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Influence of Temperature on Antimicrobial Efficacy of Various Endodontic Irrigants: An in Vitro Study

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Abstract

Background: Aim: The aim of this study was to compare the antimicrobial efficacy of 5.25% NaOCl, 2% CHX, and 17% EDTA at 25oC, 45oC, and 60oC in extracted bovine incisor teeth. **Methods**: Two hundred dentin tubes were prepared and infected for 28 days with Enterococcus faecalis. The samples were divided into the following groups: Group I (n=20): 5.25% NaOCl at 25oC, Group II (n=20): 2% CHX at 25oC, Group III (n=20): 17% EDTA at 25oC, Group IV (n=20): 5.25% NaOCl at 45oC, Group V (n=20): 2% CHX at 45oC, Group VI (n=20): 17% EDTA at 45oC, Group VII (n=20): 5.25% NaOCl at 60oC, Group VIII (n=20): 2% CHX at 60oC, Group IX (n=20): 17% EDTA at 60oC, Group X (n=20): Sterile dentin tubes. Dentin chips were collected with round burs in YG broth. After culturing on tryptic soy agar (Difco) for 48 h at 37oC, bacterial colonies were counted and recorded as colony-forming units (CFU). Variables were expressed as means ± standard deviation. Tukey's multiple post hoc test was used for comparisons among the groups. Significance level was set at $p<0.05$.**Results**: The maximum number of CFUs was seen in NaOCl (49.514 at 20oC), thus meaning lower antimicrobial efficacy. The least amount of mean bacterial content was seen for 2% chlorohexidine (12.779 at 60OC). Tukey test showed significant difference between 5.25% NaOCl at 25oC and 45oC, 2% CHX 25oC and 17% EDTA 45oC ($p>0.05$). **Conclusion**: All the irrigating solutions showed a significant reduction in the CFU numbers over a temperature rise.

Keywords: Antimicrobial Efficacy, Enterococcus Faecalis, Endodontic Irrigant, Sodium Hypochlorite.

INTRODUCTION

The major goal of any root canal treatment is the complete removal of microbes from the canal space in order to achieve a healthy periapical tissue.[1] The removal of microorganisms mainly depends upon on chemo mechanical treatment of the radicular pulp space.[2] Due to complex anatomy of endodontic space like numerous irregularities and accessory canals, total disinfection is impossible. This leads to persistence of infection as it serves as ecological niches to microorganisms.[3] Those areas that cannot be mechanically approached to control microorganisms are disinfected using endodontic irrigants.[4]

The most common irrigants used are sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX) and

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ethylenediaminetetraacetic acid (EDTA). According to infection status of root canal, the irrigants can be used in combinations.[5]

Sodium hypochlorite is the most common irrigant used in endodontics due to their antibacterial, tissue-dissolving and lubricating properties.[6] Also they have many advantages like good shelf life, inexpensive and easy availability in market.[7]NaOCl has been recommended to be used in concentration of 5.25%, 2.5% and 1.25%.[8,9]

Chlorhexidine gluconate is another more commonly used irrigant. It is potent antimicrobial irrigant, especially against E.faecalis.[10] It is used in concentration of 2% as a final rinse. CHX is highly effective against intra-radicular microorganisms in cases of apical periodontitis.[11] EDTA has a property to react with calcium ions in dentin, thus forming calcium chelates which has the ability to remove smear layer.[12] It is used in concentration of 17% for a duration of one minute.[13]
Very few studies have evaluated the influence of temperature on endodontic irrigants in terms of antibacterial effectiveness and dynamic viscosity.[14,15] Hence, the purpose of this study is to evaluate the influence of temperature (25oC, 45oC and 60oC) on the antibacterial effectiveness of 5.25% NaOCl, 17% EDTA and 2% CHX.

