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A Comparative Analysis of Antimicrobial Efficacy on Enterococcus Faecalis and Candida Albicans using Various Irrigants along with Laser and Ultrasonic Device: An in Vitro Study

S Sudhakar Naidu[1*] V Rajasekhar[2] M Varma Nadimpalli[3] Abitha Seshadri[4] Nagesh Bolla[5] T.B.V.G.Raju[6]

[1]MDS, Department of Conservative Dentistry, Sree Sai Dental College & Research Institute, Srikakulam, AP, India.
[2]MDS, Department of Conservative Dentistry, Sree Sai Dental College & Research Institute, Srikakulam, AP, India.
[3]MDS, Department of Conservative Dentistry, Sree Sai Dental College & Research Institute, Srikakulam, AP, India.
[4]MDS, Department of Conservative Dentistry, Sree Sai Dental College & Research Institute, Srikakulam, AP, India.
[5]MDS, Department of Conservative Dentistry, Sree Sai Dental College & Research Institute, Srikakulam, AP, India.
[6]MDS, Department of Conservative Dentistry, Sree Sai Dental College & Research Institute, Srikakulam, AP, India.

ABSTRACT

Aim: To evaluate the efficacy of different concentration of different irrigants and their antimicrobial efficacy.

Materials and Methods: 240 single rooted human permanent teeth with straight canals were collected and decoronated upto 15 mm from the apex. Cleaning and shaping of all teeth was done using step down method. The diameter of the orifice opening of all teeth was standardized. The specimens were infected with the microorganisms (E.faecalis and C.albicans). Later teeth were randomly grouped and irrigated with their respective irrigating solutions (Sodium hypochlorite and Chlorhexidine) and agitation devices (Laser and ultrasonic). After irrigation the samples were processed and carried to the microbiological lab and cultivated for viable organisms where colony forming units were observed indicating the presence of organisms.

Results: Kruskal-Wallis one-way ANOVA test and Mann-Whitney U-test was done to statistically analyze antimicrobial efficacy of two irrigants at various concentrations. There was significant difference among the groups.

Conclusion: 2 % Sodium hypochlorite agitated with laser showed better antimicrobial efficacy. Least antimicrobial efficacy was seen with 0.5 % Chlorhexidine without any agitation.

Keywords: Sodium hypochlorite, Chlorhexidine, Laser, Ultrasonic, E.faecalis, C.albicans.

INTRODUCTION

Elimination of microorganisms and complete removal of pulp tissue from the root canal system are of dominant magnitude during endodontic therapy. Microorganisms are the main causative factor of pulpal and periapical inflammation and disease. Failure to effectively eliminate them and their by-products might result in persistent irritation and impaired healing. Sodium hypochlorite at different concentrations is the irrigating solution most accepted worldwide as a result of its chemical properties of pulp dissolution, effective antibacterial action, dissolution of organic material, clearing, transformation of amines into chloramines, and deodorizing effects.[1] Chlorhexidine (CHX) is widely used as a mouth rinse in the prevention and treatment of periodontal diseases and dental caries, and it has been suggested as an irrigating solution

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*Correspondence Dr. S Sudhakar Naidu.
Department of Conservative Dentistry, Sree Sai Dental College & Research Institute, Srikakulam, AP, India.
Email: sudhakarnaidu19@gmail.com

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or intracanal dressing in endodontic therapy.[2] CHX has also been shown to be more effective against gram-positive organisms than gram-negative organisms.[1]

Bacteria has long been recognized as the primary aetiological agent in the development of periapical bone lesions (Kakehashi et al. 1965). Enterococcus faecalis is the most commonly isolated species from the canals of teeth presenting posttreatment disease (Peciuliene et al. 2001). This microorganism has demonstrated the capacity to survive in an environment in which there are scant available nutrients and in which commensality with other bacteria is minimal (Sundqvist et al. 1998).[3]

Although yeasts have occasionally been reported in untreated cases (Sen et al. 1995), they have been more associated with cases of failed endodontic treatment (Nair et al. 1990). To what extent Candida species may be of significance in the persistence of periapical infection is unknown. However, it may be significant that such species are present and may not respond to conventional cleaning and shaping procedures in the same way as other organisms.[4]

Therefore, the aim of the present study was to evaluate the effect of the new 980-nm diode laser wavelength irradiation on antimicrobial efficacy at different concentrations after irrigation with Saline, NaOCl, or CHX on E.faecalis and C.albicans.

