Comparing The Antibacterial Efficacy of NaoCL, Chlorhexidine, NaOCL/MTAD Chlorhexidine/MTAD Against *E. Faecalis*

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Abstract: Aim of study: This study aims at comparing the anti-microbial efficacy of irrigation with Biopure MTAD against irrigation with 5.25 % NaOCl, CHX, NaOCl/MTAD, and CHX/MTAD in *E. faecalis*-root canals. Materials and Methods: Thirty (30) human single-rooted lower premolar teeth were extracted and de-coronated at the cemento-enamel junction (CEJ). Then, the roots were instrumented by k-file until the size is 40. Furthermore, these teeth were exposed to bacterial contamination in brain heart infusion (BHI) for 48 h. The contaminated samples were divided into 5 groups (MTAD, NaOCl, CHX, NaOCl / MTAD, and CHX / MTAD). The dentin chips in the canals of the samples were removed using a sterile low-speed handpiece round bur and transferred to BHI. The media was cultured, and the bacterial colonies were counted and recorded as CFU/mL. Results: NaOCl / MTAD showed a significantly higher antimicrobial activity compared to 5.25 % NaOCl, MTAD, CHX, and CHX / MTAD. Conclusion: The antimicrobial activity of a combined NaOCl and Biopure MTAD was strongest against *E. faecalis* compared to the other irrigant solutions.

Keywords: NaOCl, CHX, *E. faecalis*

1. Introduction

One of the basic aims of root canal treatment (RCT) is to eradicate microbes from infected root canals and prevent re-infection. Achieving a successful RCT depends on an efficient cleaning and shaping of root canals using suitable irrigants; it also depends on complete obturation of the canal system (blerim et al., 2017). Irrigation is a crucial factor that determines the result of RCT. During RCT, an irrigant is used to flush debris out from the instrumented root canals; it is also used to dissolve the organic tissue remnants, lubricate the working site, and disinfect the root canal space without unnecessarily irritating biological tissues (sheetal et al., 2017). Sodium hypochlorite (NaOCl) is the commonly used irrigating solution owing to its effectiveness against microbes. It is also an excellent organic solvent and a good lubricant. However, at high concentrations, NaOCl is highly irritating to periapical tissues (prabhakar et al., 2010).

Another antimicrobial agent that holds substantivity and low-grade toxicity is Chlorhexidine gluconate (CHX). At low concentration (0.2%), it is bacteriostatic, but at high concentration (2%), it is bactericidal and can adsorb to dental tissues which can cause a prolonged intermittent release at therapeutic levels. However, CHX is not suitable for dissolving pulp tissue and may leave out debris on canal walls which can obstruct the dentinal tubules (rakesh et al., 2013). MTAD is an endodontic irrigant comprising of a mixture of a tetracycline isomer, Tween 80, and citric acid.

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It is used for the disinfection of the root canal system and represents an innovative technique for a simultaneous complete root canals disinfection and endodontic smear layer removal. Clinically, it has been proven biocompatible and effective with a potential anti-bacterial substantivity (Sandeep et al., 2017). This study aims to evaluate NaOCl, CHX, NaOCl/MTAD, and CHX/MTAD for anti-bacterial activity against *E. faecalis*.

Biopure MTAD (Dentsply, Tulsa OK) is a mixture oftetracycline isomer (doxycycline) an acid (citric acid), anda detergent (Tween 80). When used as a root canal irrigant, MTAD has been reported to safely remove the smear layerand effectively eliminate Enterococcus faecalis. Studieshave found E. faecalis to be a commonly recoveredmicrobe in failing root canals (Sandeep et al., 2017).

It has also been shown that mechanical instrumentationwithout irrigation reduces but does not predictablyeliminate bacteria in the canal. Thus, a root canal irrigantis needed to aid in the debridement of the canals. Variousconcentrations of sodium hypochlorite (NaOCl) have beenused as root canal irrigants for many decades. The mainadvantages of NaOCl are its ability to dissolve necrotictissues and its antibacterial properties against most microorganisms (kokak et al., 2008).

