Available online at www.easletters.com

ISSN No: 2349-2864

Entomology and Applied Science Letters, 2016, 3, 5:104-108

An *In Vitro* Study to Compare Antibacterial Efficacy of Various Root Canal Irrigants Used In Endodontics

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ABSTRACT

The aim of the study was to compare antibacterial efficacy of various root canal irrigants used in department of endodontics. One hundred and fifty freshly extracted mandibular & maxillary molars were stored in normal saline at room temperatures .The teeth were deroofed & their wider canals that is distal canal in mandibular molars & palatal canal in maxillary molars were selected. Working lengths of specimens were recorded. All teeth were washed & autoclaved. Widening of canals was done using the K-file with a step back technique and divided into 5 groups (30/group).The teeth were then inoculated with 10 microliter of bacterial suspension of enterococcus faecalis fixed at 0.144 absorbance value (550nm wavelength) and incubated for 48-hrs. There was evident statistically significant difference in the antibacterial efficiency between 5% sodium hypochlorite (NaOCl) (positive control) (Group A) 0.12% chlorhexidine (CHX) (Group B), 2% Iodine (Iodine potassium iodide) (Group C), Sodium Chloride (NaCl) (Group D) and distilled water (Group E) (negative control) ($\rho<0.05$). Group A where 5ml of 5% NaOCl was used at initial & final rinse as root canal irrigants showed the maximum antibacterial efficiency against streptococci followed by Group B, Group C, Group D & Group E (Control Group).

Key Words: Root canal irrigants, NaOCl, chlorhexidine, NaCl, Iodine

INTRODUCTION

An endodontic therapy is influenced by the presence of microbial load; therefore elimination of all the microorganisms is necessary from the root canals of the effected tooth for an effective treatment[1].

Majority of microbes from the canals are eliminated using hand & rotary files. Mechanical action by the rotary instruments is not sufficient for the preparation of a root canal satisfactorily, due to the complexity of internal dental anatomy which increases the incapability to access apical deltas, lateral canals and accessory canals[2]. Thus a need of alternatives arises of both physical and chemical actions by the irrigating solutions[1].

During canal preparation of a tooth, irrigation is essential due to many reasons which include cleaning of canal, lubricating the files during mechanical preparation, flushing of debris, dissolving the dentinal tissue and eradicating bacteria without affecting the periapical tissues. An ideal irrigant should have two major qualities i.e. it should have antibacterial effect as well as lack toxicity of the periapical tissues.

NaOCl has a capability to act as both oxidizing and hydrolysing agent with an antibacterial and proteolytic effect[3]. Since 1915 NaOCl has been profoundly used as a wound irrigant due its high antibacterial quality and then later by 1920 as an endodontic irrigant [4-6]. NaOCl is a relatively cheap endodontic irrigant, which has a combinational effect of being virucidal as well as bactericidal [6, 7].A wide range of NaOCl concentrations is acceptable when used as an irrigant (1-5.25 %).

CHX having a wide range of antimicrobial spectrum[8] and is also proposed to be used as endodontic irrigant, due to its high effectiveness against gram-negative and gram-positive microorganisms commonly found in endodontic infections as well as against yeast [8]. CHX has an ability to attack the bacterial cytoplasmic or inner membrane or the yeast plasma membrane by penetrating cell wall or outer membrane [9].As a general concept there is an increase in antibacterial effectiveness with a higher concentration of CHX, nearly about 2% is proposed to be used as an irrigant [9, 10] this is due to the concentrations of CHX ranging from 0.2-2% are considered as toxicologically safe [11, 12].

Iodine being a strong oxidizing agent penetrates the microorganisms reacts with the free sulfhydryl group enzymes and attacks the molecules such as fatty acids, proteins and nucleotides, which results to cell death [11]. Therefore it has profound effect against several root canal microbes. Aqueous iodine solutions are unstable, whereas the molecular iodine (I_2) has remarkable antimicrobial activity, therefore iodine potassium iodide (2% iodine in 4% potassium iodine) is used in endodontic therapy [8].

Saline provides gross debridement & lubrication. A few investigators have supported isotonic saline solution as an irrigation solution to minimize tissue irritation & inflammation. In isotonic concentration, saline produces no documented tissue damage & has been confirmed to flush debris from the canals as thoroughly as NaOCl. Irrigation with saline alone forgoes chemical destruction of microbiologic matter & dissolution of mechanically inaccessible tissue e.g.; tissues in accessory canals.

