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An in Vitro Comparison of Antimicrobial Efficacy and Residual Antimicrobial Activity of Root Canal Irrigants Qmix, 3% Sodium Hypochlorite, 2% Chlorhexidine against Enterococcus Faecalis

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Abstract: ***Aim: To evaluate the antimicrobial activity and the residual antimicrobial activity of Qmix, 3% NaOCl and 2% chlorhexidine (CHX) against E.faecalis in human root dentin in vitro. Method: Assessment of antimicrobial activity was done with a dentindisk put into 2 ml Eppendorf tube containing 200µl E.faecalis suspension in BHI broth.The tubes were incubated under anaerobic conditions at 37°C for 3 weeks. The different root canal irrigants of 200 µl each was put in each tubes. After 2 min incubation, the dentin blocks were removed and dried with absorbent paper. The biofilm biomass was measured by crystal violet (CV) staining and the absorbance measurement values were obtained by spectrophotometry at 600 nm. Assessment of residual antimicrobial activity of different groups was done by measuring the absorbance values at different intervals of 0 h, 24 h and 48 h by spectrophotometryat 600nm. Results: Qmix showed highest antimicrobial activity followed by 3% NaOCl and 2% CHXagainst E.faecalis biofilm. The residual antimicrobial activities of the irrigants 2% CHX showed maximum reduction in E.faecalis biofilm at 0 hr. CHX residual antimicrobial activity became significant at 48 h. Qmix showed reduction in E.faecalis biofilm at the end of 48 h NaOCl did not show any residual antimicrobial activity. Conclusion: In conclusion, our study showed that 3% NaOCl, 2% CHX and Qmix could kill E. faecalis in matured biofilm. The antibacterial activity of QMix excelled among the irrigants included in the study and the residual antimicrobial activity of 2% CHX was better than QMix at 24h and 48h respectively. Clinical Significance: Antibacterial activity and residual antimicrobial activity are important properties of root canal irrigants against persistent microorganisms like E.Faecalis***

Keywords: Root canal irrigants; Enterococcus Faecalis; Sodium hypochlorite; Chlorhexidine; QMix root canal irrigant;

1. Introduction

It is well established that bacteria and their products play a crucial role in the development of periradicular diseases. Sundqvist demonstrated the important role of bacteria play in periradicular lesions. (1) Endodontic infections have a polymicrobial nature, with obligate anaerobic bacteria conspicuously dominating the microbiota. E.faecalis has been in focus as a recognized pathogen in endodontics, isolated both in mixed microbiota and in monocultures. (2) It has the ability to colonize the root canal in a biofilm-like style, invade dentinal tubules, and resist endodontic treatment. The complex anatomy of the root canal system (i.e., isthmuses, accessory canals, and dentinal tubules) enables the survival of bacteria after conventional cleaning. The current techniques in endodontics have been the use of irrigating solutions with strong antibacterial activity along with mechanical preparation of root canal system. The purpose of irrigants used during endodontic treatment is to be antimicrobial, flush out debris, to dissolve organic matter in the canal and provide lubrication to the dentinal walls. (4) Antimicrobial substantivity is the prolonged association between a material and a substrate. The use of irrigants with this property helps ensure residual antimicrobial activity, avoiding the negative impact that bacterial invasion would have on the success of an endodontic procedure. (5) Hence, the present study aims to evaluate the antimicrobial activity and the residual antimicrobial activity of newer irrigant Qmix with 3% sodium hypochlorite and 2% chlorhexidine against E. faecalis.

2. Materials & Methods

A total of 60 extracted non carious single rooted teeth were obtained and stored in distilled water. 60 dentin disks (1cm × 0.5cm × 1 mm [width × length × height]) were prepared by sectioning the coronal one-third of the roots from the cementoenamel junction with a low speed; precision cut diamond saw (Minitom, USA) under continuous water coolant. The dentin disks were steam autoclaved for 30 min under 15 psi pressure at 121 °C to ensure that no bacteria remained. All samples were preserved in 37 °C brain heart infusion for 24 h to test for the presence of bacteria. After confirming complete sterilization, all dentin blocks were stored in sterile saline. Standard strains of E.faecalis preserved at −80°C was thawed. They were inoculated into freshly prepared BHI and cultivated under anaerobic condition at 37°C for 24h. Then, a bacterial suspension with 1×10^7/ml E.faecalis was obtained by dilution with BHI broth. The dentin blocks with E.fecalis were randomly divided into different groups with 16 samples each. The samples were divided into Group I: Control, Group II: Sodium hypochlorite, Group III: Chlorhexidine, Group IV: QMix

