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ORIGINAL RESERACH

Assessment of Impact of Various Root Canal Irrigants on the Adherence of the Gelatinase-producing and the Gelatinase-deficient *E. faecalis* Strains to Dentin

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ABSTRACT

Aim: Present study was planned to assess the impact of various root canal irrigants on the adhesion of different Enterococcus faecalis (*E. faecalis*) strains to the dentinal surface.

Materials and methods: A total of 80 freshly extracted first and second molars were used in the present study. Preparation of dentin discs was done followed by a random division into four study groups and one control group. Four study groups included; 2.5 % sodium hypochlorite (NaOCl) , 2 % chlorhexidine (CHX), 2.5 % NaOCl + saline + 2 percent CHX and 2.5 % NaOCl + 17 % ethylene diamine tetra-acetate (EDTA) + 2.5 % NaOCl group respectively. In the control group (E), sterilized dentin discs were incubated with sterile TSB solution. Division of all the groups into two subgroups were done depending upon the type of strain of *E. faecalis* used. Incubation of all the specimens was done followed by assessment with XTT assay and measurement of Optical density (OD). All the results were compiled and analyzed by Statistical Package for the Social Sciences (SPSS) software.

Results: Among the groups containing gelatinase producing strains; maximum score was exhibited by 2.5 percent sodium hypochlorite solution followed by 2.5 % NaOCl + 17 % Ethylene Diamine tetra-acetate (EDTA) + 2.5 % NaOCl group (group D). On comparing the OD values among various study groups incubated with Gelatinase producing strain, significant results were obtained. Gelatinase-producing *E. faecalis* showed a significantly higher amount of adherence to dentin, in comparison to the gelatinase-deficient *E. faecalis* strains.

Conclusion: Lesser quantity of bacteria is recovered from specimens in whom CHX was added to the irrigation protocol.

Clinical significance: Production of gelatinase by *E. faecalis* might lead to an increase in adhesiveness of *E. faecalis* to the dentin.

Keywords: *Enterococcus faecalis,* Irrigation, Root canal, Sodium hypochlorite.

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INTRODUCTION

Microorganisms generally incline toward the establishment of biofilm which provides the ability to survive tough growth and environmental conditions. It is likewise shown that the resistance of microscopic organisms in biofilm is 2 to 1,000 times greater in comparison to that of the planktonic structures. Moreover, biofilms can revamp themselves in the wake of being halfway influenced.[1,2] For the success of endodontic therapy, it is notable that microbial biofilm in the contaminated root canal ought to be wiped out, since these microbes are largely responsible for pulpal and peri-apical infections, in addition to the failure of root canal therapy.[3]

Among the entire root canal microbial flora, Enterococcus faecalis (*E. faecalis*), a facultative anaerobic gram-positive coccus, is one of the most resistant microorganisms. It is most commonly encountered species in the failed cases of endodontically treated teeth. One of the important steps necessary for the success of root canal therapy is root canal irrigation.[4,5]

Antimicrobial capability of various root canal irrigant solutions has been widely studied in the past literature; however, there is paucity of data that have analyzed the effectiveness of various irrigant protocols on the attachment of *E. faecalis* to dentin.[6] Hence, present study was planned to assess the impact of various root canal irrigants on the adhesion of different *E. faecalis* strains to the dentinal surface.

MATERIALS AND METHODS

The present study was conducted in the department of conservative dentistry and endodontic of the dental institute. Ethical approval was taken from the institutional ethical committee before the commencement of the study.

Preparation of Dentin Discs

A total of 80 freshly extracted teeth were included in the present study. Only non-carious first and second molars were used. Only those teeth were selected which were extracted due to periodontal disease or prosthetic need.