MATERIALS & METHODOLOGY

Sample Preparation

240 single rooted teeth with single straight canals were obtained from an existing collection of extracted human permanent teeth. As it took 3 months to collect the teeth, the properties of soft tissue inside the root canals had to be made as similar as possible. For this purpose, the teeth were soaked in 10% formalin immediately after extraction and then stored in distilled water 7 days before the start of the study.

All teeth were intact and had complete root development. The teeth were decoronated transversely apical to the cemento enamel junction using a diamond disc at a distance of 15mm from the apex.

Then #10 and #15 K-files were used to make sure that the roots had only one canal and the canal was patent. All canals were prepared in a similar manner to decrease the number of confounding variables. In the first step, an appropriate-sized Hedstrom file was used to remove pulp remnants and debris from the canals. Then a #15 or #20 file was inserted into the canals so that the tip of the file was visible at the apical foramen. Then the working length was determined 1 mm short of the file penetration into the canal.

All canals were instrumented in a sequential manner with Protaper series upto F_3 . The canals were irrigated with 3% NaOCl solution between filings. Debridement and shaping of the canals were completed with the protaper files. The specimens were placed in 17% EDTA for 10 minutes, followed by 3% sodium hypochlorite for 10 minutes. The specimens were finally immersed in sterile distilled water for 10 minutes. Specimens were placed in capped laboratory containers and put in an autoclave at 121ºC and 15 psi for 20 minutes for sterilization. Subsequent to sterilization all the specimens were transported and manipulated under aseptic conditions using sterile instruments and equipment.

Bacterial Preparation and Inoculation

Pure cultures of E.faecalis (MTCC 439) and C.albicans (MTCC 227) were previously cultivated in BHI broth for 24hr at 37^{0}C. The turbidity of the medium during the incubation indicated bacterial growth. The purity of the cultures was confirmed by Gram staining, catalase production, colony morphology on BHI agar ± blood. Under aseptic conditions the sterile tooth were placed in eppendorf tube containing nutrient agar medium for the microorganisms. Microorganisms were inoculated in the tooth with the sterile 25 guage needles and the eppendorf tube lid was closed. These specimens were kept at 37^{0}C for 24 hrs in an incubator.

Laser Irradiation and Ultrasonic Cleaning

The infected teeth were removed from eppendorf tube for irrigation procedures with the sterile tweezer. In each root canal, either 2% or 1% NaOCl or 1% or 0.5% CHX was filled into the root canal orifice. Either an Diode laser fiber was

Groups 1, 2, 3, 4

Table 1:

ENTEROCOCCUS FAECALIS , ULTRA SONIC											MEAN ±SD Score	K-W test H-Value	P value
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**			
	Colony forming units per ml												
2 % NaOCl	1	0	1	0	2	0	2	1	0	1	0.80±0.79	34.730	<0.0001 Significant
1 % NaOCl	3	5	3	4	3	4	3	3	4	4	3.60±0.70		
1 % CHX	6	6	6	7	6	7	6	7	6	7	6.40±0.52		
0.5 % CHX	8	8	8	8	6	6	8	7	8	6	7.30±0.95		

Groups 5, 6, 7, 8

Table 2:

