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EVALUATION OF ANTIBACTERIAL EFFICACY OF 2% CHLORHEXIDINE GEL, CALCIUM HYDROXIDE AND LINCOSAMIDE BASED ANTIBIOTIC AGAINST ENTEROCOCCUS FAECALIS- AN IN VITRO STUDY

Dental Science

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ABSTRACT

AIM: Aim of the present study is to evaluate the antibacterial efficacy of 2% Chlorhexidine gel (CHX), calcium hydroxide (CH), Clindamycin 1%w/v (CLI), and combination of Clindamycin 1%w/v (CLI) with Calcium Hydroxide (CH) against Enterococcus faecalis (EF).

MATERIALS AND METHODS: Enterococcus faecalis strains were mixed with peptone water and the turbidity was adjusted equal to the McFarland's turbidity standard tube No: 0.5. The inoculum obtained were used to make lawn cultures on the agar plates. A total of 30 agar plates were prepared, with each plate having four wells containing the four medicaments. The plates were incubated and evaluated for zones of inhibition after 24 hours. The results were statistically evaluated by paired t-test, ANOVA and Post-hoc analysis using Turkey's HSD.

RESULTS: The difference between values of the zones of inhibition around each medicaments after 24 hours was found to be statistically significant. The maximum mean value of the zones of inhibition after 24 hours was shown by group I CHX (11.19 ± 0.75), while the minimum was shown by group II CH (6.03± 0.67).

CONCLUSION: Within the confines of the study, it can be concluded that clindamycin can be used as an alternate to calcium hydroxide dressing as an intracanal medicament. Combination of calcium hydroxide and clindamycin showed synergistic action as proven in the present study.

KEYWORDS

Chlorhexidine, Clindamycin, Calcium Hydroxide, Intracanal Medicaments

INTRODUCTION

Elimination of bacteria and their by-products from the root canal system is one of the main goals of root canal treatment. Even though a number of instrumentation and irrigation techniques exist, debris is often left behind in the root canal system. Chemo-mechanical instrumentation can eliminate most of the infecting bacteria but complete disinfection of the canal is not achieved because of root canals anatomical complexity and bacterial variety. Use of intracanal medicaments to disinfect the root canal system has been suggested for achieving long term successful outcome of the treatment (1).

Root canals of the infected teeth have a complex microbial flora consisting of cocci, rods, spirochetes, filaments and sometimes fungi. Enterococcus faecalis has been isolated mostly from asymptomatic and persistent root canal infections. It also found in 77% of failed endodontic cases, and in 50% cases with chronic apical periodontitis (2,3). Enterococcus can survive in the root canals as a single organism without the support of the other bacteria (4,5).

Intra canal medicaments have a long history of use as an inter appointment dressings to reduce pain, lower bacterial count, prevent regrowth and render the canal contents inert (6).

Calcium hydroxide (CH) is used as an intracanal medicament due to its bactericidal properties, high pH (of about 11–12.5) has destructive effect on cell membrane and protein structure of the bacteria there by leading to cell lysis (7).

Chlorhexidine (CHX) has broad spectrum of action against gram positive and negative organisms (8). The substantivity property of Chlorhexidine prevents the microbial colonization on the dentinal surface (9). It is widely used as an intracanal medicament and is effective against various intracanal micro flora (8).

Previous studies have reported that Enterococcus faecalis present with in the dentinal tubules are resistant to calcium hydroxide intracanal dressing over 10 days (10) but Chlorhexidine have shown superior results(11).

Clindamycin (CLI) is a bacteriostatic lincosamide known for its efficacy against a broad spectrum of endodontic bacteria (i.e., gram-positive aerobes and most anaerobic bacteria) and is effective in the treatment of acute infection, flare-ups and abscesses (12). Clindamycin is a choice of drug if the patient is allergic to penicillin or if a change in an antibiotic is indicated. Clindamycin and penicillin are highly effective in the treatment of odontogenic infections (13). Because of its broad spectrum of activity clindamycin is evaluated as an intracanal medicament against E faecalis in the present study.

The aim of study is to evaluate the antibacterial efficacy of CHX, CH, CLI, and CLI+ CH on EF at time intervals of 24 hours using the agar diffusion method.

MATERIALS AND METHODS

The study was conducted in the Department of Conservative Dentistry and Endodontics, in association with the Department of Microbiology, SVS institute of dental sciences, Mahbubnagar. Agar plates were prepared on the sterilized glass petri dishes and were left overnight at 37°C. EF strains were mixed with peptone water and the turbidity was adjusted equal to the McFarland's turbidity standard tube No: 0.5. The inoculum was obtained to make lawn cultures on the agar plates using sterile cotton swabs. Following this, wells that were three millimeters in diameter and four millimeters in depth were punched on the agar plates. A total of 30 agar plates were prepared, each plate had four wells, into which each of the four medicaments were placed.

Group I (CHX) - 2% Chlorhexidine gel

Group II (CH) - Calcium hydroxide
Group III (CLI) - Clindamycin 1% w/v
Group IV (CLI+CH) Clindamycin 1% w/v and Calcium hydroxide
The samples were incubated at 37°C and evaluated for zones of inhibition after 24 hours and measured with a vernier caliper. The readings corresponding to each medicament were statistically evaluated by graph pad prism software version 6.01 using repeated one way ANOVA and Post-hoc analysis.