This in *vitro* study was performed to compare the efficacy of various irrigants which are commonly available & used in dental practices.

MATERIALS AND METHODS

Ethical approval was obtained from the Ethics Committee of the Faculty of Dentistry, Baqai University, Karachi, Pakistan for extracted teeth collection. One hundred and fifty extracted human mandibular and maxillary molars were obtained after taking written consent from patients at the Baqai University hospital, Karachi, Pakistan. The exclusion criteria for the selection of teeth used in this study was i) Pre–root treated teeth ii) wisdom molars iii) teeth with dilacerations or morphological anomalies, or with open apices and iv) with root caries. The inclusion criterion was distal canals of extracted mandibular molars and palatal canals of maxillary molars were selected for the experiment.

Teeth were de-roofed using straight fissure diamond bur #21 (DiaBurs.China).Distal canal in the mandibular molars and the palatal canals in the maxillary molars were selected as they are wider canals. Working lengths of the specimens were recorded by using # 20 k files (Mani. China).The canals were prepared upto the file no # 45 as Master Apical File (MAF) and step back technique was performed using K-file no # 50, 55, 60.Rating of the commonly found root canal bacteria was carried first, by incubating the paper points taken from the canals as sample; in the 5% Blood Agar Culture Medium (Oxoid, U.K) for 24 hours at 37℃. Microscopic reading revealed the presence of Streptococcal *E. faecalis* colonies in abundance. The teeth were washed and autoclaved at 121°C under 15 lbs pressure for 15 mins.

The *E. faecalis* as potential pathogen, colonies were cultured in BHI broth (Oxoid, U.K), the colonies were used to prepare a stock solution for further use. Later the stock solution was standardized by UV spectrophotometer at absorbance value of 0.144 (550 nm). The suspension was inducted in each sterile tooth by employing the pipette of 10 µl. All one hundred and fifty teeth were incubated for 48 hours at 37 ℃ and divided equally into six groups of 30 each (15 mandibular ,15 maxillary molars) designated as A) 5% NaOCl (positive control),B) 0.12% CHX,C) 2% Iodine, D) Isotonic NaCl and E) sterile distilled water (negative control).

Group A (5% NaOCl)
In this group 5% NaOCl was used as a root canal irrigant. Throughout the instrumentation, 3ml of NaOCl solution was used for irrigation followed by a final rinse with 2ml of solution for 3 minutes. Removal of excessive moisture from the canals was carried out by paper points and a sterile point of 40# was retained in the irrigated canal up to the previously determined working length for 15 seconds. These points were transferred into sterile test tubes, used to inoculate the bacteria in plates of 5% Blood Agar and incubated for 48 hours at 37℃. Colony counter was used finally for results.

Same procedure was performed for group B, group C, group D & group E as mentioned above for group A.

RESULTS

After 48-hrs of incubation the agar plates were analysed on colony counter (Rocker, Taiwan) to calculate the colony forming units (CFU/ml) after treatment with the respective irrigant in Table 1. NaOCl was found to be the most effective anti-bacterial irrigant reducing the CFU count to maximum value followed by CHX >2% Iodine > Isotonic NaCl> sterile distilled water. The data was analysed using IBM SPSS (Chicago, USA) version 21.

Table 1: CFU count of *E. faecalis* retained on human teeth treated with respective irrigants after incubation of culture plates for 48-hrs at 37°C

Groups	Mean ± SD
Group A	16.88 ± 3.86^{A}
Group B	39.47 ± 6.33^{B}
Group C	96.11 ± 7.12^{C}
Group D	239.33 ± 8.73^{D}
Group E	283 ± 9.48^{E}

The CFU count was expressed as Mean ± SD for the respective treatment group. 5% NaOCl (Group A) used as positive control, 0.12% CHX (Group B), 2% Iodine (Group C), Isotonic NaCl (Group D) and sterile distilled water (Group E) negative control. Where the number of determinants (n)=30

$^{A-E}$different capital letters mean statistical significant difference between the CFU count/ ml of the five respective treatment groups of irrigants.

