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"ANTIMICROBIAL SUSCEPTIBILITY OF ASSISTING CHEMICAL SUBSTANCES ON ENTEROCOCCUS FAECALIS AND S. MUTANS IN ROOT CANALS"

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ABSTRACT

The anatomic complexity of the root canal system, organic tissues and some bacteria such as Enterococcus faecalis, which are commonly, located in dentinal tubules as well as in isthmus and ramification areas. This microorganism was chosen because of its ability to penetrate dentinal tubules and colonize the root canal system. The purpose of this study was to compare in vitro the effectiveness of chlorhexidine digluconate and sodium hypochlorite (NaOCl) associated with root canals of human teeth infected with Enterococcus faecalis and S. mutans. Freshly extracted human tooth's [n=40] were taken and preserved in formalin and solution was changed after 72 hours. E. faecalis and S. mutans were cultured in blood agar and brain heart infusion medium. Chlorhexidine digluconate 0.2-2%, NAOCL 5.25%, was used to irrigate canals. Chlorhexidine digluconate showed better result against E. faecalis.

INTRODUCTION:

Microorganisms play an important role in the etiology factor of pulp and peri-apical diseases (Kakehashi et al. 1965; Sundqvist, G, 1992) and Elimination of microorganisms from infected root canals is a difficult task. Numerous measures have been described to reduce the numbers of root canal microorganisms, including the use of various instrumentation techniques, irrigation regimens and intra-canal medicaments. There is no evidence in the literature to show that mechanical instrumentation alone results in a bacteria-free root canal system (Bystrom A, Sundqvist G, 1981). Considering the complex anatomy of the root canal pulp space (Hess, W. 1925; Peters OA, Laib A, Gohring TN et al, 2001) this is not surprising. It is assumed, but not demonstrated, that any pulp tissue left in the root canals can serve as bacterial nutrient. Furthermore, tissue remnants also impede the antimicrobial effects of root canal irrigants and medicaments. Therefore some sort of irrigation / disinfection is necessary to remove tissue from the root canals and to kill microorganisms. Simply, chemical treatment of the root canal can be arbitrarily divided into irrigants, rinses, and inter-visit medicaments. The ability to dissolve pulp especially from inaccessible areas such as fins and isthmuses is important because the presence of infected tissue within root canals produces persistent periradicular inflammation (Moller et al. 1981). The ability of alkaline solutions of hypochlorite to rapidly oxidize, decarboxylate, and deaminate primary and secondary alpha amino acids has been shown (Langheld, K., 1909 & Crane, AB., 1920) and the antimicrobial efficacy of sodium hypochlorite (NaOCl) is well recognized (Shih, et al. 1970; Zehnder et al. 2002).

2% Chlorhexidine gluconate (CHX) has been suggested as an alternative irrigating solution that could replace NaOCl. CHX is a cationic bisguanide that seems to act by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components. At low concentration, CHX has a bacteriostatic effect and at high concentration it has a bactericidal effect because of precipitation and/or coagulation of intracellular constituents (McDonell G, Russell AD, 1999). Its optimal antimicrobial activity is at pH 5.5-7.0 (Russell AD, Day MJ, 1993). CHX has a broad spectrum antimicrobial activity, targeting both gram positive and gram-negative microbes (Delany et al. 1982). In general, in vitro studies suggested that CHX and NaOCl have comparable antibacterial effect when used in similar concentrations (Jeanssone MJ, White RR, 1994). In addition, CHX appeared to be a promising agent as a final irrigant. In a clinical study, (Zamany et al., 2003) showed that a 2% CHX solution, used as a final irrigant, significantly decreased bacterial loads in root canals that had been irrigated with sodium hypochlorite during canal preparation. Additional advantages of CHX are its retentive character in root canal dentin, (Rosenthal et al. 2004) its relatively low toxicity, (Filho et al. 2002) more tolerable odor and taste and non bleaching effect. Despite its advantages, CHX activity is pH dependent and is greatly reduced in the presence of organic matter. Unlike sodium hypochlorite, it lacks tissue dissolving properties (Naenni N, Thoma K, Zehnder M. 2004).

