An *In Vitro* Evaluation of Antimicrobial Effects of Three Different Root Canal Irrigating Solutions against *Enterococcus faecalis* and *Streptococcus mutans*

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ABSTRACT

Aim: The present study was conducted to evaluate and compare antimicrobial effect of 5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX) and *N-Acetylcysteine* (NAC) irrigating solutions against *Enterococcus faecalis* (*E. faecalis*) and *Streptococcus mutans* (*S. mutans*).

Materials and methods: The present study was conducted on 40 freshly extracted noncarious permanent mandibular incisors teeth of both genders (males–12, females–14). In all teeth, root canal preparation was done up to size 40 K-file. Roots were sterilized and microbial suspension of mixed culture of the tested microorganisms was inoculated into canals and incubated for 48 h. Teeth were divided into four groups, group I (5.25% sodium hypochlorite), group II (2% chlorhexidine), group III (200 mg/mL *N-Acetylcysteine* NAC) and group IV (sterile distilled water). The antimicrobial effect in each group was compared.

Results: Statistical evaluation was completed using statistical software Statistical Package for the Social Sciences (SPSS). Planktonic *S. mutans* bacterial count was lowest in group III followed by group I, group II and group IV. *E. faecalis* count was 6.14 ± 0.12 in group I, 5.76 ± 0.44 in group II, 3.88 ± 0.08 in group III and 11.98 ± 1.04 in group IV. The difference was significant ($p < 0.05$). The proportion of dead cell found to be 0.04 ± 0.01 , 0.72 ± 0.06 , 0.01 ± 0.06 and 1.02 ± 0.11 in groups I, II, III and IV respectively. The difference was significant ($p < 0.05$).

Conclusion: NAC proved to be effective against *E. faecalis* and *S. mutans*. This solution can be considered alternative in root canal infections in addition with CHX and NaOCl.

Clinical significance: Effectiveness of three different irrigating solutions was compared and NAC found to be more efficient in

decreasing bacterial count. Hence, NAC can be precisely used in irrigating root canals to achieve optimal clinical outcomes particularly regarding reoccurrences of infections. Furthermore, NAC could be proved as a promising innovation in future endodontic methodologies.

Keywords: Chlorhexidine, Confocal laser scanning microscopy, Irrigating solution, *Streptococcus mutans*.

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INTRODUCTION

Endodontic treatment (ET) or root canal treatment (RCT) is a common treatment modality in endodontics. The basic ideology and principle is the complete eradication of root canal irritants, cleaning, and shaping, obturation of root canals and post-endodontic restoration. It majorly involves access opening, cleaning the canals using various instruments along with the use of irrigating solution for instance sodium hypochlorite. Subsequently, the obturation of prepared canals is completed using obturating materials like gutta-percha. After completion of root canal treatment, the tooth is restored with various permanent restorative materials like amalgam, composite or GIC depending upon clinical condition.¹ As a proven fact, root canal treatment (RCT) significantly reduces the severity of pain. However, the persistence of pain in RC treated teeth could be possibly due to the penetration of pulpal tissues, dentinal debris, microorganisms and irrigants to the periapical tissues leading to periradicular infection and inflammation. The successful endodontic treatment largely depends on the absolute removal of

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microorganisms from the root canals and prevention of reinfection.² Some of the prominent bacterium, such as *E. faecalis* and *S. mutans* play an imperative role in persistence root canal infection. These bacteria frequently lead to root canal treatment failure solely because of their incomplete eradication. It has also been observed that several other traits of *E. faecalis* are persistently present in root canals.³ This is mainly because of their invasion into dentinal tubules and frequent adaptation to adverse conditions. Therefore the role of root canal irrigating solutions is of paramount importance undoubtedly. Sodium hypochlorite (NaOCl), chlorhexidine (CHX) and NAC (*N-Acetylcysteine*) are some of the prominent irrigating solutions those are widely used in the field of endodontics.⁴ This study was conducted to evaluate and compare the antimicrobial effect of sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX) and NAC irrigating solutions against *E. faecalis* and *S. mutans*. The presence of dead bacterial cells in the biofilm was also assessed by confocal laser scanning microscopy (CLSM). Here authors had genuinely attempted to explore the existing outcomes by processing the observations and other relevant findings.

