RESEARCH
www.commprac.com
ISSN 1462 2815

EFFECT OF VARIOUS ROOT CANAL DISINFECTION PROCEDURES ON EXPRESSION OF ENTEROCOCCUS FAECALIS VIRULENCE FACTOR – A PCR STUDY

Dr. Vandana J *[1], Dr. Gautham S.P [2], Dr. Charanya C [3], Dr. Balagopal S [4] and Dr. Dheepshi M [5]

[1] Professor, Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai, Tamil Nadu, India.
*Corresponding Author Email: vandnajames@yahoo.com
[2] Assistant Professor, Department of Dental Surgery, P.S.G Institute of Medical Science, Peelamedu, Coimbatore, Tamil Nadu, India.
[3] Reader, Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai, Tamil Nadu, India.
[4] Professor and Head, Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai, Tamil Nadu, India.
[5] Post Graduate, Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai, Tamil Nadu, India.

DOI: 10.5281/zenodo.12186241

Abstract

Objective: To compare and evaluate antimicrobial effects of 2% Chlorhexidine (CHX) versus 0.1% Octenidine Dihydrochloride (OCT) as root canal irrigant with and without Laser activation against *Enterococcus faecalis* virulence factor - *E. faecalis* endocarditis antigen (efaA) using real time polymerase chain reaction. **Aim:** To evaluate the effect of various root canal disinfection procedures on expression of *Enterococcus faecalis* virulence factor (efaA) using real time PCR. **Methods and Materials:** Forty single rooted premolars were taken and decoronated to standardize the root length as 14 mm. The canals were instrumented up to F3 Protaper Gold and teeth were autoclaved at 121°C for 20 minutes at 15 psi. 0.1 mL of the bacterial suspension was injected into the root canals, and the samples were incubated at 37°C and 100% humidity for 21 days. The samples were randomly divided into four groups: Group I – 2% Chlorhexidine, Group - II 2% Chlorhexidine with Laser, Group III – 0.1% Octenidine dihydrochloride, Group IV- 0.1% Octenidine dihydrochloride with Laser. After the irrigation protocol, Paper points were used to transfer the contents of the canal. PCR was performed to detect the presence of Enterococcus faecalis virulence factor (efaA). **Results:**Octenidine (0.1%) was more effective than 2% Chlorhexidine against *E. faecalis*. Group II and IV showed significant difference compared to Group I and III with statistically significant difference ($p<0.001$). Laser activation enhanced the antimicrobial action of the irrigants. **Conclusion:** Octenidine (0.1%) was more effective than 2% Chlorhexidine against *E. faecalis*. Laser activated irrigation proved to enhance the antimicrobial action of the irrigants.

Keywords: Octenidine Dihydrochloride, Chlorhexidine, Vireulence Factor, E. Faecalis.

INTRODUCTION

The success of root canal treatment relies on complete debridement, asepsis and three dimensional obturation. Literature shows that various primary and secondary microorganisms are present in the root canal. The most common bacteria discovered in persistent/secondary infections has been confirmed as E. faecalis. (1) *Enterococcus faecalis* (E. faecalis) is an extremely resistant strain of microorganism that persists in the root canal even after treatment, frequently causing 24% to 77% of endodontic failures. However, E. faecalis has been found in cases where there have been no apical periodontitis lesions. (2) Molecular analysis supported this, leading to the conclusion that it may not be necessarily the major pathogen related with post-treatment illness.(3) The reason for the presence of E. faecalis in cases with and

without disease is unknown, however it could be connected to variances in virulence capacities across strains of the species. Mechanical instrumentation cannot sufficiently disinfect root canals and hence irrigating solutions and intracanal medicaments are required to eradicate microorganisms. The irrigation plays a pivotal role in the asepsis. Over a period of time, a variety of chemicals have been introduced. An endodontic irrigant should ideally exhibit powerful antimicrobial activity, disinfect the root canal space, and have no cytotoxic effects on periradicular tissues. Some of the gold standard irrigating solutions are sodium hypochlorite and chlorhexidine.