2. Materials and Methods

Thirty (30) fresh human single-rooted premolar teeth were extracted from patient's age 16-25 year and placed in distilled water. Then, piezon Master 400scaler (EMS, Swiss) was used to mechanically remove the soft tissue remnants and calculus on the external root surface. The specimens were inspected for root fractures or any defect under a stereomicroscope (Motic, China, x10 magnification) to ensure the complete formation of apices. Each tooth was decoronated at the CEJ using a diamond sectioning disc (Brasseler, Germany). Pulp tissues were removed using a barbed broach, while the patency of root canal was confirmed using a No.10 K-type file. The same No.10 K-type file was also used to determine the working distance of each root canal. The instrument was positioned in the root canal under a stereomicroscope until its tip emerges at the apical foramen. Then, the actual working distance of each root canal was determined by subtracting 1.0 mm from the measured file length (kokak et al., 2008). Root canal irrigation was carried out with 2 mL of 2.5 % hypochlorite solution before and after each instrumentation (kumar et al., 2008) 7. After the biomechanical preparation process, the dentinal smear layer on the specimens was removed using 2 mL of 17 % EDTA for 1 min, followed by 2 mL of 5.25 % hypochlorite solution. Finally, the samples were autoclaved to ensure sterility (kho P et al., 2006).

2.1 Isolation of Microorganisms

2.1.1 Selection of Patients

Four (4) patients diagnosed with apical periodontitis of single root teeth via clinical examination and radiograph were selected for this study.

2.1.2 Access Opening Preparation

Initially, access cavity was prepared using a sterile round and straight turbine fissure bur until reaching the roof of the pulp chamber. Prior to the perforation of the pulp chamber, the tooth was first isolated using a cotton roll and a sucker tip before obtaining the access opening using a sterile handpiece large round bur. K-files of size 25, 30, and 35 were inserted into the root canal as the initial canal enlargement before disinfecting the root canal with an irrigant solution to ensure the microorganisms

are not killed. A 25-sized paper point was placed in the root canal and allowed for 1 min before being removed and placed into BHI for 30 minutes.

2.1.3 Isolation of E. faecalis

A loopful of the inoculated BHI broth was collected using a cotton swab, evenly spread on Enterococcus selective media, and incubated aerobically in an anaerobic jar for 48 h at 37◦C. At the end of the incubation period, the surface of the media was observed for reddish-pink Enterococcus colonies (srikumar et al., 2007).

2.1.4 Root Canal Contamination with E. faecalis

A colony of the isolation E. faecalis was picked and injected into the root canal. The canal was incubated anaerobically at 37°C for 24 h. Then, the irrigant solutions {Biopure MTAD (Dentsply Tulsa Dental USA), NaOCl 5.25, CHX 2%, NaOCl/ MTAD, CHX /MTAD} were used to irrigate the samples. Normal saline was first used to irrigate the samples before using MTAD as the final irrigant. The irrigant was allowed in the canal for 5 min as per manufacturer's recommendation before drying the canals using sterile paper points. For samples irrigated with a combination of NaOCl/ MTAD and CHX /MTAD, NaOcl or CHX was first used to irrigate the samples before using MTAD as a final irrigant. The irrigant was also left in the canal for 5 min before drying the canal using sterile paper points. Canals irrigated with the other groups were first irrigated with 5 mL of 5.25% NaOCl, 2% CHX, or normal saline before drying the canal using sterile paper point. After the drying process, a sterile handpiece round bur was inserted into the root canal to cut the internal canal wall. The dentine chips were collected and seeded into BHI broth and allowed for 30 min. Later, 0.1 mL of the broth was collected and add to 0.9 mL of new BHI broth. From this suspension, 0.1 mL was drawn and added into 0.9 mL of fresh broth to make a final volume of 1 mL. Then, this final 1 mL broth volume containing the microorganism was inoculated on a selective media and incubated for 48 h at 37°C. Finally, the colonies were checked and recorded as CFU/mL. (srikumar et al., 2007) (Maryam et al., 2017).