RESULTS

The effect of different medicaments against Enterococcus faecalis after 24 hours was found to be statistically significant ($p<0.0001$) (Fig 1). Group 1 Chlorhexidine showed maximum inhibitory zone (11.19 mm) while group 2 Calcium hydroxide showed least inhibitory zone (6.03 mm). Group 3 Clindamycin 1% w/v showed better results (7.20 mm) compared to calcium hydroxide. In group 4 the combination of clindamycin and calcium hydroxide showed even more better results than clindamycin (8.41mm).

A comparison between the four groups after 24 hours [Table 1] (Post-hoc analysis using Turkey's HSD) showed that each group differed significantly from the others. Among all the medicaments used, calcium hydroxide showed least antibacterial effect.

Table: 1 Comparison between four groups at 24 hours using ANOVA test

Groups	n	Minimum	Maximum	Mean	SD	P-Value
2% Chlorhexidine gel	30	10.00	12.42	11.19	0.75	P <0.0001
Calcium Hydroxide	30	4.92	7.64	6.03	0.67	
Clindamycin	30	6.24	8.21	7.20	0.57	
Clindamycin + Calcium Hydroxide	30	7.22	9.82	8.41	0.74	

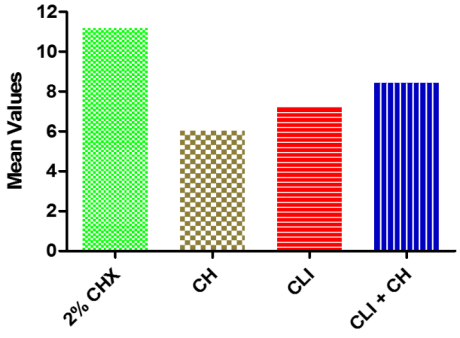

Fig 1: Mean inhibitory zone for Enterococcus faecalis

DISCUSSION

The present study investigated the antimicrobial efficacy of 2% Chlorhexidine, calcium hydroxide, clindamycin and their combination against Enterococcus faecalis Most of the intracanal medicaments shows their antibacterial efficacy within 24 hours (11). Therefore, this study was conducted to check the efficacy of the materials after 24 hours.

The present study uses blood agar as the culture media, since they are easily available and commonly used media for Enterococcus faecalis. Blood agar consists of a base containing a protein source like tryptones, soybean protein digest, sodium chloride, agar and 5% sheep blood (14). E faecalis causes a gamma hemolysis when grown on a blood agar (15). The agar diffusion method presents a zone of inhibition around the wells containing the medicament.

Calcium hydroxide was tested in this study because it is most commonly used intracanal medicament (16). The antimicrobial property of calcium hydroxide is due to its high pH 12 which causes the release of hydroxyl ions in an aqueous environment (17). High pH causes destruction of bacterial cell membrane and protein structure (18). In the present study CH was the least effective against EF, this is in accordance with previous studies by Evans et al.(19) The mean zone of inhibition of calcium hydroxide after 24 hours of incubation is (6.03± 0.67) .When CH is placed in agar, its high pH starts to precipitate, preventing its diffusion (17).

Furthermore, the release of Ca and OH ions decreases the pH of the media, enhancing growth of the organisms being tested (20). These factors may be responsible for its lack of effectiveness against EF in blood agar. Moreover, proton pump of EF carries protons to the interior of the cell, acidifying its cytoplasm in situations of increased alkalinity when subjected to CH (19).

Chlorhexidine (CHX) is known for its broad spectrum of action against gram-positive and negative organisms. CHX was found to be effective against EF after 24 hours (11.19 ± 0.75) of incubation on agar plates. It is in accordance with the results obtained by previous studies (11,21). Antimicrobial effects of Chlorhexidine is due to attraction between CHX (cation) and negatively charged bacterial cell wall (22). When Chlorhexidine is absorbed onto the organism's cell wall, it disrupts the integrity of the cell membrane and causes the leakage of intracellular components of the organisms. Unlike the conventional medicaments, the positively charged molecules of Chlorhexidine can adsorb into the dentinal tubules and prevent microbial colonization on the dentine surface for a longer period this is due to its substantivity (3,10).

In general clindamycin is used in endodontics as a systemic antibiotic because of its higher bioavailability in bone. It also has better anti biofilm effect as an intracanal medicament in comparison with tetracycline, doxycycline, Chlorhexidine and propolis (23). It inhibits bacterial protein synthesis by binding to 50s rRNA. Clindamycin causes changes in the cell wall surface, which lowers adherence of bacteria to host cells and increases intracellular lysis of organisms. In the present study clindamycin was found to be effective against EF after 24 hours (07.20 ± 0.57) of incubation in agar.

When clindamycin and calcium hydroxide combined, calcium hydroxide acts on bacterial cell membrane and clindamycin acts intercellularly leading to synergistic action (8.41± 0.74).

A study revealed that clindamycin had the ability to penetrate into dentinal tubules. It has the potential to serve as an effective intracanal medicament in persistent infections where other medicaments fail (24,25). In persistent infection (ie, infections in which CH or other medicaments are not effective in eradicating the bacteria) clindamycin can be an alternative to disinfect the dentinal tubules.

Limitations of the study is that agar diffusion method does not differentiate between micro biostatic and microbicidal properties of dental medicaments, neither does it provide information about microbial viability after the test (26).

The test results depend upon the medicament's solubility and diffusability in agar, rather than its actual efficacy against the organism.

However, there has not been an in-vivo study yet evaluating the effectiveness of Clindamycin as an intra-canal medicament against Enterococcus faecalis or any other organism.

CONCLUSION

Within the confines of the present study, it can be concluded that clindamycin can be used as an alternate to calcium hydroxide dressing as intracanal medicament. Combination of calcium hydroxide and clindamycin showed synergistic action as proven in the present study.

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