal irrigation reported that the treated teeth with 5.25%

NaOCl /15 % EDTA reported greater bacterial reduction and antimicrobial efficacy (0 CFU/ml) as compared to those treated with 1.3% NaOCl/Biopure MTAD (19±15 CFU/ml) with statistically significant difference (p=0.01<0.05). The results of the present study are inconsistent with this previous study as present study showed that there was no statistically significant difference between the antimicrobial efficacy of 1.3% NaOCl/MTAD and 5.25% NaOCl/ 17% EDTA. (p=0.404>0.05).

Shabahang and Torabinejad[11] conducted a study on comparison of antimicrobial efficacy against E. Faecalis of 1.3% NaOCl/MTAD, 5.25% NaOCl/17% EDTA and 5.25% NaOCl for root canal irrigation showed that the teeth treated with 1.3% NaOCl/ MTAD showed greater antibacterial efficacy (0 growth) vs 5.25% NaOCl / 17% EDTA (8 samples showed growth) and versus 5.25%NaOCL with statistically significant difference (p=0.002<0.05).but there is no statistically significant difference in the antibacterial efficacy between 5.25 % NaOCl/ 17% EDTA and 5.25 % NaOCl (p=0.849>0.05) The results of antimicrobial efficacy between 1.3% NaOCl/MTAD and 5.25% NaOCl/ 17% EDTA in the present study are inconsistent to this previous study as present study showed no significant difference in antimicrobial efficacy between 1.3% NaOCl/MTAD and 5.25% NaOCl/ 17% EDTA (p=0.404>0.05) , but The results of antimicrobial efficacy between 1.3% NaOCl/ MTAD and 5.25 % NaOCl is consistent with this previous study as we also found significant difference(p=0.001< 0.05).The results of antimicrobial efficacy when comparing the antimicrobial efficacy of 5.25% NaOCl / 17%EDTA with 5.25% NaOCl is also inconsistent with this previous study as we found significant difference. (p=0.001<0.05).

CONCLUSION

In conclusion, there is no difference in antibacterial efficacy of 5.25% NaOCl/17% EDTA and 1.3% NaOCl/MTAD against E. faecalis. Sodium hypochlorite alone is not sufficient in achieving complete canal root canal disinfection, EDTA and MTDA have important role in root canal disinfection.

Author's Contribution:

Concept & Design of Study:	Uzair Aftab
Drafting:	Madiha Zaighum, Darshana
Data Analysis:	Talha Asad Khan, Rafia Khan, Sheba Ramzan
Revisiting Critically:	Uzair Aftab, Madiha Zaighum
Final Approval of version:	Uzair Aftab

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Persoon IF, Özokcorresponding AR. Definitions and Epidemiology of Endodontic Infections. Curr Oral Health Rep 2017;4(4): 278–285.
2. Song M, Kim HC, Lee W, Kim E. Analysis of the cause of failure in nonsurgical endodontic treatment by microscopic inspection during endodontic microsurgery. J Endod 2011;37: 1516–9.
3. Johnson EM, Flannagan SE, Sedgley CM. Coaggregation interactions between oral and endodontic Enterococcus faecalis and bacterial species isolated from persistent apical periodontitis. J Endod 2006;32: 946–950.
4. Endo MS, Ferraz CC, Zaia AA, Almeida JF, Gomes BP. Quantitative and qualitative analysis of microorganisms in root-filled teeth with persistent infection: Monitoring of the endodontic retreatment. Eur J Dent 2013;7:302–9.
5. Agrawal Vineet S, Rajesh M, Sonali K, Mukesh P. A Contemporary Overview of Endodontic Irrigants – A Review. J Dent App 2014;1: 105-115.
6. Abuhaimed TS, Abou Neel EA. Sodium Hypochlorite Irrigation and Its Effect on Bond Strength to Dentin. Biomed Res Int 2017; 1930360.
7. Guneser MB, Arslan D, Dincer A, Gamze Er. Effect of sodium hypochlorite irrigation with or without surfactants on the bond strength of an epoxy-based sealer to dentin. Clin Oral Investig 2017;21(4):1259-1265.
8. Faras F, Abo-Alhassan F,Sadeq A, Burezq H. Complication of improper management of sodium hypochlorite accident during root canal treatment. J Int Soc Prev Community Dent 2016;6(5): 493–496.
9. Mohammadi Z, Shalavi S, Jafarzadeh H. Ethylenediaminetetraacetic acid in endodontics. Eur J Dent 2013;7(Suppl 1): S135–S142.
10. Jaju S, Jaju PP. Newer Root Canal Irrigants in Horizon: A Review. Int J Dent 2011;1-9.
11. Torabinejad M, ChoY, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J Endod 2003;29:233-239.
12. Shabahang S, Torabinejad M. Effect of MTAD on enterococcus faecalis-contaminated root canals of extracted human teeth. J Endod 2003;29:576–9.
13. Davis JM, Maki J, Bahcall JK. An In Vitro Comparison of the Antimicrobial Effects of Various Endodontic Medicaments on Enterococcus faecalis. J Endod 2007;33:567–9.
14. Kho P, Baumgartner JC. A Comparison of the Antimicrobial Efficacy of NaOCl/Biopure MTAD versus NaOCl/EDTA against Enterococcus faecalis. J Endod 2006;32:652–5.
15. Johal S, Baumgartner JC, Marshall JG. Comparison of the antimicrobial efficacy of 1.3% NaOCl/Biopure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation. J Endod 2007;33: 48-51.