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Relative assessment 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 done to evaluate and compare antimicrobial effect of 2% chlorhexidine (CHX) and N-Acetyl cysteine (NAC) irrigating solutions against two different bacterium species.
Materials & Methods: This study was conducted on 60 newly extracted non carious permanent maxillary incisors teeth of both genders. Biomechanical preparation was done 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 21. S. *S. mutans* bacterial colony count was highest in group III and lowest in group II. Specifically it was 6.54±0.67 in group I, 3.76±0.34 in group II, 11.34± 2.21 in group III. *E. faecalis* count was found maximum in group III and minimum in group II. Efficiently it was 5.34± 0.43 in group I, 4.65± 0.89 in group II, 12.32± 2.54 in group III (Table 3). The difference was significant ($P< 0.05$).
Conclusion: N-Acetylcysteine showed to be exceptionally 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 efficiency of two irrigating solutions wherein N-Acetylcysteine found to be more efficient in lowering bacterial count. N-Acetylcysteine may possibly be proved as promising innovation in endodontic.

Keywords: Chlorhexidine, irrigating solution, *Streptococcus mutans*

Introduction
Disinfection of the root canal system is essential for successful endodontic treatment. Root canals are usually infected by multiple bacteria. In general, 2.5% sodium hypochlorite and 2% chlorhexidine are used as irrigating solutions in endodontics. Sodium hypochlorite has been used as an irrigant because of its broad antimicrobial spectrum and ability to dissolve necrotic tissue remnants while 2% chlorhexidine has been used as an irrigant due to its broad antimicrobial activity, substantivity, and low cytotoxicity. Root canal treatment 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 assess 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 (Pyrex 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 28.78, 28.32 and 29.72 respectively. Standard deviation was noted least for group II (0.165) and maximum for group I (0.292). Standard error was noted minimum for group II (0.101) while it was maximum for group III (0.502). *S. mutans* bacterial colony count was highest in group III and lowest in group II. Specifically it was 6.54±0.67 in group I, 3.76±0.34 in group II, 11.34± 2.21 in group III. *E. faecalis* count was found maximum in group III and minimum in group II. Efficiently it was 5.34± 0.43 in group I, 4.65± 0.89 in group II, 12.32± 2.54 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.

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

Discussion

Polymicrobial infection encountered inside the root canal system is one of the major challenge that is faced by an endodontists. Although variety of microbes are cultured from the infection of root canal system depending upon the type of infection e.g. primary endodontic infection is seen colonized mainly by Bacteroides, Porphyromonas, Prevotella, Fusobacterium, Treponema, etc, whereas in secondary infection, enterococcus, Actinomyces, streptococcus and candida species are seen [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 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 slowdowns 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* [27-33]. No published studies have assessed microbial load reduction in the root canal system with a combination of reciprocating instrumentation and irrigation with ozonated water. Reciprocating instrumentation shapes the root canal system rapidly, thus enabling high-flow, large-volume irrigation, while the biocompatibility and properties of ozonated water make it an interesting alternative irrigant. Within this context, the present study sought to evaluate the antimicrobial effect of 2% chlorhexidine (CHX) and N-Acetylcysteine (NAC) irrigating solutions against two different bacterium species.

Conclusion

N-Acetylcysteine showed to be exceptionally 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 efficiency of two irrigating solutions wherein N-Acetylcysteine found to be more efficient in lowering bacterial count. N-Acetylcysteine may possibly be proved as promising innovation in endodontic. Moreover, our study results may be considered as evocative for estimating clinical outcomes for such therapeutic circumstances. Nevertheless, we expect other large scale studies to be conducted that can further establish certain concrete norms in this prospect.