Under the constant flow of water irrigation, removal of the sound enamel structure was done using high speed cutting instruments. Cutting was continued until a standardized anatomic crown width of 5 mm was reached. With the help of a rotary diamond saw, dentin discs of approximately 1.2 mm thickness were obtained just beneath the deepest portion of the dentinoenamel junction. Thus, a bilaterally grounded dentin disc was obtained. From a single tooth, only one dentine disc was prepared. Stereomicroscopic verification of the disc was done to check any remnant of removed enamel on the disc surface. Sandpaper was then used for final grinding of the disc with the aim of preparing smooth surfaces. This was followed by reducing the final disc size to 1 mm. Smear layer was thus formed on all the surfaces of the disc as a consequence of this procedure.

Culturing of Specimens

For the present study, two different strains of E. faecalis were obtained; one was gelatinase producing (GP strain) while the other was gelatinase deficient (GD strain) from FICCI Research and Analysis Centre, India. At 37 degree centigrade, both the strains were incubated under aerobic conditions for the overnight period by keeping them in tryptone soy broth (TSB). Incubation of *E. faecalis* was done in five milliliters of TSB at 37°C. Using a spectrophotometer, standardization of the optical density of the bacterial suspension was done.

Treatment with Different Irrigants

Pre-numbered Eppendorf tubes were used, consisting of phosphate-buffered saline (PBS, pH = 7.2), and all the individual dentin discs were relocated into them. This was followed by autoclaving at one hundred twenty-one degree centigrade for twenty minutes. All the 80 specimens were divided into four study groups and one negative control group with 16 specimens in each group as shown in Table 1.

Incubation of sterilized dentin discs was done in the sterile TSB solution was categorized as a negative control. Following irrigation of the dentin discs with various irrigation protocols, five ml of distilled water was used for rinsing the discs. In group 1, 5 mL of 2.5% sodium hypochlorite (NaOCl) was used for 15 minutes. In group 2, 2 % c hlorhexidine (CHX) was used for 15 minutes. In group 3, 5 mL of 2.5% NaOCl + 5 mL of normal saline + 5 mL of 2* CHX was used in sequence. Each irrigant was used for 5 minutes. In group IV, 5 mL of each 2.5% NaOCl, 17% EDTA and 2.5% NaOCl was used in sequence for 5 minutes each.

Subdivision of all the groups was done with eight specimens in each group depending upon the type of bacterial strain used (GP and GD). Placement of the treated dentin discs was done in sterile culture plates. Standardization of the bacterial cells was done to 1×10^8 cells/ ml in TSB. Transferring of the bacterial suspension was done into each culture plate followed by aerobic incubation at 37°C for 48 hours. Refreshing of the bacterial suspension was done after 24 hours with one ml of fresh media. Bacterial adhesion was quantitatively measured by XTT-reduction assay, as described previously in literature.[7,8]

Carboxanilide (XTT) Test

Reduction of 2,3-bis (2-methoxy-4 –nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) molecules to formazan (soluble salts) by metabolic active cells, as detected by the absorption of the soluble product by its absorbance at 480 nm forms the basis of XTT test. Spectrophotometer was used for assessment of calorimetric alteration and measurement of Optical Density (OD) in the XTT reduction assay.

Statistical Analysis

All the results were analysed by SPSS software. Chi-square test, One way ANOVA and post hoc Tukey tests were used for assessment of level of significance. P- value of less than 0.05 was taken as significant.

RESULTS

A total of 80 specimens were included in the present study and were divided into four study groups and one control group (Table 1). In the control group, there was absence of bacterial growth on the specimens (OD value = 0). Among the groups containing gelatinase producing strains, maximum score was exhibited by 2.5 percent sodium hypochlorite solution (group A) (OD score = 0.331) followed by 5 ml of each 2.5 % NaOCl, 17 % Ethylene Diamine tetra-acetate (EDTA) and 2.5 % NaOCl (group D) (OD score = 0.208). OD of group B was 0.027 and in group C was 0.026. (Table 2). Significant results were obtained while comparing the OD in between various study groups incubated with Gelatinase producing strain (p-value < 0.05). Among the specimens of Gelatinase deficient E. Faecalis study groups, maximum OD score was obtained in the NaOCl group (OD score= 0.156). While comparing the OD score in between various study groups in Gelatinase deficient specimens, significant results were obtained. In group A, it was 0.156, in group B it was 0.023, in group C 0.020 and in group D, it was 0.123. (Table 3). Gelatinase-producing *E. faecalis* showed significantly higher amount of adherence to dentin than gelatinase-deficient *E. faecalis* strains. (P- value < 0.05) (Table 4).