Assessment of antimicrobial activity of different root canal irrigants - One Dentin dentin disk was put into each 2 ml Eppendorf tube and 200µl E.faecalis suspension in BHI broth was added and sealed. The tubes were incubated under anerobic conditions at 37°C for 3 weeks. The dentin disks were removed and placed in new 2 ml Eppendorf tubes. The different root canal irrigants of 200 µl each was put in each tubes. After 2 min incubation, the dentin blocks were removed and dried with absorbent paper. A Biofilm biomass

measurement by crystal violet (CV) staining was performed. An aliquot of 190 µL of 0.01% CV aqueous solution was added to the tubes containing dentin disks and incubated at room temperature for 30 min. Then, CV solution was removed and wells were washed three times with 200 µL of phosphate buffered saline. During this wash step care was taken not to disturb the biofilm. Then dentine blocks was left to dry for 30 min at 50 °C. Next, 200 µL of 96 –99% ethanol was added to each sample and biofilm was detached by vigorous vortexing. The Absorbance measurement value at 600 nm was obtained by spectrophotometry.

Assessment of residual antimicrobial activity of different root canal irrigants - The remaining dentin disks were incubated in each 2 ml Eppendorf tube and 200µl E.faecalis suspension in BHI broth. The different root canal irrigants of 200 µl was put in each tubes. After 2 min incubation the dentin blocks were removed and dried with absorbent paper. The formed biofilm was washed once with 200 µL of phosphate-buffered saline (PBS). Turbidity was measured at 600nm with plain PBS as reference at different intervals 0 h, 24 h and 48 h by spectrophotometry.

The data obtained was analysed using Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Descriptive analysis expression of Optical density values using Mean & SD for each study groups. One-way ANOVA test followed by Post hoc Tukey's Test was used to compare the mean OD values at 600nm by crystal violet method and Residual Antimicrobial activity after treatment at different time intervals between the study groups. The level of significance [P-Value] was set at P<0.05.

3. Results

The mean values were statistically analyzed using One-way ANOVA Test and Multiple comparison of mean differences at 600nm using Tukey's Post hoc Analysis in Tables 1and 2.It was statistically significant (p<0.001) indicating all irrigants eradicated E.faecalis biofilm.

Table 3-8 show Multiple comparisons of mean differences done for groups at various time intervals of 0 hours, 24 hours and 48 hours respectively using Tukey's Post hoc Analysis. Group II: Sodium hypochlorite showed the maximum reduction followed by Group IV: Qmix at 0 hours and was statistically significant (P<0.001) among all the groups. Group III: Chlorhexidine showed statistically significant reduction between various time intervals of 0 hours, 24 hours and 48 hours respectively demonstrating its residual antibacterial activity. This indicates the irrigant Group IV: Qmix has good antibacterial activity against E.faecalis along with substantivity. However the substantivity of Group III: Chlorhexidine was better among all the groups.

Table 1: Comparison of mean OD values at 600nm by crystal violet method after treatment between study groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group I	16	1.3456	0.1114	1.209	1.563	<0.001*
Group II	16	0.1151	0.0161	0.081	0.145	
Group III	16	0.1099	0.0115	0.089	0.138	
Group IV	16	0.0213	0.0127	0.002	0.050	

Table 2: Multiple comparison of mean differences at 600nm by crystal violet method after treatment using Tukey's Post hoc Analysis

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI of the Diff.		P-Value
			Lower	Upper	
Group I	Group II	1.2305	1.1791	1.2819	<0.001*
	Group III	1.2358	1.1844	1.2871	<0.001*
	Group IV	1.3243	1.2729	1.3757	<0.001*
Group II	Group III	0.0053	-0.0461	0.0566	1.00
	Group IV	0.0938	0.0424	0.1452	<0.001*
Group III	Group IV	0.0886	0.0372	0.1399	<0.001*

*Statistically significant

Table 3: Comparison of mean OD values at 600nm by residual antibacterial activity test at 0 hr between study groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group I	16	0.0906	0.0112	0.078	0.112	<0.001*
Group II	16	0.0149	0.0069	0.003	0.026	
Group III	16	0.0239	0.0047	0.003	0.021	
Group IV	16	0.0157	0.0089	0.003	0.036	

Table 4: Comparison of mean OD values at 600nm by residual antibacterial activity test at 24 hr between study groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group I	16	0.1044	0.0158	0.088	0.134	<0.001*
Group II	16	0.0150	0.0052	0.004	0.023	
Group III	16	0.0213	0.0050	0.013	0.031	
Group IV	16	0.0158	0.0069	0.003	0.026	

