

Comparative Evaluation of Antimicrobial Effects of Two Root Canal Irrigating Solutions Against *E. Faecalis* and *S. Mutans*: An In Vitro Study

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ABSTRACT

Aim: This study was aimed to assess and compare antimicrobial effect of 2% chlorhexidine (CHX) and N-Acetylcysteine (NAC) irrigating solutions against two different bacterium species.

Materials & Methods: This study was performed on 60 freshly extracted non-carious permanent maxillary incisors teeth of both genders. Biomechanical preparation was completed up to size 40 K- file. Well prepared microbial suspensions of mixed culture of the studied bacterium were inserted into root canals. It was further incubated for 48 hours. All 60 samples were segregated into 3 groups. Group I (Chlorhexidine), group II (N-Acetylcysteine NAC). The third group was kept as control group (sterile distilled water). The antimicrobial effect in each group was observed, documented and compared.

Results: Statistical analysis was completed using statistical software Statistical Package for the Social Sciences version 20. *S. Mutans* bacterial colony count was highest in group III and lowest in group II. Specifically, it was 5.22 ± 0.56 in group I, 2.42 ± 0.46 in group II, 10.21 ± 2.32 in group III. The difference was significant ($P < 0.05$).

Conclusion: N-Acetylcysteine showed to be extremely efficient against colonies of *E. faecalis* and *S. mutans*. Hence N-Acetylcysteine can be used as an alternative root canal irrigant. Here in this study the authors have studied effectiveness of two irrigating solutions wherein N-Acetylcysteine found to be more effective in lowering bacterial count. N-Acetylcysteine may possibly be proved as promising innovation in endodontic advancements and technologies.

Keywords: Chlorhexidine, Irrigating solution, *Streptococcus Mutans*.

INTRODUCTION

Endodontic treatment is one of the most frequent dental procedures being performed in the dentistry. Root canal treatment or Endodontic treatments are mainly performed to manage grossly decayed teeth and other clinical circumstances. RCT has been

usually divided into two major portions; biomechanical preparation and obturation. Root canal irrigation is an integral part of root canal therapy. Finally, the basic ideology and principle is complete eradication of root canal irritants, cleaning & shaping, obturation of root canals and post-endodontic restoration. Root canal treatment

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or Endodontic treatments chiefly have access opening, cleaning the canals using various instruments along with use of irrigating solutions.¹⁻² These could be sodium hypochlorite or other solutions. Consequently the obturation of prepared canals is completed using obturating materials like gutta percha. Soon after completion of root canal treatment, the tooth is restored with different permanent filling/restorative materials like amalgam, composite or GIC depending on intricate clinical requirements. As we all are aware that Root canal treatment or Endodontic treatments are usually performed as a measure of instant analgesia; to relieve painful symptoms immediately.³⁻⁴ Conversely, if it persists then the reasons could be multiple like; dentinal debris, microorganisms, incomplete root canal irrigation, encroachment of infections into periapical region leading to infection and inflammation of surrounding alveolus/jaw bone. The successful endodontic treatment largely depends on the absolute removal of micro-organisms from the root canals and prevention of re-infection. Few of the potent bacterium is *Enterococcus faecalis* (*E. faecalis*) and *Streptococcus mutans* (*S. mutans*) These are the main species those causing crucial role in continued root canal pathologies. For that reason the functions of root canal irrigating solutions is of supreme importance unquestionably.⁵⁻⁶ Chlorhexidine (CHX) and NAC (N-Acetylcysteine) are some of the famous irrigating solutions which are frequently used in field of endodontics. NAC exerts anti-inflammatory activity as it has the ability to inhibit the expression and release of a variety of Pro-inflammatory cytokines. NAC solution is found to be an effective agent and is used to protect the pulp and surrounding peri-radicular tissues. NAC inhibits growth and eradicates biofilm of *E. faecalis*. It effectively reduces extracellular polysaccharide production, disrupts mature biofilms, and therefore decreases the adhesion potential of the bacteria on surfaces.⁷⁻⁸ This study intended to evaluate and compare antimicrobial effect of 2% chlorhexidine (CHX) and NAC irrigating solutions as seen against *E. faecalis* and *S. mutans*.

MATERIALS & METHODS

Total 60 freshly extracted non carious permanent maxillary incisors teeth were selected for this study.

Samples were obtained from both male and female subjects. Total 40 subjects were considered out of which 25 were male and 15 were female. We have also focused on the etiologies of the extractions. First and foremost, teeth extracted due to orthodontic or periodontal matters were selected for the study. The study was presented and cleared by institutional ethical committee. At first, we have planned to cut/section the teeth through neck area. This was completed with diamond disc driven by high speed air rotor headpiece. Dimensional assessment of working length for each root was done radio-graphically. Biomechanical preparation was done using step back technique exactly as stated by pioneer authors. Biomechanical preparation of root canals were done up to 40 K sized file. Firstly, plane distilled water was used for the purpose of root canal irrigation. After Biomechanical preparation of root canal, the apical foramen was closed off using auto curing acrylic resins (Rapid Repair, Dr Jagdish Lal Sethi and Co., Delhi, India). Following Biomechanical preparation, the roots were entirely sterilized. The microbial suspension of mixed culture of the tested microorganisms was inserted into prepared root canals. All intended tested samples were incubated for 48 hours. We have further divided the sample teeth arbitrarily into 3 groups of 20 each based on irrigating solutions used. Group I samples were subjected to 2% Chlorhexidine (CHX), group II samples were subjected to 200 mg/mL N-Acetylcysteine (NAC) solution and group III samples were subjected sterile distilled water. In the beginning, bacteriological sample was taken from root canal lumen. In these micro-bacteriological samples, the total number of Colony Forming Units (CFU) of tested bacterium was calculated. To accelerate the procedure and make it more user friendly, authors have cut down each root along with their root span. These maneuvers have completely exposed the root canal and the canal was entirely seen without any hindrance. The sectioned pieces were dipped into microbial suspensions for minimum 48 hours. We have also ensured to control the relative quantity of irrigation solution used in each group. It was kept maintained at 5 ml with immersion time of 5 minutes. Results thus obtained were subjected to statistical analysis wherein P value less than 0.05 was considered significant.