MATERIALS AND METHODS

This study was conducted in the Department of Conservative Dentistry and Endodontics of the Institute. The studied samples were 40 freshly extracted noncarious permanent mandibular incisors teeth of both genders. All 40 samples were belonging to 12 male and 14 female subjects. Primarily, teeth extracted because of orthodontic or periodontal reasons were selected for the study. The study was being presented and approved by the institutional ethical committee. Initially, all sample teeth were sectioned through cervical region using with a diamond disc with air rotor headpiece. Measurements of working length for each root were completed radiographically with 10 K sized file. Using step back technique, thorough preparation of root canals were done up to 40 K sized file. In the beginning, the root canal irrigation was done with plain distilled water. Soon after preparation of root canal, the apical foramen was sealed with self-curing acrylic resins (DPI Self Cure, Bombay Burmah Trading Corporation, Limited, Mumbai, India). Roots were completely sterilized, and inoculation of microbial suspension of mixed culture of the tested microorganisms was done into canals. The complete sets of tested samples were incubated for 48 hours. For the ease of study and analysis, the sample teeth were arbitrarily divided into four groups of 10 each based on irrigating solutions used. Group I samples were subjected to 5.25% sodium hypochlorite (NaOCl), group II samples were subjected to 2% chlorhexidine (CHX), group III

samples were subjected to 200 mg/mL *N-Acetylcysteine* (NAC) solution, and group IV samples were subjected sterile distilled water.

First and foremost, bacteriological samples were obtained from root canal milieu. In these bacteriological samples, the total number of colony forming units (cfu) of the tested bacterium was calculated. After that, each root was cut down in a lengthwise manner to expose the pulp canal (Fig. 1). A standard length of 4 mm was cut and obtained from the middle portion of either side sectioned root piece. To avoid any superimposition and overlapping of bacterial colonies, the sectioned pieces were completely sterilized. The premixed and prepared microbial suspensions were used to inoculate these sectioned pieces for minimum 48 hours. The quantity of irrigation solution used in each group was 5 mL with an immersion time of 5 minutes. The relative presences of dead bacterial cells in the formed biofilm were also identified and studied by C LSM) at 10X magnification in the fluorescent mode. Results thus obtained were subjected to statistical analysis wherein *p* value less than 0.05 was considered significant.

RESULTS

All the observational findings were compiled and sent for statistical evaluation using statistical software SPSS version 21 (IBM Inc., Armonk, New York, USA). The resultant data was subjected to relevant statistical tests to obtain *p* values and other inferences. The irrigating solution used was 5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), 200 mg/mL *N-Acetylcysteine* (NAC) and Sterile distilled water in group I, II, III and IV respectively (Table 1). There were 10 teeth in each group. Planktonic *S. mutans* bacterial count was highest in group IV and lowest in group III. Precisely, it was 3.84 ± 0.28 in group I, 4.82 ± 0.86 in group II, 3.12 ± 0.76 in group III and 12.91 ± 2.14 in group IV. *E. faecalis* count was found the maximum in group IV and minimum in group III. Quantitatively

Fig. 1: Sample teeth showing exposed root canal lumen

Table 1: Types of irrigating solutions used in study

Groups	Group I	Group II	Group III	Group IV
Solutions	5.25% Sodium hypochlorite (NaOCl)	2% Chlorhexidine (CHX)	200 mg/mL N-Acetylcysteine (NAC)	Sterile distilled water
No. of teeth	10	10	10	10

Graph 1: Graphical representation of planktonic bacterial count in each group **Fig. 2:** Confocal laser scanning microscopic image confirming higher proportions of dead cells (10X magnification in the fluorescent mode) in group IV specimen**Table 2:** Planktonic bacterial count in each group

