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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF ANTIBACTERIAL ACTIVITY OF CHLORHEXIDINE, SODIUM HYPOCHLORITE AND NORMAL SALINE WITH PASSIVE ULTRASONIC IRRIGATION AGAINST ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

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ABSTRACT

Introduction: The purpose of the present in vitro study was to compare and evaluate the antimicrobial efficacy of 6% sodium hypochlorite, 2% chlorhexidine and 0.9% normal saline using passive ultrasonic irrigation technique against enterococcus faecalis in root canals.

Material and Method: Total forty extracted human single rooted teeth were selected and divided into experimental and control group. Access cavities were prepared and specimen decoronated to standardized root length. ATCC 35550, MTCC code 4399 of pure culture of Enterococcus Faecalis has been used in this study. All selected cases were inoculated with *E.Faecalis* and subjected to Passive Ultrasonic Irrigation using aforesaid solutions. Four mm of root apex were cut with sterile disc and the cut apical third were dropped directly into Trypton Soya broth agar for 48 hours. Results were subjected to statistical analysis and 6% sodium hypochlorite was found best against *E.Faecalis* when used with passive ultrasonic irrigation.

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INTRODUCTION

Successful endodontics is based on the debridement, disinfection and obturation (Young *et al.,* 2007). Biologically, all irritating agents must be removed from the root canal by chemomechanical preparation without damaging the periapical tissues (Rosenfeld *et al.,* 1978). Sodium hypochlorite (NaOCl) is commonly used as an endodontic irrigant due to its tissue-dissolving and antibacterial properties (Berutti *et al.,* 1997).The 6% concentration of sodium hypochlorite is used in this study. Its disadvantages include toxicity, odor, discoloration, and corrosion of dental equipment. Chlorhexidine has been found to be the most effective antibacterial substance when compared with 5.25% NaOCl, 17% EDTA, $Ca(OH)_2$, H_2O_2, and saline. In addition to its broad spectrum of antimicrobial properties, chlorhexidine has demonstrated substantivity. Irrigation with 2% chlorhexidine has been shown to prevent microbial activity with residual effects for up to 48 h and for at least 7 days (Stuart *et al.,* 2006). In another study, Jeansonne and White found no difference in antimicrobial activity between 2% chlorhexidine and 5.25% NaOCl, but NaOCl has the added advantage of tissue dissolution. Chlorhexidine is an excellent irrigant for NaOCl-allergic patients and teeth with open apices. Normal Saline is a commonly used sterile, nonpyrogenic solution for fluid and electrolyte replenishment. It contains no antimicrobial agents. The pH is 5.0 (4.5 to 7.0). It contains 9 g/L Sodium Chloride with an osmolarity of 308 mOsmol/L. It contains 154 mEq/L Sodium and Chloride.It is indicated as a source of water and electrolytes and widely used in endodontics (Dunavant *et al.,* 2006). The use of ultrasonics has been found to eliminate bacteria from the canal more efficiently than hand instrumentation alone (Jensen *et al.,* 1999). After hand instrumentation, passive activation of sonic or ultrasonic files for 2 min with 6% NaOCl resulted in significantly cleaner canals than with hand instruments alone. Cheung and Stock showed that ultrasonic energy increases the debridement and antimicrobial activities of NaOCl. However, it is not known whether the use of ultrasonics increases the substantive antibacterial properties of chlorhexidine or affects the antibacterial properties of sodium hypochlorite (Cheung and Stock, 1993). The issue of whether sodium hypochlorite demonstrates residual antimicrobial activity has not been addressed. The purpose of this study was to evaluate the effect of passive ultrasonic activation of 2% chlorhexidine, 6%

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NaOCl and 0.9% Normal salineirrigant on residual antimicrobial activity in root canals.