The aim of this study is to evaluate the effect of various root canal disinfection procedures on expression of *Enterococcus faecalis* virulence factor (efaA) using real time PCR.

METHODOLOGY

Figure 1: 40 single rooted mandibular premolar

40 single-rooted mandibular premolars extracted for periodontal reasons were included in this study. Teeth with root caries, root fracture and developmental anomalies were excluded. The teeth presented straight root canals, fully formed apices and root canal length was standardized to 14mm by decoronating with a diamond disc. Apical patency was established with a #10-K file (Mani Inc., Japan). The orifice enlargement was done using Gates Glidden drills #6, 5, 4 (Dentsply Maillefer, USA), followed by biomechanical preparation upto F3 (Protaper gold, Dentsply Maillefer, USA), to working length [WL]. 5% NaOCl (Prime Dental Products Pvt., Ltd., India), 0.9% saline (Fresenius Kabi Private Limited, India), 17% EDTA (RC Help, Prime Dental Products Pvt. Ltd., India) irrigation were carried out between each instrumentation. The apex of all specimens were made impermeable with two layers of epoxy adhesive (LOCTITE Pvt., Ltd., India).(4) To obtain a closed root canal system that mimicked clinical in vivo scenarios and to ensure easy handling, a customized model was fabricated for each tooth by mounting into a 1.5-mL polypropylene Eppendorf tube (Eppendorf Private Limited, India) filled with silicone rubber impression

material.(5) (Figure 2) These specimens were then autoclaved at 121 °C, 15 lbs for 30 minutes.

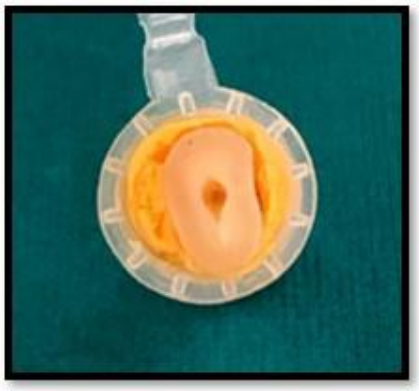

Figure 2: Customized mould

Biofilm formation

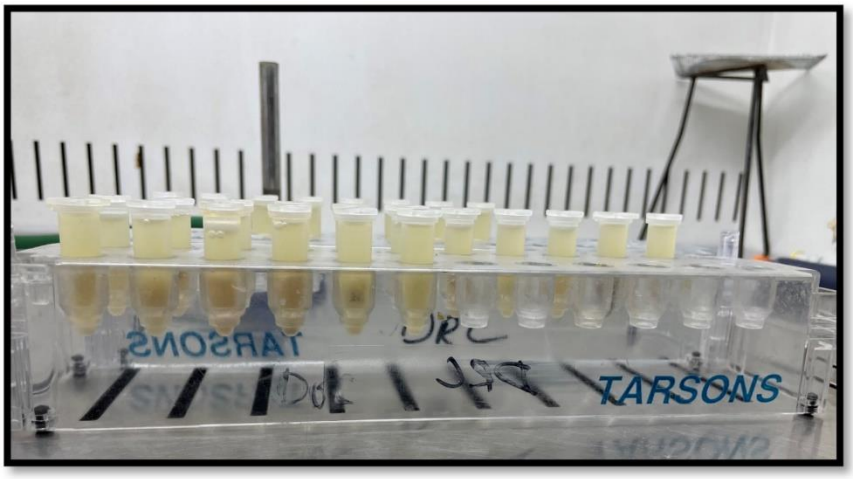

Figure 3: E.faecalis inoculation and incubation

Pure standard strain of ATCC 29212 Enterococcus faecalis 50µL was inoculated in 5ml of brain heart infusion (BHI) broth for 24 hrs at 37℃. The cell suspension was adjusted spectrophotometrically to match the turbidity of 3.0×10^8 CFU/mL (equivalent to 1.0 McFarland standard). Under laminar air flow chamber ensuring asepsis, a sterile needle was used to inoculate 0.1-mL aliquots of E. faecalis culture into each canal. (Figure 3) Fresh BHI broth (100µl) was replaced every 24 h, for seven days. During the experimental period, the entire set-up was incubated at 37°C. Samples were randomly divided in five groups (n = 10).