*Statistically significant

Table 5: Comparison of mean OD values at 600nm by residual antibacterial activity test at 48 hr between study groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group I	16	0.1181	0.0126	0.098	0.136	<0.001*
Group II	16	0.0145	0.0049	0.007	0.022	
Group III	16	0.0158	0.0054	0.012	0.032	
Group IV	16	0.0158	0.0043	0.010	0.023	

Table 6: Multiple comparison of mean differences at 600nm by residual antibacterial activity at 0 hr after treatment using Tukey's Post hoc Analysis

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI of the Diff.		P-Value
			Lower	Upper	
Group I	Group II	0.1036	0.0958	0.1115	<0.001*
	Group III	0.0942	0.0864	0.1020	<0.001*
	Group IV	0.1024	0.0945	0.1102	<0.001*
Group II	Group III	-0.0094	-0.0173	-0.0016	0.01*
	Group IV	-0.0013	-0.0091	0.0066	0.99
Group III	Group IV	0.0082	0.0004	0.0160	0.04*

*Statistically significant

Table 7: Multiple comparison of mean differences at 600nm by residual antibacterial activity at 24 hr after treatment using Tukey's Post hoc Analysis

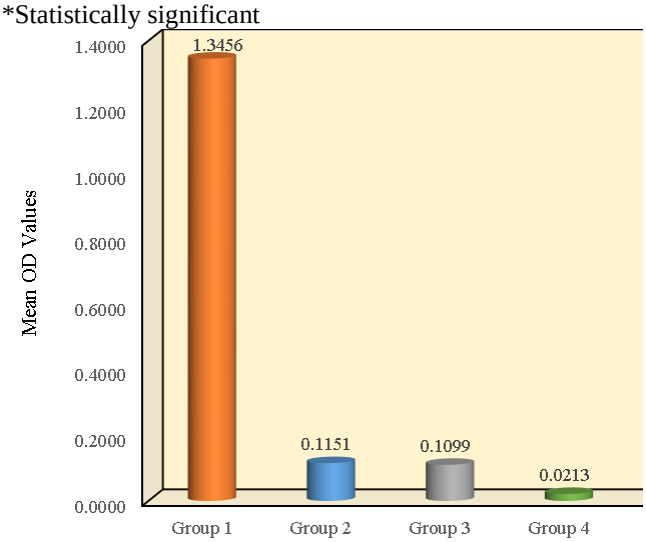
(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI of the Diff.		P-Value
			Lower	Upper	
Group I	Group II	0.0894	0.0806	0.0981	<0.001*
	Group III	0.0831	0.0743	0.0918	<0.001*
	Group IV	0.0886	0.0798	0.0973	<0.001*
Group II	Group III	-0.0063	-0.0151	0.0025	0.27
	Group IV	-0.0008	-0.0096	0.0080	1.00
Group III	Group IV	0.0055	-0.0033	0.0143	0.41

*Statistically significant

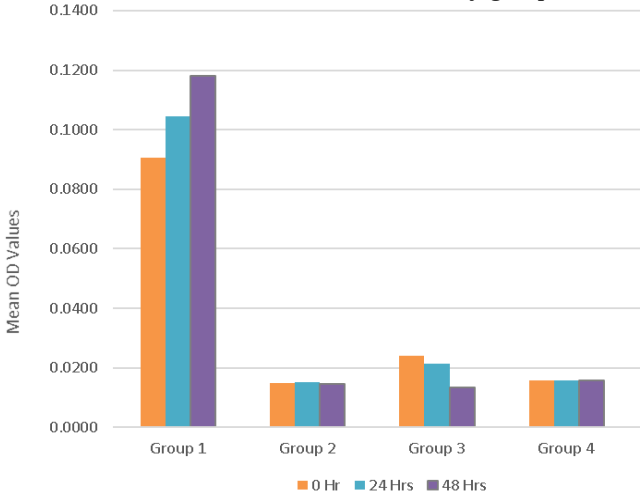
Table 8: Multiple comparison of mean differences at 600nm by residual antibacterial activity at 48 hr after treatment using Tukey's Post hoc Analysis

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI of the Diff.		P-Value
			Lower	Upper	
Group I	Group II	0.0757	0.0668	0.0846	<0.001*
	Group III	0.0771	0.0682	0.0860	<0.001*
	Group IV	0.0749	0.0660	0.0838	<0.001*
Group II	Group III	0.0014	-0.0075	0.0103	0.99
	Group IV	-0.0008	-0.0097	0.0082	1.00
Group III	Group IV	-0.0022	-0.0111	0.0067	0.96

*Statistically significant



Graph 1: Mean OD values at 600nm by crystal violet method after treatment between study groups



Graph 2: Mean OD values at 600nm by residual antibacterial activity method at different time intervals between various study groups