ENTEROCOCCUS FAECALIS , LASER											MEAN ±SD	K-W test H-Value	P value
	1	2	3	4	5	6	7	8	9	10			
	Colony forming units per ml												
2 % NaOCl	0	1	0	2	1	1	0	1	1	0	0.70±0.67	21.025	0.0001 Significant
1 % NaOCl	1	2	1	0	2	2	1	0	2	2	1.30±0.82		
1 % CHX	3	0	2	0	3	2	2	2	2	3	1.90±1.10		
0.5 % CHX	1	3	4	4	3	3	4	3	3	4	3.20±0.92		

Groups 9, 10, 11, 12

Table 3 :

ENTEROCOCCUS FAECALIS , CONTROL											MEAN ±SD	K-W test H-Value	P value
	1	2	3	4	5	6	7	8	9	10			
	Colony forming units per ml												
2 % NaOCl	7	5	6	7	6	5	7	6	6	7	6.20±0.79	36.848	<0.0001 Significant
1 % NaOCl	9	11	10	10	11	9	11	10	10	11	10.20±0.79		
1 % CHX	16	14	16	17	17	16	17	15	16	16	16.00±0.94		
0.5 % CHX	22	26	24	23	24	26	22	23	24	23	23.70±1.42		

Groups 13, 14, 15, 16

Table 4:

CANDIDA ALBICANS , ULTRA SONIC											Mean±SD	K-W test H-Value	P value
	1	2	3	4	5	6	7	8	9	10			
	Colony forming units per ml												
2 % NaOCl	2	2	0	0	0	0	0	2	1	0	0.70±0.95	3.584	0.278 Not significant
1 % NaOCl	2	3	1	0	1	0	0	1	2	1	1.10±0.99		

1 % CHX	2	0	0	2	0	0	0	1	0	2	0.70±0.95		
0.5 % CHX	3	0	0	2	1	2	3	2	2	0	1.50±1.18		

Groups 17, 18, 19, 20

Table 5:

CANDIDA ALBICANS , LASER											Mean±SD	K-W test H-Value	P value
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**			
Colony forming units per ml													
2 % NaOCl	0	0	1	1	0	0	2	1	0	1	0.60±0.70	8.301	0.046 Significant
1 % NaOCl	1	1	1	1	0	0	0	1	1	0	0.60±0.52		
1 % CHX	1	0	0	2	3	1	2	1	2	1	1.30±0.95		
0.5 % CHX	0	0	1	1	3	2	2	2	3	2	1.60±1.07		

Groups 21, 22, 23, 24

Table 6:

CANDIDA ALBICANS , CONTROL											Mean±SD	K-W test H-Value	P value
	1	2	3	4	5	6	7	8	9	10			
	Colony forming units per ml												
2 % NaOCl	13	11	11	13	11	13	13	12	11	13	12.10±0.99	36.792	<0.0001 Significant
1 % NaOCl	15	17	17	15	14	19	19	17	19	15	16.70±1.89		
1 % CHX	23	21	23	31	31	29	29	21	25	27	26.00±3.92		
0.5 % CHX	33	35	35	35	37	37	37	36	35	38	35.80±1.48		

inserted for laser irradiation or a file-type tip of the ultrasonic cleaner was inserted to generate oscillation.

Control group

Group 9,10,11,12 (with E.faecalis), 21,22,23,24 (with C.albicans) served as the negative control, after root canal preparation, irrigating solutions were used respectively, but without any agitation device. In the laser irradiation group [groups 5,6,7,8, (with E.faecalis) 17,18,19,20 (with C.albicans)], the Diode Laser was used. The device generates the laser with a maximum output of 6 W and a frequency of 5 to 100 pps. In the present study, the output was set at 3 W, the frequency at 15 pps, and the irradiation time 5 s. The fiber tip was positioned about 1 mm above the apical seat, and as irradiation began, the fiber tip was moved up and down within a range of about 5 mm from the apex.