Group1- No treatment (Negative control)

Group 2- 2% CHX (Asep-RC, Anabond Stedman Pharma Research, India) without activation (Positive control)

Group 3- 2% CHX with LASER activation (IMDSL Automatic Dental Diode Laser - 10W, Macromed, India)

Group 4- 0.1% OCT (octenisept; Schulke & Mayr, Nordersdedt, Germany) without activation

ISSN 1462 2815

Group 5- 0.1% OCT with LASER activation

For all the non-laser activation groups, the respective irrigating solutions were used. A sterile needle (26 gauge) was introduced into the root canal until 1 mm short of WL and 4 mL of the specific solution was irrigated, for 2 min. Thereafter, the root canals were washed with 2 mL of sterile saline solution.

For the laser activation groups, a sterile needle was introduced into the root canal until 1 mm short of the WL and 2 mL of the specific solution was injected, for 2 min. LASER activation by diode laser, delivered by 400 µm fiber tip kept 1-2 mm away from the apex was activated at 25 uJ Energy; 1 W power; Pulse mode; 20 sec- 3 cycles in sweeping motion from apical to coronal third for 1 min. Then, irrigation was done with respective irrigating solutions (2 ml) again for 2 min. Finally, the canals were flushed with 2 ml of sterile saline solution. (Figure 4)

Figure 4: LASER activation

After the disinfection protocol, sterile paper points were introduced into the root canal and the contents of the canal were absorbed to determine the expression of specific virulence factors of E.faecalis using real-time RT-PCR method.

RNA extraction:

Extraction of RNA was performed using the mRNA-ONLY ™ prokaryotic mRNA isolation kit (Epicenter; Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. The obtained RNA was refrigerated at a temperature of −70°C for the synthesis of cDNA.

cDNA production:

The cDNA synthesis was performed according to the RevertAid first-strand cDNA synthesis kit protocols (Fermentas, Vilnius, Lithuania). In order to control the cDNA, its expression was investigated by the RT-PCR method and 16S rRNA primers. The real-time RT-PCR technique was used to study the expression of the target genes. Using the data from the real-time RT-PCR and the ddCt formula, the ratio of changes in the expression of target genes was calculated in comparison with the control sample.

STATISTICAL ANALYSIS

The values were entered into an Excel sheet (Microsoft) for calculation. Data was tabulated and the results were presented in mean ±SD. One way analysis of variance followed by Tukey's post-hoc tests were used to compare ratio of changes among the groups. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

RESULTS

PCR determines the results in Ct. Ct is inversely proportional to the amount of target DNA and hence, the number of microorganisms in the sample. DNA load for all the different groups of root canal disinfection were compared with the values attained in the control group and was found to be statistically significant ($P < 0.005$).

A significant increase in the expression of ***efa*** virulence factor in group 1 in comparison with experimental groups indicating that the disinfection protocol effectively decreases the expression of virulence factor.

The expression of ***efa*** factor was greater in group 2 in comparison with group 4 but the difference was not statistically significant. While comparing the groups 3& 5, expression of ***efa*** virulence factor is lesser in group 5 compared to group 3 which is statistically significant indicating that Octenidine dihydrochloride is better than chlorhexidine and LASER act synergistically in supressing the expression of ***efa*** virulence factor.

Thus, the expression of ***efa*** virulence factor between experimental groups showed that group 3 & 5 showed significant suppression among all indicating that LASER activation has boosted the antimicrobial effect of disinfection procedure. (Graph 1)

DISCUSSION

Complexities of the root canal anatomy, polymicrobial nature of infection and biofilm formation by microbes is the major challenges faced by endodontists in routine clinical practice. E. faecalis, a gram-positive facultative anaerobe can be encountered even in a perfectly obturated root canal and produce the most resistant biofilm.(6) Hence in the present study the antimicrobial efficacy of irrigants was tested against the E. faecalis.