Planktonic bacterial	Group I	Group II	Group III	Group IV	p value
<i>S. Mutans</i>	3.84 ± 0.28	4.82 ± 0.86	3.12 ± 0.76	12.91 ± 2.14	0.01
<i>E. Faecalis</i>	6.14 ± 0.12	5.76 ± 0.44	3.88 ± 0.08	11.98 ± 1.04	0.02

p < 0.05 significant

it was 6.14 ± 0.12 in group I, 5.76 ± 0.44 in group II, 3.88 ± 0.08 in group III and 11.98 ± 1.04 in group IV (Graph I and Table 2). The difference was significant ($p < 0.05$). CLSM was used to identify and proportionate the relative presences of dead bacterial cells in the formed biofilm. The CLSM evaluation revealed that the relative presence of a bacterial proportion of dead cell in biofilm was maximum in group IV (Fig. 2) and minimum in group III (Graph 2 and Table 3). Quantitatively they were 0.04 ± 0.01 in group I, 0.72 ± 0.06 in group II, 0.01 ± 0.06 in group III and 1.02 ± 0.11 in group IV. The difference was significant ($p < 0.05$). The overall results indicated that NAC had maximum antibacterial activity as compared with other irrigating solutions including sterile distilled water.

DISCUSSION

Literature has well evidenced that the overall success of endodontic treatment is healthily appreciated by

Graph 2: Graphical representation of proportions of dead cells in each group

patients. There are few factors which affect success rates such as inadvertently missed strands of pulp tissue, failure to adequately seal the access cavity and even non-cooperation of the patient with respect to postoperative

Table 3: Proportions of dead cells in each group

	Group I	Group II	Group III	Group IV	p value
Proportion of dead cell	0.04 ± 0.01	0.72 ± 0.06	0.01 ± 0.06	1.02 ± 0.11	0.01

p < 0.05 significant

instructions. Moreover, any potent insult or injury to the periapical tissue during endodontic treatment results into more rigorous secretion of inflammatory mediators. These inflammatory mediators are also considered as mediators of pain for example prostaglandins, serotonin, leukotrienes, histamine, and bradykinin.⁵ It was postulated that bacteria in the root canals forms biofilm by attaching to root canal walls or may occur as single cells as free-floating or attached. Most commonly encountered bacteria in root canals are *E. faecalis* and *S. mutans*. *E. faecalis* is a versatile pathogenic bacterium which is one of the leading causes of endodontic failures in most of the cases.⁶ Stuart et al suggested that the prevalence of *E. faecalis* ranges from 24 to 77%.⁷ It is a gram-positive facultative anaerobic cocci may occur singly or in a pair. It has the property of invading into dentinal tubules and competes with other microorganisms. Kishen et al. conducted an in vitro study and suggested that *E. faecalis* initiates biofilm formation on root canal dentine which calcifies over the years and becomes a full-fledged calcified layer.⁸ Silva et al. found the invasion of *E. faecalis* in dentinal tubules. The author observed that bacteria itself may be the infectious agent in root canals. *S. mutans* is a gram-positive aerobic bacterium which shows its presence in root canals of teeth. It has been observed that *S. mutans* species in root canals contribute to microbe-microbe interactions which ultimately enhance total biofilm formation. Combination of *S. mutans* and *E. faecalis* forms a strong biofilm.⁹ This interaction poses difficulty for antimicrobials agents to show effect. Different species of *Streptococcus* has been isolated in multiple situations. Chavez observed *Streptococcus* species in 47% of retreatment cases.¹⁰ Some of the other pioneer studies have also shown the presence of bacteria during intracanal dressings, before and after instrumentation.^{11,12}

In the present study, we used 5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), 200 mg/mL *N-Acetylcysteine* (NAC) and sterile distilled water as an irrigating solution. We observed that planktonic bacterial count was significantly higher in group IV in which sterile distilled water was used as compared to groups I–III. This is in agreement with the study by Darrag.¹³ Arias et al. found that 5.25% sodium hypochlorite is more effective as compared to 2% chlorhexidine.¹⁴ NaOCl has higher antibacterial efficacy and it prevents attachment of bacteria to other bacteria and dentinal walls due to its organic tissue solvent nature. Similarly, in the present study, bacterial count was lower in group I as compared to groups II–IV. We used 2% chlorhexidine as it has a bacteriostatic action and bactericidal effect at higher concentrations. Commonly available sterile distilled water is a absolutely nonreactive solution with no antimicrobial effect. 200 mg/mL of NAC solution was used as at this concentration, the important effect is observed. Berber and