In the ultrasonic cleaning groups [groups 1,2,3,4 (with E.faecalis), 13,14,15,16 (with C.albicans)], an ultrasonic root canal cleaner was used, and as the tip, a #30 K-file (ultrasonic oscillator) was used with the power level set at 2.0. The file tip was positioned about 1 mm above the apical seat, and once oscillation began, the tip was moved up and down within a range of about 5 mm from the apex for 5 s to clean the root canal.

After irrigation the dentin shavings were taken from the root canal with the help of GG drills. The dentin shavings were placed in the 5 ml sterile container filled with phosphate buffer saline solution. These sterile containers were carried to the microbiological lab where further investigation was carried.1 ml of solution was taken from the container and cultivated for viable organisms to grow on petri dishes. After 48 hrs, colony forming

units were observed, indicating the presence of organisms.

Statistical Analysis

The data obtained were statistically analyzed by using Kruskal-Wallis test and Mann-Whitney U-test. The Kruskal-Wallis test was used to statistically analyze the differences between groups (1 to 4), (5 to 8), (9 to 12), (13 to 16), (17 to 20), (21 to 24). The Mann-Whitney U-test was additionally used statistically to compare within the groups of (1 to 4), (5 to 8), (9 to 12), (13 to 16), (17 to 20), (21 to 24).

Inference

In the present study 2% NaOCl shows better antimicrobial efficacy on Enterococcus faecalis when irradiated with laser device, when compared with other groups. Similarly 2% NaOCl showed better antimicrobial efficacy on Candida albicans when irradiated with laser device, when compared with other groups. 2% NaOCl and 1% CHX showed similar antimicrobial efficacy on Candida albicans when irradiated with ultrasonic unit, but lesser than the laser device.

DISCUSSION

The sanitization process of an infected root canal is the objective of endodontic therapy. Chemical and mechanical cleaning and shaping significantly reduce the number of microorganisms, but do not eliminate them.[7,26] Hypochlorite has the unique capacity to dissolve necrotic tissue and the organic components of the smear layer.[8] Higher concentrations give more rapid tissue dissolution, but are more toxic if they come in contact with vital tissue.[9,21]

Estrela et al. discussed that the physicochemical characteristics of NaOCl are essential for the explanation of its mechanism of action. Saponification, neutralization of amino acids and chloramination reactions that occur in the presence of microorganisms and organic tissue aid the antimicrobial and tissue dissolution processes.[7,18]

Chlorhexidine was the most potent of the tested bisguanides. Chlorhexidine is a strong base and is most stable in the form of its salts. The original salts were chlorhexidine acetate and hydrochloride, both of which are relatively poorly soluble in water. Hence, they have been replaced by chlorhexidine digluconate.[8,19,20] The antimicrobial effect of CHX is related to the cationic molecule binding to negatively charged bacterial cell walls, thereby altering bacterial osmotic equilibrium. A disadvantage of CHX is that it does not dissolve organic tissues.[2,24]

Ultrasonic activation of sodium hypochlorite has also been advocated, as this would "accelerate chemical reactions, create cavitational effects, and achieve a superior cleansing action".[8,25] Acoustic streaming, as described by Ahmad et al, has been shown to produce sufficient shear forces to dislodge debris in instrumented canals. When files were activated with ultrasonic energy in a passive manner, acoustic streaming was sufficient to produce significantly cleaner canals compared with hand filing alone. This seems to improve the efficacy of irrigation solutions in removing organic and inorganic debris from root canal walls.[10,23,27]

Different laser wavelengths have different absorption coefficients with the primary dental tissue components of water, pigment, blood contents, and mineral, and laser energy can be transmitted or absorbed based on the composition of target tissue.[11,25]

Enterococcus faecalis, a facultative anaerobic gram positive cocci, has been implicated in persistent root canal infections and has been used in several previous studies on the efficacy of endodontic irrigants, especially for its high level of resistance to a wide range of antimicrobial agents.[1,29] It is one of the intra-canal bacteria which are most resistant to elimination by disinfecting agents.[12] Although yeasts have occasionally been reported in untreated cases (Sen et al. 1995), they have been more associated with cases of failed endodontic treatment (Nair et al. 1990).[4]