It has been considered that the virulence factor is responsible for the clinical resistance of this microorganism. These virulence factors may be related not only in E.faecalis adhesion to host tissues, invasiveness, and abscess formation, but also in the production of several compounds that aid biofilm formation. They can also influence the inflammatory responses of the host.(7) The virulence factors aggregation substance (AS), serine protease (sprE), enterococcal polysaccharide antigen (epa), gelatinase (gelE), enterococcal surface protein (esp), and general stress protein (gls24) have all been found in clinical isolates; however, gelE, sprE, and esp have not all been found in clinical isolates systematically.(8)

E.faecalis endocarditis antigen (efaA) is a powerful virulence factor detected in E.faecalis strains isolated in the root canals of treatment-resistant endodontic infections.. (9) Pathogenicity of the microorganism depends on the expression of Virulence Factor. RT PCR was used in this study to check the presence of particular virulence factor efaA which is responsible for the failure of endodontic treatment.

The gold standard of oral antiseptics is chlorhexidine digluconate (CHX).(10) CHX has antibiofilm activity and high substantivity lasting for at least 24hr.(11) Therefore, CHX is used as root canal irrigant or intracanal dressing.(12) However, there are some side effects to CHX use, including tooth discoloration and taste disturbance, which prevent their long term use.(13) Hence, there has been a growing interest in new antimicrobial compounds in the past decades.

Octenidine dihydrochloride (OCT) is a bispyridine cationic antiseptic developed in the 1980s at the Sterling-Winthrop Research Institute.(14) OCT has been marketed as a product with a similar antimicrobial spectrum like CHX.(15) OCT interferes with cell wall and membrane of microorganisms showing bactericidal and fungicidal activity.(16) Furthermore, OCT inhibits biofilm formation and disrupts fully formed biofilm even in presence of serum protein. (17) OCT has a similar effect to 5.25 % NaOCl against C. albicans and E. faecalis at concentrations of 0.03 %, 0.05 %, and 0.10 %, and a higher effect than 0.12 % and 2% CHX. (18) Additionally, OCT has greater antimicrobial activity against E. faecalis biofilms compared with CHX, and presents faster ability to produce intratubular disinfection than NaOCl and CHX.(19) Furthermore, OCT antibacterial activity against cariogenic microorganisms was shown in several in vitro studies. OCT was even 3 to 4 times more effective than CHX depending on the bacterial species.(20)

Diode lasers penetration depth into the dentine upto 750 µm and also have lower thermal risks.(21) There are only few literature data about the antimicrobial effectiveness of diode lasers in endodontics. The amount of bacterial reduction using a 830-nm diode laser with output power of 1.5 W is 97.56%. It is claimed that diode laser can be considered as an alternative technique for root canal disinfection.(22) The use of diode laser in occluding the dentinal tubules particularly in the apical third area after smear layer removal could help in decreasing the risk of reinfection.(23) The results of a study by Mehrvarzfar et al is in accordance with this study stating that diode laser has the potential to significantly reduce the microbes when compared with the non-laser groups.(24)

Live and dead microbes and Penetration depth of microbes are unknown. Further in-vivo studies are necessary to evaluate the efficacy of irrigants and diode lasers against polymicrobial scenario which may interfere and affect the antimicrobial efficacy of root canal irrigants. In order to eliminate the possible interfering factors, there is a need to create controlled conditions in ex-vivo studies. There is no doubt that more clinical studies on the efficacy of this solution in comparison with other disinfecting irrigants are necessary. Further studies are necessary to know the effect of penetration depth of the octenidine dihydrochloride.