4. Discussion

The primary goal of endodontic treatment is to optimize root canal disinfection and to prevent its re-infection. It has been shown that bacteria persisting within the root canal system are the major cause of endodontic treatment failures. (2, 4) E. faecalis has been frequently found in persistent intraradicular infections after failed endodontic treatments. (6) The ability of E. faecalis to penetrate dentinal tubules enables it to escape the action of endodontic treatments and irrigants used during chemomechanical preparation. (7) It is resistant to mechanical instrumentation and intracanal antimicrobial agents and has the ability to adapt to the harsh environmental condition. (8) The ideal properties of root canal irrigants described by Zehnder are broad antimicrobial spectrum, high efficacy against anaerobic and facultative microorganisms organized in biofilms, dissolve necrotic pulp tissue remnants, inactivate endotoxin, prevent the formation of a smear layer during instrumentation or to dissolve the latter once it has formed. (9) Surface tension is considered as one of the most important factors in determining the wettability of a solution. Wettability is the property of a fluid to spread over or adhere to a solid surface. This property is required for the chemical solution to penetrate the main and lateral canals, as well as the dentinal tubules and it depends on the surface tension. (10) Several studies have generally concurred that sodium hypochlorite has a broad-spectrum antimicrobial activity. Despite its excellent tissue-dissolving and antimicrobial abilities. NaOCl possesses some drawbacks. One of its major drawbacks of NaOCl is its toxicity. Another major drawback is its high surface tension, which limits it penetration into canal irregularities and the depth of dentinal tubules. (11) Any new concepts and techniques to be used in the clinic should ideally be assessed against their respective gold standards. Hence, our study compared the antimicrobial activity of newer irrigant Qmix against gold standard sodium hypochlorite.

QMix™ 2 in 1 (Dentsply a Dental Specialties, Tulsa, OK, USA), contains a mixture of a bisbiguanide antimicrobial agent (2% chlorhexidine), a polyamino carboxylic acid, calcium chelating agent (17% EDTA); saline; and a surfactant (cetrimide).The rationale of adding a surface active agent in QMiX is because of its ability to lower surface tension of solutions and increase their wettability (12, 13) This novel irrigant has been introduced to both remove smear layer and kill bacteria. This irrigant has a proprietary formulation and method of preparation, and has been shown to remove smear layer and kill resistant bacteria, such as E. faecalis, in one application, Mixing EDTA and CHX is known to produce a white precipitate. (14) In QMiX, this is avoided because of its chemical design. A study by Al Khatani et al demonstrated its biocompatibility and also reported that compared to sodium hypochlorite, QMix is less aggressive and more acceptable to living tissues. (15) Stojicic et al demonstrated that this irrigant has antibacterial activity comparable to NaOCl and superior activity against Enterococcus faecalis in planktonic form and biofilm compared to 2% CHX. (16) . The long-term antimicrobial effects rely on whether or not the particular agent has any properties of substantivity. Chlorhexidine is one antimicrobial agent that features this characteristic of

substantivity. (17) The microorganisms may penetrate into the root canal via any defects in the coronal aspect of the tooth and the temporary restorations. Furthermore, re-infection of the root canal can occur because of re-growth of any residual microorganisms that have survived the endodontic treatment procedures. Hence, the use of antimicrobial agents that exhibit substantivity, that is, agents that can have a therapeutic effect for a prolonged period. (18)

Our study also compared the residual antimicrobial activity of the irrigants used in the study to assess the property of substantivity. Group III -2% Chlorhexidine showed maximum reduction in E.faecalis biofilm at 0 hr. CHX showed less residual antimicrobial activity at 24 h, while its residual antimicrobial activity became significant at 48 h. This is similar to results by Rosenthal et al which evaluated the substantivity of 2% CHX solution within the root canal system after 10 min of application and they reported that the CHX was retained in the root canal dentine effective amounts for up to 12 weeks. (19)

Qmix showed reduction in E.faecalis biofilm at 0 hr. At 24 h, few residual antimicrobial activities were detected in the QMix group. However, at 48 h, QMix group remained unchanged. This may be because the unique combination of Chlorhexidine and EDTA produces synergistic antibacterial activity helps reduction of bacteria in a single step. This result is similar to studies by Zhang et al which observed QMix had less substantivity compared to CHX. (20)

5. Conclusion

Our study showed that irrigants 3% sodium hypochlorite, 2% chlorhexidine and Qmix could kill E.faecalis in matured biofilm. The antibacterial activity of QMix was best among the irrigants included in the study followed by 3% sodium hypochlorite and 2% chlorhexidine. The residual antimicrobial activity of 2% chlorhexidine was better than QMix at 24h and 48h respectively.

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