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ANTI-MICROBIAL ACTIVITY OF IRRIGANTS ON ENTEROCOCCUS FAECALIS: IN-VITRO ANALYSIS

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ABSTARCT:
INTRODUCTION: The contribution of microorganisms to the development of pulpal and periapical disease has been well documented. The bacteria associated with primary endodontic infections are mixed but are predominantly gram-negative anaerobic rods, whereas the bacteria associated with secondary infection comprise only one or a few bacterial species-the most important of which is Enterococcus faecalis. The purpose of this study was to compare and evaluate the antimicrobial efficacy of ChloroQuick with Sodium Hypochlorite and 2% Chlorhexidine gluconate on E.faecalis.

MATERIALS AND METHODS: Thirty extracted human lower bicuspids with a single root canal, checked by radiographs were selected. E. faecalis suspensions were injected into the canals of all teeth using sterile insulin syringes under aseptic conditions. In group 1, 10 root canals were irrigated with 0.9% NaOCl In group 2, 10 root canals were irrigated with ChloroQuick. In group 3, 10 root canals were irrigated with 2% CHX. Sterile paper points were selected to sample the bacteria from root canals after cleaning and shaping the root canal. The paper points were left in the canal for 1 minute and the samples were transported into plates containing TSA with 5% sheep blood and placed in an incubator at 37 degree celsius for 48 hours. The samples were retrieved from the incubator after 48 hours to be evaluated bacteriologically as to the presence of microorganisms and observation of the colonies formed.

RESULTS*: Bacterial growth was seen only in one sample of ChloroQuick, but in 3 of CHX group and 2 of NaOCl group. Chi-Square test showed no statistical difference between groups.*

CONCLUSION: *Based on the results of this study, it seems that all three solutions have acceptable antimicrobial effect on E. faecalis.*

Keywords: *Antimicrobial, Chlorhexidine Gluconate, ChloroQuick, Enterococcus faecalis, Sodium Hypochlorite*

I. INTRODUCTION:

It is well recognized that the primary aim of the treatment of periapical disease consists of eradication of polymicrobial infections from the involved root canal system. Numerous clinical studies have sought to evaluate the antimicrobial effectiveness of treatment strategies. Mechanical instrumentation alone appears not to reduce the bacterial load effectively or permanently. The use of antimicrobial agents as adjuncts for irrigation and medication of root canals has been shown to help reduce the bacterial counts further. [1] These studies also demonstrate that despite the use of such antimicrobial agents, bacteria may still persist. The genera most frequently implicated as persistent are streptococci, enterococci, staphyloccocci, fusobacteria, peptostreptococci and lactobacilli.

Among the procedures involved in the control of endodontic infection, irrigation can play an important role in the elimination of microorganisms from the root canal. Irrigants are used during the endodontic treatment to flush out loose debris, to lubricate the dentinal walls, to dissolve organic matter in the canal, and to be antimicrobial. [2]

Enterococcus faecalis is more likely to be found in failed cases. The genus Enterococcus includes more than 17 species, although only a few cause clinical infections in humans. Since the beginning of the antibiotic era, they

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have posed major therapeutic challenges. These species are facultative anaerobic microorganisms that grow in high salt concentrations. [3] Enterococcus faecalis and Enterococcus faecium are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates. Enterococcus faecalis is non motile and resists detergents , bile salts , heavy metals ,ethanol and azides. E. faecalis is resistant to commonly used anti microbial agents such as cephalosporins. [4]

Regardless of the NaOCl concentration used as irrigant, studies have demonstrated that microorganisms may survive the effects of mechanical preparation. Remaining microorganisms can jeopardize the outcome of the endodontic therapy. If they gain access to the periradicular tissues, treatment can inevitably result in failure. Other irrigants and different strategies have been recommended to enhance both the cleaning and antimicrobial capabilities of irrigation. [5] For instance, the combination of NaOCl with other substances, such as hydrogen peroxide, citric acid, or EDTA has been a commonly recommended strategy.

The biocompatibility problems associated with the use of concentrated NaOCl have led to the use of substances with known antimicrobial properties and less toxicity, such as chlorhexidine. Chlorhexidine is an effective antiplaque agent and is routinely used in periodontal therapy and caries prevention. [4][6] Its effectiveness against Streptococcus mutans and Lactobacillus is the basis for its use in caries prevention. Chlorhexidine gluconate is a cationic molecule that acts by adsorbing onto the cell wall of the microorganism, disrupting the integrity of the cytoplasmic membrane and causing leakage of the intracellular components. [7]

Newer instruments and techniques have also been purposed for root canal preparation. Rotary nickel-titanium instruments with increased tapers and different designs have been recently developed. Although it has been demonstrated that these newer instruments and techniques improve the shaping of the root canal, few studies have evaluated their capability in eliminating the root canal infection. [8]

E. faecalis is completely resistant to intra-canal medications and is one of the microorganisms which resist against antimicrobial properties of calcium hydroxide and is able to survive in root canals without synergistic effect of other bacteria. Different kinds of antimicrobial rinses have been introduced for disinfecting the root canal system, all these solutions have disadvantages such as limited antimicrobial activity, non-selectivity for host cells, inability to penetrate into dentinal tubules and a risk of allergy and toxicity in patients. [3][9]

Different irrigation regimens have been proposed to enhance the effectiveness of NaOCl in disinfecting the root canal system. Grossman (1943) suggested the alternate use of NaOCl and hydrogen peroxide (H2O2) for the irrigation of the root canal. This association caused effervescence, which may improve the debridement and disinfection of the root canal. Martin (1976) has proposed irrigation with NaOCl solution during ultrasonic instrumentation of the root canal system. He claimed that ultrasonic waves accelerate chemical reactions and potentiate the bactericidal efficiency of NaOCl. Studies have demonstrated that ultrasonication of NaOCl solution increases its cleaning and antibacterial effects. [5][10]

The purpose of this study was to compare and evaluate the in-vitro intracanal bacterial reduction produced by using ChloroQuick with NaOCl and CHX.