3. Results

Table 1: Descriptive statistics of the irrigant solutions

Material	N	Mean	SD	SE	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
MTAD	6	5.0000	2.64575	1.52753	-1.5724	11.5724	2.00	7.00
NaOCl	6	10.6667	2.51661	1.45297	4.4151	16.9183	8.00	13.00
CHX	6	14.0000	2.00000	1.15470	9.0317	18.9683	12.00	16.00
NaOCl/MTAD	6	0.3333	0.57753	0.33333	-1.1009	1.7676	0.00	1.00
CHX/MTAD	6	7.3333	1.15470	0.66667	4.4649	10.2018	6.00	8.00
Total	30	7.4667	5.12510	1.32330	4.6285	10.3049	0.00	16.00

SD = standard deviation, SE = standard error

According to table (1) the highest antibacterial activity was the combined irrigant solution NaOCL/MTAD against E. faecalis, the 2nd irrigant solution was MTAD and then followed by CHX/MTAD and then followed by Naocl and finally the weakest irrigant solution against E. faecalis was the CHX.

Table 2: One-way ANOVA comparison of the materials that showed statistically significant differences between groups ($p < 0.05$).

Material	Sum of squares	Df	Mean square	F	Sig.
Between groups	329.733	4	82.433	21.693	0.000
Within groups	38.000	10	3.800		
Total	367.733	14			

According to table (2) there is a significant differences between all groups ($p<0.05$) the Naocl/MTAD was the best irrigant solution with highest antibacterial activity against E. faecalis in comparison to the other groups(MTAD , CHX , NaOCL , CHX/MTAD) and the weakest one was the CHX.

Table 3: presents the antibacterial activity of the irrigants used in this study against E. faecalis. The Duncan test was used as a post hoc comparison to show significant differences between the antibacterial activities of the irrigant solutions.

Material	N	Duncan's grouping* mean **				
		A	B	C	D	E
NaOCl/MTAD	6	0.3333				
MTAD	6		5.0000			
CHX/MTAD	6			7.3333		
NaOCl	6				10.6667	
CHX	6					14.0000

*Significant results indicated by different letters.

** mean of observed antibacterial activity.

Accordind to table (3) Duncan test we can see the significant difference between results that's indicated by different letters , letter A mean the highest antibacterial activity against E.faecalis and related to the combined irrigant solution NaOCL/MTAD , letter B mean the 2nd irrigant solution in regarding the antibacterial activity against E.faecalis and it was MTAD , letter C mean the 3rd irrigant solution in regarding the antibacterial activity against E.faecalis it was CHX/MTAD , letter D mean the 4th irrigant solution in regarding the antibacterial activity against E.faecalis it was NaOCL , letter E mean the most weak irrigant solution in regarding the antibacterial activity against E.faecalis it was CHX.

4. Discussion

The outcome of this study showed NaOCl/MTAD to exhibit the strongest antibacterial activity against E. faecalis, followed by MTAD, CHX/ MTAD, NaOCl, and CHX. This result agreed with previous studies that compared the antibacterial activity of 1.3 % NaOCl/BioPure MTAD versus 5.25 %

NaOCl/17% EDTA in the apical 5 mm E. faecalis-infected teeth. After irrigation, E. faecalis in dentinal tubules were exposed by resecting and pulverizing the root canal apexes. The report of the study observed no significant differences in the antibacterial activity of irrigation with 5.25% NaOCl-17% EDTA versus 1.3% NaOCl/BioPure MTAD in E. faecalis-infected 5-mm apical dentinal tubules (Maryam et al., 2017) (clegg et al., 2006).

A similar study reported only 6% and 3% NaOCl to have disrupted and removed biofilm, while 1.6% NaOCl/BioPure MTAD only disrupted but couldn't completely eliminate bacteria (Sandeep dubey etal 2017). Furthermore, a comparative study of MTAD, NaOCl and the consecutive use of the irrigants (instead of combination) have been reported. Here, 1.3% NaOCl was used, and the results suggested MTAD as the most potent in E. faecalis elimination. Meanwhile, no significant difference was noted between NaOCl and N+M likely due to the varying NaOCl concentrations, bacterial species used, or the slight variation in incubation conditions used (shenoi et al., 2016) (Tay et al., 2006) (Madhya et al., 2016).