CONCLUSION

Octenidine dihydrochloride (0.1%) was more effective than 2% Chlorhexidine against *E. faecalis* virulence factor (efA). Laser activated irrigation proved to enhance the antimicrobial action of the irrigants. Diode laser in combination with 2% CHX and 0.1% OCT solution had significant effects in reducing the expression of E. faecalis virulence factor in comparison to other experimental groups. It was also evident that irrespective of the irrigating solution used, all the laser activated groups had statistically significant results when compared to their non- laser counterparts.

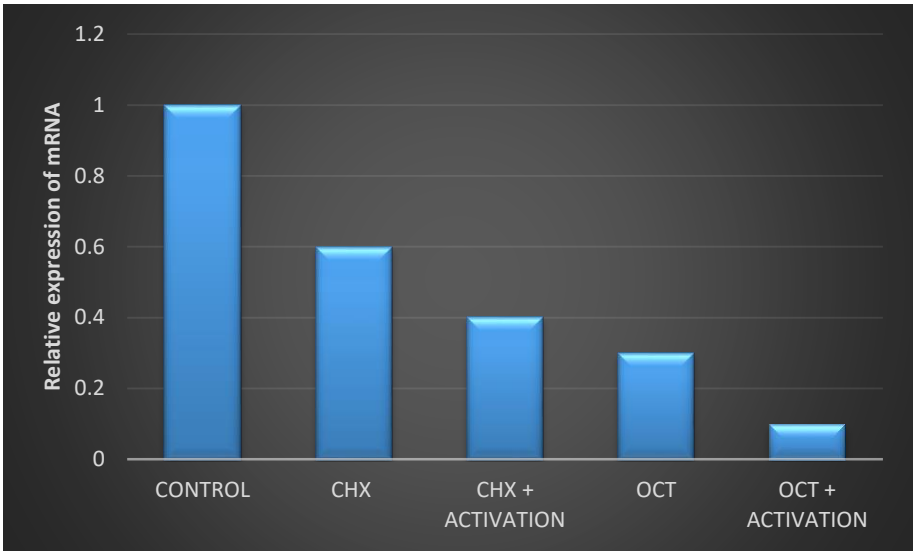

Table 1: Effect Of Different Disinfection Procedures On The E.Faecalis Virulence Factor

References

1) Rocas, I.N., Siqueira Jr., J.F., Santos, K.R.N., 2004. Association of Enterococcus faecalis with different forms of periradicular diseases. J. Endod. 30, 315-320.

2) Molander, A., Reit, C., Dahle n, G., Kvist, T., 1998. Microbiological status of root-filled teeth with apical periodontitis. Int. Endod. J. 31, 1-7.

3) Zoletti, G.O., Siqueira Jr., J.F., Santos, K.R.N., 2006. Identification of Enterococcus faecalis in root-filled teeth with or without periradicular lesions by culture dependent and independent approaches. J. Endod. 32,722-726.

4) De Almeida J, Cechella B, Bernardi A, de Lima Pimenta A, Felippe W. Effectiveness of nanoparticles solutions and conventional endodontic irrigants against Enterococcus faecalis biofilm. Indian J Dent Res. 2018;29(3):347.

5) Liu Y, Guo L, Li Y, Guo X, Wang B, Wu L. In vitro comparison of antimicrobial effectiveness of QMix and other final irrigants in human root canals. Sci Rep. 2016 Nov;5(1):17823.

6) Siqueira JF, Rôças IN, Ricucci D. Biofilms in endodontic infection: Biofilms and apical periodontitis. Endod Top. 2010 Mar;22(1):33–49.

7) Tendolkar PM, Baghdayan AS, Shankar N. Putative surface proteins encoded within a novel transferable locus confer a high-biofilm phenotype to Enterococcus faecalis. J Bacteriol. 2006. March; 188(6): 2063– 72.

8) Giard JC, Riboulet E, Verneuil N, Sanguinetti M, Auffray Y, Hartke A. Characterization of Ers, a PrfA-like regulator of Enterococcus faecalis. FEMS Immunol Med Microbiol. 2006. April; 46(3): 410– 8.