This study aimed to evaluate the in-vitro antimicrobial effectiveness of intracanal irrigants (2% chlorhexidine & 5.25% NaOCl) used in endodontic therapy on the S. mutans and E. faecalis.

MATERIALS AND METHODS

Sample collection and preparation: The samples used on this study were collected from the root canals of a patient diagnosed with dental caries by a physician. The patient was receiving treatment at the outpatient clinic, Faculty of Dental Medicine, Vananchal Dental College & Hospital (Nilamber Pitamber University) Garhwa, Jharkhand. The infected area of the root canals was swabbed with sterile cotton wool and saliva transferred to a sterile screw capped tube that contained 5.0 ml of Reduced Transport Fluid (RTF) with aseptic precautions, vortex mixed for 1 minute, to disperse the bacteria. A loopful of dispersed samples was inoculated on various media.

Isolation and characterization of *Bacteria*

The samples were collected immediately streaked on Brain Heart Infusion (BHI), Bile Esculin Agar and Blood agar, in an inhibitory media incubated at 37°C for 24, 48 and 72 h. Characteristic colonies were picked from the plates and purified by repeated sub-culturing. S. faecalis and S. mutans was identified using cultural, morphological and bio-chemical characteristics basis.

Microbiological analysis

(Delany et al. 1982) evaluated 0.2% CHX-gluconate in infected root canals. Bacteriologic samples were obtained from immediately after 24 h and instrumentation, irrigation and medication either with CHX-gluconate or with sterile saline. There was a highly significant reduction in the number of microorganisms in the CHX-treated specimens after instrumentation and irrigation. (Basson & Tait, 2001) compared the effectiveness of NaOCl and a CHX solution in disinfecting root canal systems that were infected with E. faecalis and S. mutans. (Oncag˘ et al. 2003) evaluated the antibacterial properties of 5.25% sodium hypochlorite (NaOCl), 2% CHX, after 5 min and 48 h in extracted human teeth after the canals had been infected by Enterococcus faecalis. The 2% CHX, were significantly more effective against E. faecalis than the 5.25% NaOCl at both time

periods. Two studies (Gomes et al. 2001, Vianna et al. 2004) have investigated the antimicrobial activity against endodontic pathogens of one concentration (2%) of CHX and compared them with one concentrations of NaOCl (5.25%).

RESULTS

None of the patients developed severe swelling, pain or other side effects necessitating removal from the study. Table 1 and 2, shows the mean growth of S. mutans and E. faecalis after treatment with irrigant solutions. When the mean value at individual period (24 hours, 4th and 7th days) were compared between the two groups, only at 4th days postoperatively, statistically significant difference was observed CHX 2% (M= 7.4) and more associated with NaOCl group (M= 4.2). Kirby-Bauer test for repeated measures showed that there was a highly significant change (M= 6.6 and 3.9) in the mean value over time for both the groups.

Table: 1. S. mutans, n=20 (2% CHX and 5.25% NaOCl)

S.MUTANS [n=20]	CHLORHEXIDINE DIGLUCONATE2 % [n=10]	Mean value	SODIUM HYPOCHLORITE 5.25%[n=10]	Mean value
1.	7mm	7.4	5mm	4.2
2.	8mm		6mm	
3.	6mm		6mm	
4.	9mm		4mm	
5.	6mm		4mm	
6.	7mm		5mm	
7.	8mm		2mm	
8.	8mm		3mm	
9.	7mm		4mm	
10.	8mm		3mm	

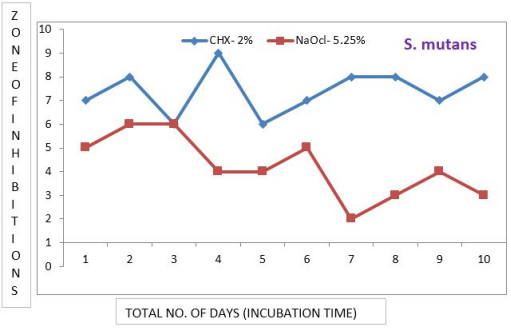
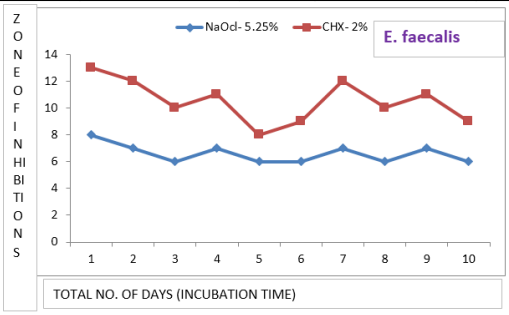