Contrarily, this in vitro study showed a superior antibacterial activity of MTAD against S. aureus, E. bacteria, and E. faecalis compared to NaOCl and M+N using the ZI method. A mixture of M+N showed the least antimicrobial activity among the studied irrigants (Mohammad et al., 2009). Another study reported 2.5% and 5.25% NaOCl and 2% CHX gel as the most effective irrigants for E. faecalis biofilms elimination at the tested time intervals compared to CHX liquid and MTAD. This could be attributed to the various strains of E. faecalis, different concentrations of doxycycline in MTAD, the incubation conditions, or the type of media used (selective or non-selective)

Sarmad etal 2020 found in his study all samples treated with MTAD showed nearly total absence of pathogens. the samples treated 3% NaOCl and 17% EDTA showed similar result to the first group, Statistical analysis of the data using dunnett t3 test showed there is no significant difference between the groups ($P > 0.05$) (sarmad et al., 2020).

Divya et al., 2020 found in his study 5% NaOCl showed the high antimicrobial activity, and herbal products showed significant antimicrobial activity against E. faecalis and can be used as an alternative to NaOCl (Divya et al., 2020).

Hatice et al., 2020 found in his study CHX, MTAD and ClO2 showed a high potential for the killing of E. faecalis, both alone and in all combinations. The EDTA, H2O2, H2O2+ EDTA, H2O2 + NaOCl and SC + NaOCl groups showed less antimicrobial activity than the other groups. The SC + CHX group showed the best antibacterial effect against E. faecalis (Hatice et al., 2020).

Nehal and walaa 2019 their results showed that during the first 10 hours, MTAD showed immediate antimicrobial effect and maintained its higher antimicrobial activity than 2% chlorohexidine. After 48, 72, 96 and 240 hours, both MTAD and 2% chlorohexidine showed the same prolonged action of post-antibiotic effect against E. faecalis with a non-significant difference. According to Antibiotic sensitivity, the results revealed MTAD is the most effective antibacterial drug, showing the highest zone of inhibition, followed by 2% Chlorhexidine and Nitrofurantoin 300 mcg which showed the same inhibitory activity. As a conclusion from the current study, it can be concluded that MTAD has a strong bactericidal effect against E. faecalis and showed the highest zone of inhibition (Nehal etal 2019).

Mohamed El SayedNikta et al., 2020 in his study, all medicaments showed variable inhibition zones for all bacterial strains except Diapex Plus which showed no antibacterial activity. NaOCl gel exhibited

the most significant inhibition zones for all bacterial strains followed by CHX gel, Ledermix, and CH. However, the effect of CHX and CH paste against S. aureus was statistically similar, while the effect of CH against E. faecalis was significantly higher than the Ledermix. Conclusion: Sodium hypochlorite gel displayed the highest antibacterial activity among tested medicaments and can be recommended as a potent intracanal medicament. Chlorhexidine gel showed a significantly higher antibacterial effect when compared with Ledermix and calcium hydroxide. Calcium hydroxide demonstrated stronger antibacterial activity against E. faecalis than Ledermix. Diapex Plus exhibited no antibacterial effect (Mohamed et al., 2020).

Muhammad Sohail et al., 2014 his study was to evaluate the antimicrobial activity of recently introduced MTAD in vitro and comparing to commonly used irrigators such as NaOCl, chlorhexidine and EDTA under similar conditions and various pH levels. The results of this in vitro study were statistically analysed using Kruskal Wallis one-way ANOVA and Mann Whitney U test. It has been concluded that MTAD has the capability to eradicate E. faecalis (Muhammad et al., 2014).

Difference in microorganism strains used, time intervals, the testing methods and the bacterial resistance may be the main reasons of these controversies (Sarmad A. et al., 2020). method of incubation (aerobic or anaerobic) (Divya Gupta et al., 2020). and type of media (selective agar or not), type of testing materials and Number of bacterial colonies. also, may be reasons for these controversies. (Nehal Sharaf et al., 2019).

5. Conclusion

According to the outcomes of this research the antimicrobial activity of a combined NaOCl and Biopure MTAD was strongest against E. faecalis compared to the other irrigant solutions the most weak irrigant solution in regarding the antibacterial activity against E.faecalis it was CHX.

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