MATERIALS AND METHODS

Freshly extracted bovine maxillary incisor teeth were used for the study. All the sample teeth were placed in 0.5% NaOCl solution for atleast 1 week. The apical 5 mm and coronal two-third were removed with a rotating diamond saw at 1000 rpm (Isomet Plus precision saw; Buehler, IL, USA). The canal was widened with a 2 mm diameter diamond bur. The root cementum was removed with a help of polishing paper (Ecomet 3, variable- speed grinder-polisher; Buehler) in such a way that outer diameter was 6mm and length of approximately 15 mm. The teeth were then cut into 4 mm blocks and enlarged with a No. 8- ISO 023 round bur (Mani, Japan) at slow speed. The samples were placed under tap water during the procedure in order to avoid dehydration.

All the 200 samples were treated with 5.25% NaOCl and 17% EDTA for 4 min each and then placed in ultrasonic bath for 10 min to remove smear layer. The tooth blocks were autoclaved for 15 min at 121oC to produce sterilized samples. Following this the sterile samples were placed in a yeast extracted-glucose broth (Yeast Extracted Oxoid, Hampshire, England, glucose 10 g/L) and incubated for 24 h at 37oC.
The experiment was carried out at 25oC, 45oC and 60oC in a water bath and checked with a thermometer (Checktemp Hanna Nord EST SRL Baranzate, MI, Italy). The specimens were randomly divided into groups:

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Group I (n=20): 5.25% NaOCl at 25oC
Group II (n=20): 2% CHX at 25oC
Group III (n=20): 17% EDTA at 25oC
Group IV (n=20): 5.25% NaOCl at 45oC
Group V (n=20): 2% CHX at 45oC
Group VI (n=20): 17% EDTA at 45oC
Group VII (n=20): 5.25% NaOCl at 60oC
Group VIII (n=20): 2% CHX at 60oC
Group IX (n=20): 17% EDTA at 60oC
Group X (n=20): Sterile dentin tubes

The test organism used for the study was Enterococcus faecalis (ATCC 29212). Stationary phase E. faecalis cells were cultured in YG Broth for 48 h at 37oC from all the groups stored at 70oC. The infectious tubules were evaluated upto 28 days under scanning electron microscope. The bacterial growth was verified by Brown and Brenn staining method histologically. The samples were removed from the broth, irrigated with 3 ml of sterile saline solution and dried with bigger size paper points. The samples were then placed over sticky wax and fixed over a cell culture plates which also closed the apical end of the root. Finally, the irrigating solutions were introduced (according to the groups) filling the whole canal. After 10 min solutions were removed from the canals with the help of paper points. The test blocks were incubated for 28 days at 37oC to maintain a humid environment. Dentin chips were collected in a test tube with help of round bur size ISO 025. The samples were cultured on tryptic soy agar (Difco) for 48 h at 37oC, and bacterial colonies were counted and recorded as colony-forming units (CFU).

Statistical Analysis:

Descriptive and comparative statistics were performed using IBM SPSS v21. Differences among the groups were analysed by Analysis of variance (ANOVA) tests. P value <0.05 was considered statistically significant for all tests. Variables were expressed as means ± standard deviation. Tukey's multiple post hoc test was used for comparisons among the groups.

RESULTS

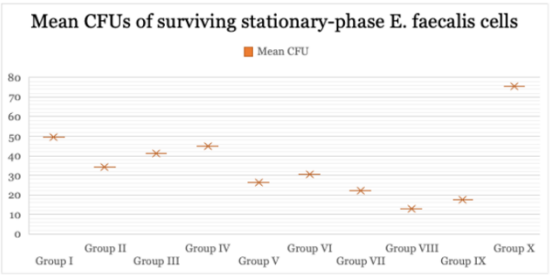

Figure 1: Variation of antibacterial effects for each irrigating solution at different temperatures.

Table 1: Descriptive statistics of the data obtained for each irrigating solution at different temperature.