DISCUSSION

Root canal therapy is one of the routinely employed procedures these days. One of its important constituent is through cleaning of the pulp canal followed by repeated irrigation protocol. Irrigating the root canal fulfils two main purposes; first is physical washing out of any remaining debris from the root canal space, and second, chemical destruction of biofilms and endotoxins released by varied microbial flora present in the infected root canal.[9-11]

Table 1: Division of study samples into different study groups

Group	*N*	*Irrigation protocol*
A	16	2.5 % Sodium Hypochlorite (NaOCl)
B	16	2 % Chlorhexidine (CHX)
C	16	2.5 % NaOCl + Saline + 2 percent CHX
D	16	2.5 % NaOCl + 17 % Ethylene Diamine tetra-acetate (EDTA) + 2.5 % NaOCl
Control (E)	16	Sterilized dentin discs incubated with sterile TSB solution
Total	80	

The microbial population residing in the root canal was observed to be significantly declined during irrigation process regardless the type of irrigant solution used due to the mechanical effect produced by the flow of the solution. The most commonly used irrigating solution is NaOCl which has excellent antimicrobial properties. It also has ability to dissolve different organic tissues. Ease of availability and cost effectiveness are few of other additional advantages offered by it along with having healthy shelf life.[12] At the same time, it is also subjected to certain drawbacks; namely, unpleasant smell and taste and varied amount of toxicity when expelled accidentally beyond the tooth apex into the peri-apical tissues. Therefore; research continues in search of more efficient and safer root canal irrigant solutions.[13,14] Hence; we planned the present study to assess the impact of various root canal irrigants on the adhesion of different *E. faecalis* strains to the dentinal surface.

Present study demonstrated that highest values of bacterial adhesion to the dentin were seen in specimens irrigated with NaOCl. We observed that in group A, the OD was 0.331, in OD of group B was 0.027, in group C was 0.026 and in group D, the OD score was 0.208. Group C showed least values among all other groups (Table 2).

It was seen that OD in specimens of Gelatinase deficient *E. faecalis* in group A was 0.156, in group B was 0.023, in group C was 0.020 and in group D was 0.123 (Table 3). Denaturation of the collagen by the effect of NaOCl further increasing the susceptibility of binding of E. Faecalis to the dentin surface might be responsible for the above results.[15,16] Also, in the Gelatinase deficient group, lesser OD score was seen (Table 4). Results of present study were in correlation with the results obtained by Guneser MB et al who also reported similar findings in their study.[16]

Impact of the smear layer on the anti-microbial quality of various disinfecting agents in the infected dentinal tubules was assessed by Wang et al. Constraining of the cells of E. Faecalis into dentinal tubules was done as indicated by a formerly settled convention. Following an incubation of three weeks of infected dentin specimens, a uniform smear layer was delivered. Preparation of 40 infected dentin blocks was done and subjected to 3 and 10 minutes of introduction to sterilizing arrangements including sterile water, 2% and 6% NaOCl, 2% CHX, 17%

Table 2: Descriptive values of OD in specimens of gelatinase producing *E. faecalis* study groups

Study groups	*OD value*	*p value*
A	0.331	0.02*
B	0.027	
C	0.026	
D	0.208	

*: Significant

Table 3: Descriptive values of OD in specimens of Gelatinase deficient *E. faecalis* study groups

