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Antimicrobial Efficacy of Various Intracanal Medicaments against *Enterococcus faecalis*

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Abstract:

Introduction: The aim of this study was to evaluate and compare the antimicrobial activity of Calcium hydroxide gel, Chlorhexidine gel, Triple antibiotic paste (TAP), and Double antibiotic paste (DAP) as intracanal medicaments against *Enterococcus faecalis*.

Methodology: Twenty four extracted intact human single rooted teeth were decoronated, and chemo mechanical preparation of the root canal was performed. After sterilization of the samples, they were inoculated with pure culture of *E.faecalis* and incubated. Then, samples were divided randomly into four groups (n=6). Each group was then exposed to various intra canal medicaments, group 1: Calcium hydroxide gel, group 2: Chlorhexidine gel, group 3: Triple Antibiotic Paste, group 4: Double Antibiotic Paste. The antimicrobial effectiveness of the various intracanal medicaments was recorded at the end of 14 days. The data was statistically analyzed using one-way analysis of variance test.

Result: The %RCC was highest for triple antibiotic paste showing 78.9% on day 14 followed by double antibiotic paste showing 54.3%, and Chlorhexidine gel showing 52.8%. Calcium hydroxide showed 20.8%.

Conclusion: Triple antibiotic paste was more effective than Double antibiotic paste, Chlorhexidine gel, and Calcium hydroxide against *E. Faecalis* at the end of 14 days.

Key Words: Calcium hydroxide, Chlrohexidine, TAP, DAP, *Enterococcus faecalis*.

INTRODUCTION:

The main objective of root canal treatment is to eradicate the microorganisms from the root canal system. The chemo-mechanical method of root canal treatment does reduces the number of bacteria, to increase the effectiveness of the method, intracanal medicaments are required to maximize the disinfection of the root canal. A wide range of intra canal medicaments have been used, a few which are common in use they are, Calcium hydroxide paste, Chlorhexidine gel, Triple antibiotic paste, and Double antibiotic paste.

Calcium hydroxide plays an important role in endodontics through its ability to induce hard tissue formation, its moderate antibacterial action and its tissue dissolving capability(1). In addition, Ca(OH)2 –Based on intracanal medicaments maintain a high pH, which is an important factor to prevent or inhibit inflammatory root resorption(2).

Chlorhexidine gluconate is used in endodontics as both irrigant and intracanal medicaments (3). Chlorhexidine has inhibitory effects on bacteria commonly found in endodontic infections (4) acting against Gram-positive and Gram-negative microorganisms (5). One of the mechanisms that explains its efficacy is based on the interaction between the positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall, which allows the chlrohexidine molecule to penetrate into the bacteria with toxic effects (2).

Triple antibiotic paste (TAP) containing metronidazole, ciprofloxacin, and minocycline has been reported to be a successful regimen in controlling the root canal pathogen and in managing non-vital young permanent tooth (6). The disadvantage of this mixture is the discoloration caused by minocycline present in it (7).Double antibiotic paste (DAP) containing metronidazole and ciprofloxacin. The purpose of this research is to check the reduction in bacterial count when the antibiotic pastes were used as intracanal medicaments.

MATERIALS AND METHOD:

Preparation of sample:

Twenty six freshly extracted single rooted teeth were collected, and cleaned with hydrogen peroxide. All the teeth were decoronated 2 to 3mm below the cemento-enamel junction with an diamond disk. The root canal shaping was done using M2 files (15-40) and was irrigated using saline. During the process of cleaning and shaping, each tooth was irrigated with 20ml of sodium hypo chlorite for 10 minutes to remove the inorganic and organic debris. The samples were autoclaved and incubated after this step. The apical end of the tooth were sealed with araldite to prevent bacterial leakage, the other end of the tooth was attached to a thin tube in which the bio film was placed.

Contamination and growth of bacteria:

E.faecalis was cultured in brain heart infusion broth. Each sample was contaminated with *E.faecalis* and was incubated at 37degree C for 21 days, specimens were verified regularly. Three days once the broth containing *E.faecalis* was changed. After incubation period, each sample was irrigated with sterile saline, dried with sterile paper points and was randomly divided into four groups (n=6).

Intracanal medicaments:
Teeth samples were submitted to following intracanal medicaments: Calcium hydroxides, 2% Chlorhexidine gel, triple antibiotic paste and double antibiotic paste. Each medicament was applied under sterile conditions with a syringe and needle until the medicament was excess to the sample. After the excess material was removed, orifices were sealed with temporary restorative cement (zinc oxide eugenol). After all this, the samples were placed into a Petri dish and covered with sterile gauze and incubated at 37degree C for 14 days.