Ferraz et al. concluded that 0.025% is a safe concentration of NaOCl to clinical use. The results showed that 5.25% was the most efficient concentration of NaOCl. However in my study 2% NaOCl irradiated with laser showed the capacity to eliminate E.faecalis. They also assessed the mechanical action of chlorhexidine gel as an endodontic irrigant, demonstrating its ability to

remove smear layer and capacity to eliminate E. faecalis from infected root canals in vitro.[13,28] But in my study 1% CHX and 0.5% CHX when agitated with ultrasonic has least efficacy when compared with laser irradiation and was more efficient than the conventional procedure/protocol/method (i.e without agitation, control groups).

Brito P.R.R et al concluded that ultrasonic activation of NaOCl for 1 minute has not been shown to be better than conventional irrigation in an in vitro study.[14,23] In my study 2% NaOCl when agitated with Ultrasonic significantly reduced the colony forming units when compared with 1% NaOCl and with control groups.

Chlorhexidine is a broad-spectrum cationic bisguanide with antimicrobial effects on gram-positive and gram-negative bacteria. Contrary to NaOCl, CHX (with a concentration of 2% in the gel and liquid forms) preserves its antimicrobial effect for some time after being used (residual effect or substantivity), but it is unable to dissolve tissues. According to Schafer and Bossman, 2% CHX is more effective compared to its lower concentrations, manifesting its influence in a shorter period of time and with a proper antimicrobial influence on E. faecalis. Ercan et al. demonstrated that 2% CHX inhibits E. faecalis in 80% of the cases, which is consistent.[12,30] However in my study 1% CHX and 0.5% CHX reduced the CFU in E.faecalis and C.albicans when irradiated with Laser, Ultrasonic, and the Control groups in an decreasing order respectively.

Giardino et al (2009) showed that 5.25% NaOCl is more effective than CHX against E. faecalis. In contrast, Vianna et al (2004) concluded that 2% CHX yields larger zones of inhibition as compared to NaOCl in different concentrations.[15] Khademi et al. found the antibacterial substantivity of 2.6% NaOCl was significantly lesser than 2% CHX and 100 mg /ml doxycycline.[16] In my study 2% NaOCl and 1% NaOCl is more effective than 1% CHX and 0.5% CHX.

Pasqualini D.et al. evaluated that Sonic activation has been shown to be an effective method to remove the oral biofilm and enhance root canal disinfection. However, the performance of subsonic agitation appears to be less effective compared with ultrasonic activation of irrigant solutions. This may be attributed to the different acoustic streaming velocity and frequency, which positively influence debris removal from the qualitative standpoint.[17,27] In my study various concentration of NaOCl and CHX with Ultrasonic agitation shown to be an effective method to eliminate E.faecalis and C.albicans, when compared with the conventional methods.

However, in my study the results showed that, better antimicrobial efficacy was when irradiation with laser device in 2% NaOCl, 1% NaOCl, 1% CHX and 0.5% CHX; when agitated with Ultrasonic, showed least efficiency than the Laser irradiation groups but more efficient than the control groups.

CONCLUSION

Sodium hypochlorite solution is one of the most economical materials that dentists use. 5.25% NaOCl and 2% CHX was shown to be the most effective irrigant solution for better antimicrobial efficacy. But 5.25% NaOCl shows toxicity to the surrounding tissues. Hence in the present study reduced concentration of irrigants along with external agitation techniques like laser and ultrasonic unit were evaluated for antimicrobial efficacy. By using the agitation devices for low concentration irrigants shows the antimicrobial efficacy similar to that of the higher concentration irrigants used alone. With different concentration of irrigating solutions and agitation methods affect the antimicrobial efficacy of irrigating solutions on Enterococcus faecalis and Candida albicans.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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