References

1. Roach RP, Hatton JF, Gillespie MJ. Prevention of the ingress of a known virulent bacterium into the root canal system by intracanal medications. J Endod. 2001; 27:657-60.
2. Hubble TS, Hatton JF, Nallapareddy SR, Murray BE, Gillespie MJ. Influence of *Enterococcus faecalis* proteases and the collagenbinding protein, Ace, on adhesion to dentin. Oral Microbiol Immunol. 2003; 18:121-6.
3. Love RM. *Enterococcus faecalis* - A mechanism for its role in endodontic failure. Int Endod J. 2001; 34:399-405.
4. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am. 2010; 54:291-312.
5. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontic: review. Int Endod J. 2009; 42:288-302.
6. Rucucci D, Siqueira JF. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod. 2010; 36:1277-88.
7. Russell AD, Hammond SA, Morgan JR. Bacterial resistance to antiseptics and disinfectants. J Hosp Infect. 1986; 7:213-25.
8. Neelakantan P, Sanjeev K, Subbarao CV. Duration-dependent susceptibility of endodontic pathogens to calcium hydroxide and chlorhexidene gel used as intracanal medicament: An *in vitro* evaluation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007; 104:e138-41.
9. Estrela C, Ribeiro RG, Estrela CR, Pécora JD, Sousa-Neto MD. Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. Braz Dent J. 2003; 14:58-62.
10. Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza E Filho FJ. *In vitro* assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. J Endod. 2001; 27:452-5.
11. Charles Stuart H, Scott Schwartz A, Thomas Beeson J, Christopher Owatz B. *Enterococcus faecalis*: It's Role in Root Canal Treatment Failure and Current Concepts in Retreatment. JOE. 2006; 32:93-8.
12. Koch S, Hufnagel M, Theilacker C, Huebner J. Enterococcal infections: host response, therapeutic, and prophylactic possibilities. Vaccine. 2004; 22:822-30.
13. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006; 32:93-8.
14. Kishen A, George S, Kumar R. *Enterococcus faecalis*-mediated biomineralized biofilm formation on root canal dentine *in vitro*. J Biomed Mater Res. 2006; 77:406-15.
15. Quah SY, Wu S, Lui JN, Sum CP, Tan KS. N-acetylcysteine inhibits growth and eradicates biofilm of *Enterococcus faecalis*. J Endod. 2012; 38:81-5.
16. Olofsson AC, Hermansson M, Elwing H. N-acetyl-L-cysteine affects growth, extracellular polysaccharide production, and bacterial biofilm formation on solid surfaces. Appl Environ Microbiol. 2003; 69:4814-22.
17. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against *Enterococcus faecalis* biofilm formed on tooth substrate: An *in vitro* study. J Endod. 2010; 36:83-6.
18. Silveira LF, Baca P, Arias-Moliz MT, Rodríguez-Archilla A, Ferrer- Luque CM. Antimicrobial activity of alexidine alone and associated with N-acetylcysteine against *Enterococcus faecalis* biofilm. Int J Oral Sci. 2013; 5:146-9.
19. Da Silva LA, Nelson-Filho P, Faria G, de Souza-Gugelmin MC, Ito IY. Bacterial profile in primary teeth with necrotic pulp and periapical lesions. Braz Dent J. 2006; 17:144-8.
20. Chavez de Paz L. Gram-positive organisms in endodontic infections. Endod Top. 2004; 9:79-96.
21. Vijaykumar S, GunaShekhar M, Himagiri S. *In vitro* effectiveness of different endodontic irrigants on the reduction of *Enterococcus faecalis* in root canals. J Clin

Exp. Dent. 2010; 2:169-72.
22. Estrela C, Silva JA, Goncalves De, Alencar AH, Leles CR, Decurcio DA. Efficacy of sodium hypochlorite and chlorhexidine against *Enterococcus faecalis*ea systematic review. J Appl Oral Sci. 2008; 16:364-8.
23. Pérez-Giraldo C, Rodríguez-Benito A, Morán FJ, Hurtado C, Blanco MT, Gómez-García AC. Influence of N-acetylcysteine on the formation of biofilm by *Staphylococcus epidermidis*. J Antimicrob Chemother. 1997; 39:643-6.
24. Portenier I, Haapasalo H, Rye A, Waltimo T, Ørstavik D, Haapasalo M. Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin. Int Endod J. 2001; 34:184-8.
25. Chen L, Wen YM. The role of bacterial biofilm in persistent infections and control strategies. Int J Oral Sci. 2011; 3:66-73.
26. Darrag AM. Antimicrobial efficacy of endodontic irrigation solutions against planktonic microorganisms and dual-species biofilm. Tanta Den J, 2013, 129-37.
27. Arias-Moliz MT, Ferrer-Luque CM, Espigares-Garcy´a M, Baca P. *Enterococcus faecalis* biofilms eradication by root canal irrigants. J Endod. 2009; 35:711-4.
28. Maniglia-Ferreira C, de Almeida Gomes F, Ximenes T, Neto MAT, Arruda TE, Ribamar GG *et al*. Influence of reuse and cervical preflaring on the fracture strength of reciprocating instruments. Eur J Dent. 2017; 11:41-7.
29. Goztas Z, Onat H, Tosun G, Sener Y, Hadimli HH. Antimicrobial effect of ozonated water, sodium hypochlorite and chlorhexidine gluconate in primary molar root canals. Eur J Dent. 2014; 8:469-74.
30. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ *et al*. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. Int Endod J. 2001; 34:424-8.
31. Weber CD, McClanahan SB, Miller GA, Diener-West M, Johnson JD. The effect of passive ultrasonic activation of 2% chlorhexidine or 5.25% sodium hypochlorite irrigant on residual antimicrobial activity in root canals. J Endod. 2003; 29:562-4.
32. Okino LA, Siqueira EL, Santos M, Bombana AC, Figueiredo JA. Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. Int Endod J. 2004; 37:38-41.
33. Pinheiro SL, Azenha GR, Democh YM, Nunes DC, Provasi S, Fontanetti GM *et al*. Antimicrobial activity of photodynamic therapy against *Enterococcus faecalis* before and after reciprocating instrumentation in permanent molars. Photomed Laser Surg. 2016; 34:646-51.