On statistical analysis using One-way ANOVA, with the irrigants as a fixed variable and CFU count as a dependent variable, statistically significant difference was observed between the negative control and the respective treatment groups. On further analysis the difference in the CFU count was statistically significant between the positive control and the three treatment groups (B, C and D).

DISCUSSION

In this study in order to determine the efficacy of various root canal irrigants, bacterial strains were isolated from various endodontic infections. Blood agar culture plates tend to give highly accurate results in such microbial investigations [13-17].These microbes were found to have a remarkably different behaviour as compared to planktonic [18].

Effect of antimicrobial agent of high relevance in clinical endodontics was compared. The effect is dependent on variable factors such as the response of biofilms microbial flora in relation to their growth phase, dose and the exposure to endodontic irrigant[16-19].

In this study modification of Siqueira*et al*, model was adopted in which they allowed the growth of microbial film on a membrane which was later placed in the antimicrobial agent in order to determine its efficacy [20].Most relevant *in vitro* study is the one in which the root canal surface of an extracted tooth are used to develop the biofilms and later treated with antimicrobial irrigants [13, 21,22]. This creates a method similar to that of an *in vivo* canal preparation process in which some factors such as complexity and variability of root canal system lead to potential difficulty in achieving a direct contact between the biofilms, antimicrobial agents and the canal preparation files. Being an uncontrolled variable it will not provide the antimicrobial efficacy of that particular root canal agent.

The antimicrobial agents selected included NaOCl, iodine potassium iodide, CHX, Sodium chloride (NaCl) & distilled water. The strains of Streptococci found on sample collection initially were used in the study.

This study claims that the vulnerability of bacteria varies in degrees, to a wide range of antimicrobial agents and that the time of exposure could be decisive. NaOCl seemed to be most effectual followed by CHX and IDI while NaCl was not effective. *Streptococcal* species has been habitually caught up as a survivor of root canal treatment regimes [23-28]. Regardless of the occurrence of this species in continual infections, it is not normally utilized in antimicrobial efficacy tests. Briseno *et al* used *Streptococcus mutans* in infective extracted tooth and compared the efficiency at different concentrations of NaOCl with and without ultrasonic activation [21].Even though there were huge reductions in figures, the microbes were not entirely eliminated and the full irrigation time was not given. Siqueira *et al* matched the effectiveness of 2.25% NaOCl and 0.2% CHX using an agar diffusion test along with three streptococci [20]. Conclusions were made that NaOCl was somewhat extra efficient but dependent relatively on the species being tested. The outcome of iodine potassium iodide irrigation cannot be substantiated because no studies were reported. Nonetheless, Molander *et al* used 5% iodine potassium iodide as dressing and established that the most prime group of relentless organisms was streptococci, while *S. intermedius* was not recognized between them [29]. The speculation is that individual species may counter in a different way to a range of antimicrobial agents.

Evaluation and comparison of the effectiveness of NaOCl and CHX irrigants on natural root canal infections in extracted teeth is done in different number of studies[30-34]. Ringel *et al* compared the efficiency of 0.2% CHX and 2.25% NaOCl in 30 teeth, each with pulp necrosis and peri-apical lesions [32].It was established that NaOCl was more effectual and discrepancy was accredited to its tissue dissolving capacity. Kuruvilla and Kamath established that CHX minimized the number of microbes by almost 70% compared with 60% by NaOCl. There was a decline of 85% when both solutions were used interchangeably. Furthermore, Delany *et al* and Leonardo *et al* explored that CHX by strength 0.2% and 2% were helpful in plummeting bacterial counts when used as irrigants [30, 34].Similarly, they further concluded that these strengths were useful in decreasing inter appointment bacterial activity. The possible consequence of disrupting the connections between bacteria that sustain some species clearly has an advantageous effect past that evident from the antimicrobial tests on individual species.

CONCLUSION

Considering the morphological complexities and the microbial infections, canal debridement is a challenging procedure. Therefore an efficient and effective chemo-mechanical root canal preparation is a mandatory process in order to overcome these challenges. NaOCl being a gold standard is proficient in inhibiting the bacterial growth on irrigation.