Assessment of antibacterial activity:
At the end of the incubation period, the temporary restorative cement was removed, and the root canal was washed with sterile saline under aseptic environment. The colony counts were performed to all the samples. The antibacterial efficacy was measured by comparing the percentage reduction in colony counts (%RCC) before and after the intracanal medicaments (on day 14).
The percentage reduction in colony count was calculated by using the following formula:

$$\text{Percentage reduction in colony} = \frac{\text{initial colony count} - \text{final colony count}}{\text{Initial colony count}} \times 100$$

RESULTS:

Calcium hydroxide showed the least percentage of bacterial reduction. On evaluating the control group, all experimental groups demonstrated significantly higher percentage of bacterial reduction ($P<0.05$) among the experimental group , triple antibiotic paste showed the highest percentage of bacterial reduction (78.9 +2.1%) followed by Chlorhexidine gel (52.8+2.1%) and double antibiotic paste (54.3+1.5%) . There was no spastically significant difference between double antibiotic paste and Chlorhexidine gel ($P>0.05$).

Calcium hydroxide	Chlorhexidine gel	Double antibiotic paste	Triple antibiotic paste
20.8± 1.3 %	52.8 ±2.1%	54.3± 1.5%	78.9±2.1%

DISCUSSION:

Endodontic therapy can be based on nonspecific elimination of intraradicular microorganisms. Furthermore, some authors prefer single-visit root canal treatment (8) although many studies have shown the importance of intracanal medication between sessions in order to kill microorganisms. Intracanal medicament act beyond the root canal lumen, inside dentinal tubules and on apical resorptions (9). E.faecalis has high resistance to antibacterial substances is widely documented, and this bacterium can enter in a viable but nonculturable state during environmental stress (10).
The bactericidal effect of calcium hydroxide against E.faecalis was lower (20.8% on day 14) when compared with other medicaments. The release of hydroxyl ions in an aqueous environment is responsible for the antimicrobial activity of calcium hydroxide. Their lethal effects on bacterial cells are probably caused by the mechanisms such as damage to the bacterial cytoplasmic membrane, protein denaturation, and damage to DNA (11). It worked effectively in eliminating E.faecalis in 3 to 8 days as compared with 14 days (12), however, the endodontic literature provides discouraging information on the antibacterial effectiveness of calcium hydroxide against E. faecalis because of the buffering action of dentin (13).
Studies have suggested that CHX gel is an effective intracanal medicament due to its broad antimicrobial spectrum (14), which is in agreement of the findings of the present study. The present results showed that the 2% CHX gel produced the largest mean inhibition zones and was effective against all microorganisms. Even at the highest concentrations, it has very low toxicity. Also, it absorbs onto dental tissues and mucous membranes resulting in its prolonged gradual release at therapeutic levels (15).Furthermore, CHX may be particularly beneficial in the treatment of inflammatory root resorption (2).However, it is not an effective intracanal barrier, needing to be changed frequently as it diffuses through the dentinal tubules, leaving an empty canal (16). It is also radiolucent, making its visualization difficult inside the canal.
Double Antibiotic Paste (DAP) showed a reduction of 54.3% of colony count, double antibiotic paste plays a significant role in revascularization (or) regenerative endodontics. DAP is a combination of ciprofloxacin, and metronidazole, initially minocycline was added with these two components but the main disadvantage is that it causes discolouration of tooth. So in order to overcome this disadvantage, minocycline was removed.
Triple Antibiotic Paste(TAP) showed a 78.9% reduction in colony count, which on day 7. Among the components, metronidazole has a wide bactericidal spectrum against obligate anaerobes, which are common in the deep dentin of infected root canals. Certain bacteria are resistant to metronidazole; hence, ciprofloxacin and minocycline were mixed with it to achieve higher antimicrobial action (17) .The TAP was shown to have complete inhibition of *E.faecalis* strain on BHI blood agar plates(18) . Minocycline binds to calcium ions by chelation to form an insoluble complex causing tooth discoloration. An experimental study (7) reported that even a short application period of 24 to 48 hours found to cause tooth discoloration. Hence, TAP should be limited into the root canal.

CONCLUSION:

Under the limitations of this study, Triple antibiotic paste (TAP) showed the maximum bacterial reduction followed by Double antibiotic paste (DAP), Chlorhexidine gel, and Calcium hydroxide showed the least. There was a significant difference when compared with triple antibiotic paste and calcium hydroxide, but the difference between double antibiotic paste and Chlorhexidine gel were not that significant. As a future scope of this research, clinical trial can be done using various antibiotic regimens as intra canal medicaments in infected root canals.

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