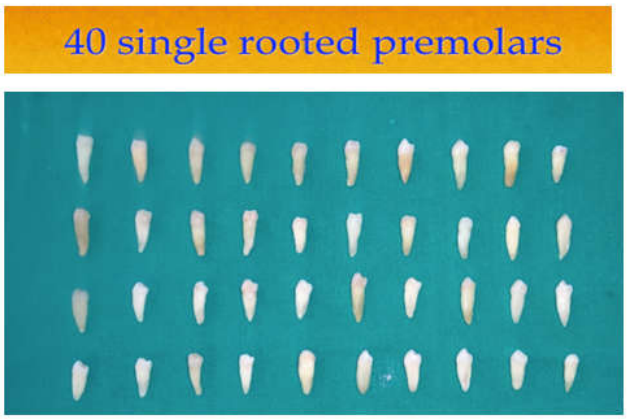
MATERIALS AND METHODS

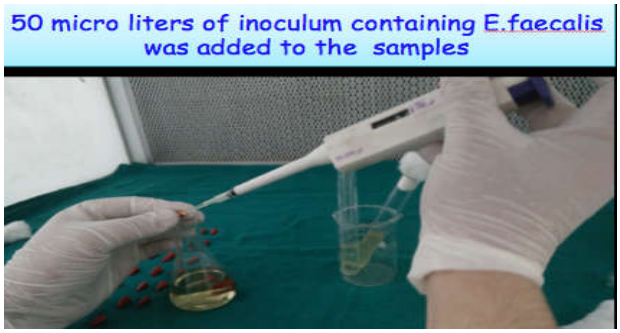
Forty freshly extracted, single-rooted human mandibular premolars with mature apices and curvatures between $0^0$and $10^0$and apical diameter confirming to #15 K-file were selected. Presence of a single canal was confirmed with radiographs. Teeth with calcified canals, canals with large apical foramina, and more than 1 canal were excluded. The teeth were divided into 3 experimental groups (n = 10) and 1 control group (n = 10). The teeth were cleaned of debris and soft tissue and stored in saline solution. Endodontic access cavities were prepared with no. 2 round bur, pulp remnants were extirpated with a fine barbed broach, and working length of the canals was established at 1 mm short of the file penetration length, when the tip of the file was just visible at the apex. Double coat of nail varnish was applied to seal any lateral or accessory canals from which the extrusion of bacteria may occur.

Broth preparation: 3 grams of broth powder (Tryptone Soya Broth) was weighed on weighing balance and added into 100ml of distilled water in the flask. The mouth of the flask was closed with a sterile cotton plug, then covered with aluminium foil followed by wrapping with blotting paper and then tying it with a thread (to prevent contamination). Then it was kept on the hot plate and gently shaken till the media powder got dissolved homogeneously and then the broth was autoclaved at 121^0C at 15psi for 15 min. Preparation of culture**:** Enterococcus faecalis MTCC code 4399 equivalent to ATCC29212, strains obtained from IMTECH, Chandigarh were used in this study. It was grown on Tryptone Soya Agar (TSA) for 72 hours. The culture was suspended in 10ml of Tryptone soya broth and incubated at 37^0C. The turbidity was adjusted to 0.5 McFarland standards.

Standardization of samples: McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. Original McFarland standards were made by mixing specified amounts of barium chloride and sulphuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. Contamination of the specimens**:** 50 micro liters of inoculum containing *E.Faecalis* was added to the samples. The samples were placed in incubator at 37^0C for 21 days. Canals were replenished with fresh bacterial suspension every 48 hrs.

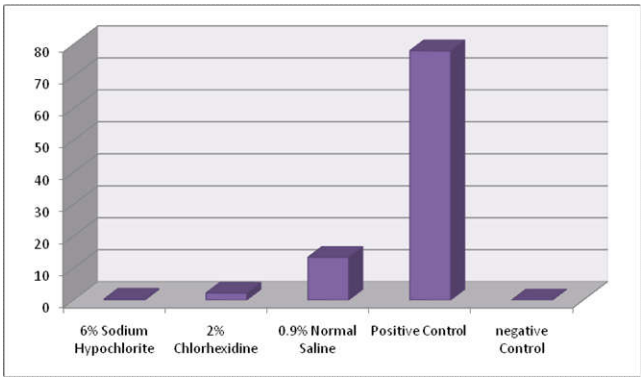
Group 1 is the group in which the antimicrobial action of 2% Chlorhexidine was evaluated using Passive ultrasonic irrigation. Ten samples after incubation for 21 days were subjected to passive ultrasonic irrigation using chlorhexidine. Canals were irrigated with 1ml, 2% chlorhexidine (NeelKanth) for 30 sec and activated with irrisafe tip size 20 length 21mm for 1 min scale power adjusted to 2 (Biosonic S1 model, Coltene, Whaledent), after 30 sec 1ml irrigant was renewed and again passive ultrasonic irrigation was done for 1 min.
Group 2 is the group in which the antimicrobial action of 6% Sodium Hypochlorite was evaluated using Passive ultrasonic irrigation. Ten samples after incubation for 21 days were subjected to passive ultrasonic irrigation using NaoCl. Canals were irrigated with 1ml, 6% Sodium Hypochlorite for 30 sec and activated with irrisafe tip size 20 length 21mm for 1 min





CFU ml^{-1}count

	1	2	3	4	5	6	7	8	9	10
6% Naocl	0.9	0.2	0.6	0.2	0.1	0.3	0.6	0.4	0.1	0.2
2% Chx	1.5	1.4	2.6	1.8	2.6	2.8	1.4	3.4	1.6	2.4
0.9% Nacl	12.2	13.6	16.4	11.9	10.4	14.3	12.9	15.4	13.4	14.2



scale power adjusted to 2 (Biosonic S1 model, Coltene, Whaledent), after 30 sec 1ml irrigant was renewed and again passive ultrasonic irrigation was done for 1 min. *Group 3* is the group in which the antimicrobial action of 0.9% Sodium chloride was evaluated using Passive ultrasonic irrigation. Ten samples after incubation for 21 days were subjected to passive ultrasonic irrigation using NaoCl. Canals were irrigated with 1ml, 0.9% Sodium chloride for 30 sec and activated with irrisafe tip size 20 length 21mm for 1 min scale power adjusted to 2 (Biosonic S1 model, Coltene, Whaledent), after 30 sec 1ml irrigant was renewed and again passive ultrasonic irrigation was done for 1 min.