9) Preethee T, Kandaswamy D, Hannah R. Molecular identification of an Enterococcus faecalis endocarditis antigen efaA in root canals of therapy-resistant endodontic infections. J Conserv Dent. 2012. October; 15(4): 319– 22.

10) Jones CG. 1997. Chlorhexidine: is it still the gold standard? Periodontol 2000. 15(1):55–62.

11) Reda B, Hollemeyer K, Trautmann S, Hannig M, Volmer DA. 2020. Determination of chlorhexidine retention in different oral sites using matrix assisted laser desorption/ionization-time of flight mass spectrometry. Arch Oral Biol. 110:104623.

12) Tanomaru Filho, M., Leonardo, M. R., & da Silva, L. A. (2002). Effect of irrigating solution and calcium hydroxide root canal dressing on the repair of apical and periapical tissues of teeth with periapical lesion. Journal of Endodontics, 28, 295–299.

13) James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, Whelton H, Riley P. 2017. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 3(3):CD008676.

14) Slee AM, Oconnor JR. 1983. In vitro antiplaque activity of octenidine dihydrochloride (WIN 41464-2) against performed plaques of selected oral plaque-forming microorganisms. Antimicrob Agents Chemother. 23(3):379–384.

15) Hubner NO, Siebert J, Kramer A. 2010. Octenidine dihydrochloride, a modern antiseptic for skin, mucous membranes and wounds. Skin Pharmacol Physiol. 23(5):244–258.

16) Tandjung, L., Waltimo, T., Hauser, I., Heide, P., Decker, E. M., & Weiger, R. (2007). Octenidine in root canal and dentine disinfection ex vivo. International Endodontic Journal, 40, 845–851.

17) Amalaradjou, M. A., & Venkitanarayanan, K. (2014). Antibiofilm effect of octenidine hydrochloride on Staphylococcus aureus, MRSA and VRSA. Pathogens, 3, 404–416.

18) Tirali, R. E., Bodur, H., Sipahi, B., & Sungurtekin, E. Evaluation of the antimicrobial activities of chlorhexidine gluconate, sodium hypochlorite and octenidine hydrochloride in vitro. Australian Endodontic Journal, 2013; 39, 15–18.

19) Bukhary, S., & Balto, H. Antibacterial efficacy of octenisept, alexidine, chlorhexidine, and sodium hypochlorite against Enterococcus faecalis biofilms. Journal of Endodontics, 2017(43); 643–647.

20) Rohrer N, Widmer AF, Waltimo T, Kulik EM, Weiger R, Filipuzzi-Jenny E, Walter C. Antimicrobial efficacy of 3 oral antiseptics containing octenidine, polyhexamethylene biguanide, or Citroxx: can chlorhexidine be replaced? Infect Control Hosp Epidemiol. 2010; 31(7):733–739.

21) De Moura-Netto C, de Freitas Carvalho C, de Moura AAM, Davidowicz H, Antoniazzi JH. Influence of Nd:YAG and Diode Laser Irradiation on Apical Sealing When Associated with AH Plus and EndoREZ Endodontic Cements. Photomed Laser Surg. 2007 Oct;25(5):413–7.

22) Ashofteh K, Sohrabi K, Iranparvar K, Chiniforush N. In vitro comparison of the antibacterial effect of three intracanal irrigants and diode laser on root canals infected with Enterococcus faecalis. 2014;6(1):5.

23) Parirokh M, Eghbal MJ, Asgary S, Ghoddusi J, Forghani F, Shahravan A. Effect of 808nm diode laser irradiation on root canal walls after smear layer removal: A scanning electron microscope study. 2007;2(2):6.

24) Mehrvarzfar P, Saghiri MA, Asatourian A, Fekrazad R, Karamifar K, Eslami G, et al. Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against Enterococcus faecalis contaminating root canals: an in vitro study. J Oral Sci. 2011;53(3):355–60.