Groups	N	Mean CFU	Standard Deviation	Standard Error
Group I5.25% NaOCl at 25oC	20	49.514	6.2313	1.3934
Group II2% CHX at 25oC	20	34.089	3.6002	0.805

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Group III17% EDTA at 25oC	20	41.2915	1.7964	0.4017
Group IV5.25% NaOCl at 40oC	20	44.691	5.0754	1.1349
Group V2% CHX at 45oC	20	26.512	2.8817	0.6444
Group VI17% EDTA at 45oC	20	30.415	2.3356	0.5222
Group VII5.25% NaOCl at 60oC	20	22.3915	1.6103	0.3601
Group VIII2% CHX at 60oC	20	12.779	1.0336	0.2311
Group IX17% EDTA at 60oC	20	17.4775	1.5993	0.3576
Group XNo treatment done	20	75.3445	6.5205	1.458

The data shows that colony- forming units (CFU) for each irrigant at three different temperatures. ANOVA showed that no significant difference was seen between 5.25% NaOCl, 2% CHX and 17% EDTA at temperature of 25oC ($p<0.05$). As the temperature a significant difference was seen in CFUs of various irrigants [Table 1]. The maximum number of CFUs was seen in NaOCl (49.514 at 20oC), thus meaning lower antimicrobial efficacy. The least amount of mean bacterial content was seen for 2% chlorohexidine (12.779 at 60OC).

Tukey test showed significant difference between 5.25% NaOCl at 25oC and 45oC, 2% CHX 25oC and 17% EDTA 45oC ($p>0.05$). All the irrigating solutions showed a significant reduction in the CFU numbers over a rise in temperature [Figure 1].

DISCUSSION

Endodontic irrigants have many properties like removal of inorganic debris accumulated during instrumentation, organic tissue dissolving property, antimicrobial activity etc.[14] Three most commonly used endodontic irrigants were compared in this in vitro study.

Due to the tissue dissolution ability, NaOCl is the one of the most commonly used endodontic irrigant.[15] Another irrigant used in this study was Chlorhexidine gluconate which is a potent antimicrobial agent, mainly against E.faecalis.[10] It is most commonly used in endodontic diseases like apical periodontitis.[11] One more irrigant used was 17% EDTA which has a ability to reduce the inorganic components of smear layer.[12] EDTA has a property to react with calcium in the tooth dentin and cause calcium chelates.[13]

Nowadays the endodontic irrigants are used in combinations like NaOCl and EDTA. EDTA acts effectively in removing smear layer when used along NaOCl. The apical third of the canal sometimes not thoroughly irrigated due to the narrow endodontic

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space which inhibits fluid movement as compared to the middle and coronal thirds.[16] However, combination of CHX and NaOCl produces pigmentation and discoloration of the teeth.[17] It is also said to produce cytotoxic products.[18]

One of the best method to increase the antibacterial activity of the root canal irrigants is heating. The results of current study showed that all the three irrigants (NaOCl, CHX and EDTA) revealed lower CFU counts at increasing temperatures of 45oC and 60oC. This may be due to the temperature rise as well as the surfactants. Various studies have evaluated the effect of heating on antimicrobial efficacy of NaOCl.[15,19] A study conducted by Mohammadi et al,[20] concluded that NaOClirrigant is most effective against microbes like E. faecalis, Candida albicans, Actinomycesisraelli, Lactobacillus casei and Pseudomonas aeruginosa. In 1936, it was noted that increased temperature of NaOCl killed Mycobacterium tuberculosis at a faster rate.[21] On heating the root canal irrigants there is a thermal agitation of the molecules, which in turn increases the flow of the irrigating solution. Also there is an double increase in antimicrobial activity with every 5oC elevation in temperature.[22] Antiseptic irrigants such as chlorhexidine gluconate also exhibits increased killing effect of microbes with heating of solution.[23]

The root canal spaces with complex anatomy, which rely primarily on irrigation rather than instrumentation, the preheating of irrigants would prove to be clinically useful.

CONCLUSION

In the conclusion it may be stated that preheating irrigating solutions can be advantageous. It improves the antimicrobial efficacy against stationary phase E. faecalis cells. It was concluded that chlorhexidine showed the best antimicrobial activity at all the temperatures.

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