colleagues compared different concentrations of NaOCl in reducing *E. faecalis* in root canal lumen.¹⁵ It was seen that disinfection of the dentinal tubules occurs at the higher concentrations. We observed that the proportion of dead cell was higher in group IV as compared to other groups. Cindy et al. in their study revealed that NAC is more efficient in reducing dead cell proportion as compared to CHX.¹⁶ We also observed reduced dead cell proportion with NAC as compared to NaOCl and CHX. It is shown that both NaOCl and CHX are less effective on smear layer due to the buffering capacity of dentin. Therefore our results were in accordance with the study results of Cindy and associates. NAC is usually considered as a strong antioxidant that slowdowns polysaccharide formation and operating on the bacterial cell wall. It prevents the attachment of bacteria on dentinal walls by inhibiting polysaccharide formation, the potent component of biofilm. Thus it shows its effect indirectly. It was observed that all solutions were effective in reducing *S. mutans* concentration as compared to *E. faecalis*. CHX is both bactericidal and bacteriostatic that primarily acting on bacterial cell wall's phospholipids. Hugo et al. showed that it shows the effect by inhibiting ATPase enzyme attachment to the bacterial cell wall.¹⁷ The production of ATP is hindered, and hence bacterial growth ceases. Vianna et al. analyzed the antimicrobial efficacy of 0.2%, 1%, and 2% chlorhexidine gluconate in gel and liquid form with 0.5%, 1%, 2.5%, 4%, and 5.25% sodium hypochlorite (NaOCl).¹⁸ They noticed that *S. aureus* and *Candida albicans* totally vanished in 15 seconds with both 2.0% gel and liquid suspensions while the gel suspensions killed *E. faecalis* in 1 minute. The timing required for 1.0% and 2.0% CHX liquid to eliminate all microorganisms was the same required for 5.25% NaOCl. Their study results were quite comparable to our inferences. Studies conducted by Anuradha et al. and Yap et al. have also shown similar comparisons.^{19,20} However, they have not emphasized on the clinical applicability of the irrigants in different clinical situations. In a recent study by Palaniswamy and colleagues, they evaluated the antibacterial efficacies of chlorhexidine and NAC wherein they concluded that NAC has almost equal antimicrobial property as chlorhexidine.²¹ Their results were quite contrasting when compared to our inferences. It could be explained on the basis of differences in the methodology particularly in terms of Agar diffusion test and incubation timings. Ghivari et al. also evaluated three irrigants wherein they found that Sodium hypochlorite irrigants have shown lowered antibacterial activity against selected single species biofilm.²² They used nitrocellulose biofilm model to estimate antibacterial efficiency of tested root canal irrigants. Their study results were comparable to our inferences. In the present study, though the authors have tried to cover maximum aspects still there are some

viable limitation like authors only studied two species of bacteria, i.e., *E. faecalis* and *S. mutans*. Moreover, apart from the other available advanced irrigating materials, only four solutions such as sodium hypochlorite, chlorhexidine, sterile distilled water, and NAC were compared. A root canal may be infected with a variety of microorganisms; hence study on multiple bacteria could have been proved fruitful.

CONCLUSION

N-Acetylcysteine proved to be effective against *E. faecalis* and *S. mutans*. It showed good antibacterial and biofilm eradication efficiencies. Hence, NAC solution can be considered as a potent alternative for root canal disinfection in addition with CHX and NaOCl. Furthermore, the maximum proportion of dead cell was seen in samples treated with sterile distilled water. Here, NAC was shown to be most effective in eradicating biofilm when compared to other tested irrigating solutions (CHX and NaOCl). Our study results can be considered as suggestive for predicting clinical outcomes for such clinical situations. Nevertheless, we expect other large scale studies to be conducted that could further establish certain concrete guidelines in this prospect.

CLINICAL SIGNIFICANCE

NAC is completely systemically nontoxic and has a broad antimicrobial spectrum hence highly effective against aerobic, anaerobic and other discretionary microorganisms. Therefore, NAC can be efficiently used in irrigating root canals to achieve optimal clinical outcomes particularly regarding reoccurrences of infections. Furthermore, NAC could be possibly providing some newer avenues in root canal irrigation systems in future endodontic methodologies.

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