Group 4 is the positive group having maximum number of colony forming units. The sample size was 5 in this group. The colony forming units were calculated after 21 days of incubation of the prepared samples and ranged from $69x10^8$ to $88x10^8$ with a mean of $78x10^8$.

Group 5 is the negative group having sterile root sections. The sample size was 5 in this group. The samples were double autoclaved and hence the bacterial count as suggested by the Life sciences associates was set at 0. This is in general standards to the studies carried out concerning microbes. Apical 4mm of all the root samples were cut and dropped in the tryptone soya broth agar and incubated for 24 hrs at 37^0 C. Colony forming units were obtained.

Counting of colony forming units: Colony Forming Unit (CFU or cfu) is a measure of viable bacterial or fungal cells. In direct microscopic counts (cell counting using haemocytometer) where all cells, dead and living, are counted, but CFU measures only viable cells. For convenience the results are given as CFU/mL (colony-forming units per milliliter) for liquids, and CFU/g (colony-forming units per gram) for solids. CFU can be calculated using Miles and Misra method, it is useful to determine the microbiological load and magnitude of infection in blood and other samples. Result shows that 6% NaOCl when used with Passive ultrasonic irrigation is statistically significant than normal saline and positive control group, 2% Chlorhexidine is less but not significant than hypochlorite group however it is significant than normal saline.

DISCUSSION

Endodontic irrigants permeate throughout dentinal tubules, but their effectiveness is dependent on the type of bacteria found within the tubules (Harrion *et al.,* 2010) E. faecalis was chosen because of its ability to penetrate dentinal tubules and colonize the root canal system (Siqueira *et al.,* 2005). Enterococcus faecalis is a microorganism commonly detected in asymptomatic, persistent endodontic infections and is major causative in the failure of root canal treatment (George *et al.,* 2005). Its prevalence in such infections ranges from 24% to 77%. Enterococcus faecalis is an anaerobic facultative microorganism that is highly resistant to conventional chemo mechanical preparation and is usually found in cases of failure of root canal treatment. This microorganism has several virulence factors and is able to withstand prolonged periods of nutrient limitation, persisting as a pathogen in the root canal.
Several endodontic irrigants have been used in endodontic therapy in order to promote an adequate decontamination of the root canal system (Haapasalo *et al.,* 2014). This study indicates that 6% NaOCl when used with Passive ultrasonic irrigationis statistically significant than normal saline and positive control group, Sodium hypochlorite (NaOCl) has been widely used as an endodontic irrigant for effective bactericidal and nonspecific proteolytic activity and is strongly alkaline and hypertonic. The rise in temperature and concentration of 6% when used in conjugation with PUI increases the penetration ability of Sodium hypochlorite which facilitates the removal of deep seated E faecalis which can penetrate to a depth of 1200 micrometers in dentinal tubules.

It is known to dissolve organic tissues containing fatty acids and lipids via a saponification reaction however 2% Chlorhexidine is less but not significant than hypochlorite group.2% Chlorhexidine shows a broad antimicrobial spectrum and substantivity, but this chemical auxiliary substance is not able to promote the dissolution of organic tissues that promotes decontamination of the root canal system with no damage to the tissues involved. The use of passive ultrasonic irrigation as an auxiliary resource in endodontic therapy has been suggested as an alternative to increase cleaning and disinfection of the root canal (Spoleti *et al.,* 2003). Ultrasonic energy passes through the irrigating solution and exerts its acoustic streaming or scrubbing effect on the canal wall (Siqueira, 2012). This study used passive ultrasonic activation for 1 min. Use of normal saline with pui decreases the bacterial count but this can be attributed to the action of pui through its mechanism of cavitation and acoustic streaming (Spoleti *et al.,* 2003; Thomas *et al.,* 2014; Sabins *et al.,* 2003). Normal saline does not exert any tissue dissolution nor bacteriostatic or bactericidal action, it only provides hydration and electrolytes. Previous studies have shown that the use of passive ultrasonic irrigation associated with endodontic irrigants provides a higher elimination of micro- organisms from the root canal space (Mozo *et al.,* 2012; Guneser *et al.,* 2015; Gheorghe *et al.,* 2010).

Conclusion

Within the limitations of this study it is concluded that 6% Sodium Hypochlorite is better than 2% chlorhexidine which is better than 0.9% normal saline when used with Passive ultrasonic irrigation in eliminating *E.Faecalis* from the root canal.

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