Table: 2 E. faecalis, n=20 (2% CHX and 5.25% NaOCl)

E.FAECALIS [n=20]	CHLORHEXIDINE DIGLUCONATE 2% [n=10]	Mean value	SODIUM HYPOCHLORITE 5.25%[n=10]	Mean value
1.	8mm	6.6	5mm	3.9
2.	7mm		5mm	
3.	6mm		4mm	
4.	7mm		4mm	
5.	6mm		2mm	
6.	6mm		3mm	
7.	7mm		5mm	
8.	6mm		4mm	
9.	7mm		4mm	
10.	6mm		3mm	



DISCUSSION

Complete preparation in the root canal space is one of the important stages in endodontic treatment. Irrigation is a necessary and important phase of cleaning the canal, but it leads to extrusion. Therefore, it is logical to assume that root canal irrigant be nontoxic and biocompatible with its surrounding host tissues so that it does not contribute to postoperative discomfort. However, all the currently available antimicrobial materials for irrigation have some limitations and search continues for the ideal irrigant.

Bacteria in the main root canals and the most superficial layers of an infected root canal wall may be detached by instrumentation and irrigation, whereas biofilms deeper in dentinal tubules are better protected and harder to eradicate. In vitro models of infected dentinal tubules are to study the effect of various treatment procedures on bacteria in dentin. It has been proved that in necrotic cases, the irrigant may go beyond the instrumented area, (Salzgaber RM, Brilliant JD, 1977) whereas in vital cases irrigant is forced only in the space created by instrumentation. Forced irrigation of sodium hypochlorite beyond the apex of the tooth can cause violent tissue reactions and intolerable pain. In one of the studies, (Southward et al, 1989), 2% chlorhexidine was used for sub gingival irrigation and it was not toxic to the periodontal tissues at this concentration. In a retrospective study, the flare-up rate was evaluated in patients treated in multiple visits and it was concluded that a positive correlation was present between flare-ups and teeth with necrotic pulp (Mor C, Rotstei I, Friedman S, 1992). On the contrary (Segura-Egea et al. 2009) demonstrated that root canal treatment in teeth with irreversible pulpitis and acute apical periodontitis was significantly more painful than that in teeth with necrotic pulp and chronic apical periodontitis. However (Harrison et al. 1983) reported no association with inter-appointment or post root canal treatment obturation pain and tooth diagnosis.

Within the limitations of this study, significant difference in post-operative pain was observed between 2% chlorhexidine and 5.25% sodium hypochlorite solutions only at 24 hours post-operatively, but with caution they should be considered for application or practice. Even though the pain was more at 24 hour postoperatively in 5.25% NaOCl group, its efficiency cannot be questioned because of its important property like tissue dissolving, and hence, the long-term results of root canal treatment. Further studies are needed to compare the level of postoperative pain caused by 2% chlorhexidine solution and 5.25% NaOCl in teeth with incomplete root apices where there are more chances of periapical extrusion of irrigants. Microbiological assessment of the root canals following these two irrigants should also be done to know the role of microorganisms in causing endodontic post treatment pain. The contribution of uneven distribution of pulp and periradicular conditions in both the groups to the overall results could not be investigated in this study and needs further investigation.

CONCLUSION

2% CHX solution was the more effective irrigant solution than 5.25% NaOCl against E. faecalis and S. mutans. Significant difference in the pain level was observed between 2% chlorhexidine and 5.25% sodium hypochlorite only at 24 hour postoperatively. Pain was present more in sodium hypochlorite group compared to chlorhexidine group. CHX had a wide range of activity against both Gram positive and Gram negative bacteria. The effect of CHX on microbial biofilms is significantly less than that of NaOCl. Dentine, dentine components (HA and collagen), killed microorganisms and inflammatory exudates in the root canal system may reduce or inhibit the antibacterial activity of CHX.

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