REFERENCES

[1] Patel, S. and J.J. Barnes, *Introduction*, in *The Principles of Endodontics*. **2013**, Oxford, UK. p. 1-5.
[2] Samaksamarn, T., et al., *Chulalongkorn University Dental Journal*. **31**(2): p. 125-34.
[3] Pashley, E.L., et al., *Cytotoxic effects of NaOCl on vital tissue.* J Endod, **1985**. **11**(12): p. 525-8.
[4] Dakin, H.D., British medical journal, **1915**. **2**(2852): p. 318.
[5] Crane, A.B., *A practicable root-canal technic*. **1920**: Lea & Febiger.
[6] Best, M., V.S. Springthorpe, and S.A. Sattar, *Am J Infect Control*, **1994**. **22**(3): p. 152-62.
[7] Underwood, M.A. and S. Pirwitz, *Am J Infect Control*, **1999**. **27**(2): p. 141-4.
[8] Haapasalo, M., et al., Endodontic topics, **2005**. **10**(1): p. 77-102.
[9] Schafer, E. and K. Bossmann, *American Journal of Dentistry*, **2001**. **14**(4): p. 233-237.
[10] Krautheim, A.B., T.H.M. Jermann, and A.J. Bircher, Contact Dermatitis, **2004**. **50**(3): p. 113-116.
[11] Yesilsoy, C., et al., *Journal of Endodontics*, **1995**. **21**(10): p. 513-515.
[12] Southard, S.R., et al., *Journal of periodontology*, **1989**. **60**(6): p. 302-309.
[13] Shih, M., F.J. Marshall, and S. Rosen, *Oral Surgery, Oral Medicine, Oral Pathology*, **1970**. **29**(4): p. 613-619.
[14] Ohara, P., M. Torabinejad, and J.D. Kettering, *Endodontics & Dental Traumatology*, **1993**. **9**(3): p. 95-100.
[15] Thrower, Y., R.J. Pinney, and M. Wilson, *Journal of Medical Microbiology*, **1997**. **46**(5): p. 425-429.
[16] Desai, M., et al., *Journal of Antimicrobial Chemotherapy*, **1998**. **42**(2): p. 153-160.
[17] D'Arcangelo, C., G. Varvara, and P. De Fazio, *Journal of Endodontics*, **1999**. **25**(5): p. 351-353.
[18] Wilson, M., *Journal of Medical Microbiology*, **1996**. **44**(2): p. 79-87.

[19] Pratten, J. and M. Wilson, *Antimicrobial Agents and Chemotherapy*, **1999**. **43**(7): p. 1595-1599.
[20] Siqueira, J.F., et al., *Journal of Endodontics*, **1998**. **24**(6): p. 414-416.
[21] Briseno, B.M., et al., *Endodontics & Dental Traumatology*, **1992**. **8**(1): p. 6-11.
[22] Siren, E.K., et al., *International Endodontic Journal*, **1997**. **30**(2): p. 91-95.
[23] Grahnen, H. and B. Krasse, *Odontol Rev*, **1963**. **14**: p. 167-177.
[24] Olgart, L.G., *Acta Odontol Scand*, **1969**. **27**(1): p. 91-103.
[25] Myers, J.W., F.J. Marshall, and S. Rosen, *Oral Surgery, Oral Medicine, Oral Pathology*, **1969**. **28**(6): p. 889-896.
[26] Bystrom, A. and G. Sundqvist, *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics*, **1983**. **55**(3): p. 307-312.
[27] Bystrom, A. and G. Sundqvist, *International Endodontic Journal*, **1985**. **18**(1): p. 35-40.
[28] Gomes, B.P.F.A., J.D. Lilley, and D.B. Drucker, *International Endodontic Journal*, **1996**. **29**(4): p. 235-241.
[29] Molander, A., C. Reit, and G. Dahlén, *Dental Traumatology*, **1999**. **15**(5): p. 205-209.
[30] Delany, G.M., et al., *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics*, **1982**. **53**(5): p. 518-523.
[31] Jeansonne, M.J. and R.R. White, *Journal of Endodontics*, **1994**. **20**(6): p. 276-278.
[32] Ringel, A.M., et al., *Journal of Endodontics*, **1982**. **8**(5): p. 200-204.
[33] Karuvilla, J.R. and M.P. Kamath, *Journal of Endodontics*, **1998**. **24**(7): p. 472-476.
[34] Leonardo, M.R., et al., *Journal of Endodontics*, **1999**. **25**(3): p. 167-171.