Study group	*OD value*	*P- value*
A	0.156	0.04*
B	0.023	
C	0.020	
D	0.123	

*: Significant

Table 4: Descriptive values of OD in specimens of all the study groups

	OD value		
Study group	*Gelatinase producing E. Faecalis*	*Gelatinase deficient E. Faecalis*	*P- value*
A	0.331	0.156	0.01*
B	0.027	0.023	
C	0.026	0.020	
D	0.208	0.123	

*: Significant

EDTA, and QmiX. The smear layer lessened the viability of sanitizing specialists against *E. faecalis* in infected dentin. In the present study combination of 6% NaOCl and QmiX demonstrated the most effective antibacterial action among all the irrigating agents.[17] As observed OD value of group A was highest (0.331) and group C showed least value ie 0.026. Guneser MB et al assessed whether the gelatinase producing capacity of *E. faecalis* gives any favourable position on attachment of *E. faecalis* to dentin after treatment with different irrigants and their different mixtures. Institutionalized dentin circles (discs) were arbitrarily separated into five divisions: group 1, 2, 3 4 and 5 consisted of 2.5% NaOCl, 2% CHX, NaOCl + Saline + CHX, NaOCl + EDTA + NaOCl and Qmix respectively. Following incubation of dentin plates with irrigants, each gathering was partitioned into two sub-divisions with 10 specimens in each sub-group as per the bacterial strains utilized; a gelatinase-delivering and a gelatinase-lacking strain of *E. faecalis*. XTT test was directed for bacterial adherence assessment, following incubation of the circles with the bacterial suspensions vigorously for 48 h. In comparison to the GD group, GP group clung to dentin more fundamentally in all test gatherings. Lower adherence of bacteria to dentin occurs by the addition of CHX to the irrigating protocols.[16]

Morgental RD et al. compared antibacterial efficacy of QmiX with routinely used irrigating solution in the presence or absence of dentin powder. They tested various irrigating solution 6% NaOCl, 1% NaOCl, QmiX, 2% CHX, and 17% EDTA against *E. faecalis*. Lowest bacterial count was shown by 6% NaOCl, in the absence of dentin following contact with bacterial suspension for ten seconds. From the results, they concluded that antibacterial activity of NaOCl and QmiX is delayed by Dentin.[18] Antimicrobial activity of NaOCl and CHX was assessed by Nascimento et al., either alone, or in combination with cetrimide (CTR) against E. Faecalis. They also compared these results with results obtained by QmiX. Elimination of all the micro-organisms occurred following the direct contact of these irrigating solutions with planktonic cells. From the results, they concluded that no change in the antimicrobial properties of NaOCl and CHX occurred on addition of CTR.[19] Comparison of antimicrobial activity of QmiX and various other irrigating solutions was done by Liu et al., who assessed their efficacy against *E. faecalis*. They chemo-mechanically prepared human teeth specimens contaminated with *E. faecalis* for one month, with NaOCl. From the results, they concluded that QmiX had comparable anti-microbial activity with that of EDTA/CHX and EDTA/CTR against intracanal *E. faecalis*.[20]

Present study suggested that demineralization and exposure of collagen fibrils can augment the *E. faecalis* adherence to dentin. The application of root canal disinfectants such as NaOCl remove exposed collagen fibrils and succeeding irrigation with antimicrobial such as CHX significantly reduce the adherence of *E. faecalis* to dentin. However, clinicians should take keep in mind to not mix NaOCl with CHX because this may result into formation of toxic compound, chloroguanidine. These complexes reduce dentine permeability by occluding the canal orifice, and hence decrease the efficacy of endodontic irrigants. In addition to it colored complexes are formed in the root canal which has the potential to stain dentine.

CONCLUSION

Lesser quantity of bacteria is recovered from specimens in which CHX was added to the irrigation protocol. Hence; a mixture of different irrigating solutions might prove to be more helpful in increasing the prognosis of root canal therapy.

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