II. MATERIALS AND METHODS:

Thirty extracted human lower bicuspids with a single root canal, checked by radiographs, were selected for this study. Conventional access preparations were made. Working length was established at the apical foramen. To make both handling and identification easier, the teeth were numbered 1-30. The root canals were divided into three experimental groups based on the irrigation method used.
To induce controlled and standard infections, pure E. faecalis suspensions were injected into the canals of all teeth using sterile insulin syringes under aseptic conditions.

In group 1, 10 root canals were irrigated with 0.9% NaOCl during instrumentation. In group 2, 10 root canals were irrigated with CHX. In group 3, 10 root canals were irrigated with ChloroQuick. Irrigant was delivered into the canals by using a 3-ml plastic syringe with a 23-gauge needle. Each set of instruments was used to prepare no more than three root canals.

Group 1 – 10 root canals were irrigated with 2ml of 0.9% NaOCl solution. The solution was agitated by hand with a size 40 k-type file and left in the root canal for 5 minutes.
Group 2 - 10 root canals were irrigated with 2ml 2% CHX solution. The solution was agitated by hand with a size 40 k-type file and left in the root canal for 5 minutes.
Group 3 - 10 root canals were irrigated with 2ml of ChloroQuick solution. The solution was agitated by hand with a size 40 k-type file and left in the root canal for 5 minutes.

Sterile paper points were selected to sample the bacteria from root canals after cleaning and shaping the root canal. The paper points were left in the canal for 1 minute and the samples were transported into plates containing TSA with 5% sheep blood and placed in an incubator at 37 degree celsius for 48 hours.
The samples were retrieved from the incubator after 48 hours to be evaluated bacteriologically as to the presence of microorganisms, their exact number in the liquid culture media and the observation of colonies formed. Clear test tubes demonstrated complete sterilization and blurred test tubes were considered contaminated samples. Samples were taken from the contaminated specimens using standard 0.001 aluminum loops and cultured in Bile-Esculin Agar (BEA), which is a specific culture medium for E. Faecalis.
Data obtained were analysed statistically for differences using the chi-squared test.

III. RESULTS:

	NaOCl	CHX	CHLOROQUICK
GROWTH	2 (20%)	3 (30%)	1 (10%)
NO GROWTH	8 (80%)	7 (70%)	9 (90%)

In the NaOCl group, in 2 samples bacterial growth was observed. Bacterial growth was present in 3 samples in CHX group and in 1 sample in ChloroQuick group. In all bacterial growth samples, colony count was in excess of 10^5 CFU/ml.

SPSS software were used for the statistical analysis of the results. Quantitative analysis was not performed since the colony counts were in excess of 10^5 CFU/ml.

IV. DISCUSSION:

The pathogenicity of E. faecalis in causing persistent periapical lesions and root canal failure is well recognized. In the present study, an E. faecalis–infected root canal biofilm model was established to investigate the bacterial removal efficiency of different final irrigation regimens in human root canals. The antimicrobial abilities of ChloroQuick, NaOCl and CHX were compared. To date, a thorough eradication of E. faecalis biofilm in root canals cannot be achieved with a single irrigating solution. The combined use of different irrigants is therefore necessary to enhance antimicrobial effectiveness. [11][12]

NaOCl is a commonly used intra-canal irrigating solution and its antibacterial properties are attributed to hypochlorous acid. Sjögren et al. demonstrated that approximately 40% of the canals remain contaminated subsequent to debridement with 2.5% NaOCl. According to a study by Shuping et al. up to 30% of the canals are contaminated after being irrigated with 1.25% NaOCl. Siqueira et al. demonstrated that the rate of canal contamination subsequent to the use of NaOCl is 30-40%. [6][13][14]

Chlorhexidine is a broad-spectrum cationic bisguanide with antimicrobial effects on gram-positive and gram-negative bacteria. Contrary to NaOCl, CHX preserves its antimicrobial effect for some time after being used, but it is unable to dissolve tissues. [15] According to Schafer and Bossman, 2% CHX is more effective compared to its lower concentrations, manifesting its influence in a shorter period of time and with a proper antimicrobial influence on E. faecalis. [16][17] Therefore, 2% CHX was used in the present study. Ercan et al. demonstrated that 2% CHX inhibits E. faecalis in 80% of the cases. [18]

ChloroQuick is a combination solution of stabilized Sodium Hypochlorite solution with buffer and HEDP with detergent and system activator along with other excepients. The freshly mixed solution has advantages over using multiple solutions. Contains 2 Glass Vials of 6 ml each with rubber stopper and punch seal. Vial-A contains Sodium Hypochlorite and Vial-B contains 1-HYdroxyethane 1,1 Diphosphonic Acid(HEDP), Activator. In the present study, ChloroQuick had not inhibited E. faecalis only in one sample. [19][20] However, no statistically significant differences were observed among these solutions.

V. CONCLUSION:

All three solutions have acceptable antimicrobial effect on E. faecalis in extracted human teeth. Further studies are needed to determine the effect of these findings in clinical settings.

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