RESULTS

Data obtained from above methodologies and exercises were gathered and sent for statistical evaluation using statistical software Statistical Package for the Social Sciences version 20 (IBM Inc., Armonk, New York, USA). The finalized data was subjected to suitable statistical tests to obtain p values and other related inferences and outcomes. We have used two irrigating solution; 2% Chlorhexidine (CHX), 200 mg/mL N-Acetylcysteine (NAC) while Sterile distilled water was used as control. This was performed in group I, II and III respectively. Table 1 shows group wise distribution of the samples. There were 20 teeth in each group. Table 2 shows about evaluation of Mean values with Standard Deviation & Standard Error at 95% Coefficient of interval. The measured mean for group I, II and III was 29.97, 27.45 and 28.61 respectively. Standard deviation was noted least for group II (0.136) and maximum for group I (29.97). Standard error was noted minimum for group II (0.082) while it was maximum for group III (0.631). S. Mutans bacterial colony count was highest in group III and lowest in group II. Specifically it was 5.22 ± 0.56 in group I, 2.42 ± 0.46 in group II, 10.21 ± 2.32 in group III. E. faecalis count was found maximum in group III and minimum in group II. Efficiently it was 4.73 ± 0.32 in group I, 3.18 ± 0.68 in group II, 11.21 ± 2.38 in group III (Table 3). The difference was significant ($P < 0.05$). The overall results undoubtedly pointed out that NAC had maximum antibacterial activity as compared with other studied irrigating solutions including distilled water.

DISCUSSION

Literature has well evidenced 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 to each other. Most commonly resident bacteria in root canals are Enterococcus faecalis and S. mutans. Streptococcus mutans is a gram positive aerobic bacterium.⁹⁻¹¹ It has been showed that Streptococcus mutans species in root canals contribute to microbe-microbe interactions. Such intractions eventually augment total biofilm configuration. In certain special situations, the Streptococcus mutans and E. faecalis form a strong biofilm.¹² Various species of Streptococcus has been

Table 1: Types of irrigating solutions used in study

Groups	Group I	Group II	Group III
Solutions	2% Chlorhexidine (CHX)	200 mg/mL N-Acetylcysteine (NAC)	Sterile distilled water
No. of teeth	20	20	20

Table 2: Evaluation of Mean values (M) with Standard Deviation (SD) & Standard Error (SE) at 95% Coefficient of interval

Group	n	Mean	SD	SE	95% CI for Mean	
Group I	20	29.97	0.162	0.173	19.982	20.835
Group II	20	27.45	0.136	0.082	24.635	26.537
Group III	20	28.61	0.273	0.631	22.834	23.529

Table 3: Bacterial colony counts Group wise

Bacterium genus	Group I	Group II	Group III	P value
S. Mutans	5.22 ± 0.56	2.42 ± 0.46	10.21 ± 2.32	0.02
E. Faecalis	4.73 ± 0.32	3.18 ± 0.68	11.21 ± 2.38	0.04

$p < 0.05$ significant

isolated in multiple situations. In present study, we used 2% chlorhexidine (CHX), 200 mg/mL N-Acetylcysteine (NAC) and sterile distilled water as irrigating solution. We noticed that bacterial count was significantly higher in group III in which sterile distilled water was used as compared to group I and II. Somewhat similar findings were also put forwarded by Stuart.¹³ Universally available sterile distilled water is an extremely nonreactive solution with no antimicrobial effect. 200 mg/mL of NAC solution was used as at this concentration, the important effect is observed. NAC is a thiol-containing antioxidant that possesses antibacterial property. The active moiety is the thiol site, which plays a role in free radical scavenging and

destruction of intermolecular or intramolecular disulfide bonds in proteins.¹⁴⁻¹⁷ The mechanism of antibacterial effects of NAC is due to the reaction of its -SH group with the disulfide bonds of bacterial proteins, leading to the irreversible damage of bacterial proteins that are essential for bacterial growth and metabolism.¹⁸⁻²³ The biofilm disruption action of NAC is likely due to its effect on exopolysaccharide (EPS) production, which is one of the major components in the biofilm. This can occur through several ways. First, the disulfide bonds of the bacterial enzyme involved in EPS production can be disrupted by the sulfhydryl group of NAC, or there can be an excretion through the thiol-disulfide exchange. Second, as NAC is antioxidant, it can exert an indirect effect on bacterial cell metabolism as well as on EPS production.²⁴⁻²⁶ NAC is usually considered as a strong antioxidant that slows down polysaccharide formation and operating on bacterial cell wall. It prevents 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*.²⁷

CONCLUSION

N-Acetylcysteine showed to be extremely efficient against growing colonies of *E. faecalis* and *S. mutans*. Hence N-Acetylcysteine can be used as an alternative root canal irrigant. Here in this study the authors have studied effectiveness of two irrigating solutions wherein N-Acetylcysteine found to be more effective in lowering bacterial count. N-Acetylcysteine may possibly be proved as promising innovation in endodontic advancements and technologies. Additionally, our study results may be considered as evocative for estimating clinical outcomes for such therapeutic circumstances. However, we expect other large scale studies to be conducted that can further establish certain concrete norms in this prospect.

CONFLICT OF INTEREST

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

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