ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at:www.ijdsir.com

Volume – 7, Issue – 3, June – 2024, Page No. : 189 - 193

Comparative evaluation of the antimicrobial efficacy of 0.2% polyhexanide and 2% chlorhexidine against E. Faecalis

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Citation of this Article: Dr. Anusha Sequeira, Dr. Vedavathi B., Dr. Akshata Airsang, Dr. Ambili. C, Dr. Dharam Hinduja Dr. Payal Das, "Comparative evaluation of the antimicrobial efficacy of 0.2% polyhexanide and 2% chlorhexidine against E. Faecalis", IJDSIR- June – 2024, Volume –7, Issue - 3, P. No.189 – 193.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Enterococcus faecalis often the most important culprit for endodontic failures. It is resistant to most of the intracanal irrigating solutions and medicaments. Hence, there is a quest for newer better material with better antimicrobial properties, yet which are not cytotoxic to the periapical tissues. NaOCl and CHX are being widely used during root canal treatment, however have not been found to be effective antimicrobial agents. PHMB widely used in medical field for its antimicrobial property and prolonged duration of action. It is also found to be effective against endodontic pathogens. However, antimicrobial efficacy of 0.2% polyhexanide (poly hexamethylenebiguanide- PHMB), 2% chlorhexidineand 3% NaOClagainst E.faecalis has not been evaluated.

This study evaluated and compared the antimicrobial efficacy of 0.2% polyhexanide (poly hexamethylenebiguanide - PHMB) and 2% chlorhexidine and NaOCl against E.faecalis using agar diffusion method. 0.2% polyhexanide showed better antimicrobial efficacy, followed by 2% chlorhexidine and 3% sodium hypochlorite. Within the limitations of this study, it can be concluded that 0.2% PHMB with good antimicrobial property can be used as a better root canal irrigant in treating cases with failed root canal treatment.

Keywords: Chlorhexidine, Endodontic irrigants, Poly hexamethylenebiguanide, Polyhexanide.

Introduction

Bacteria within the root canal system are present not only in the planktonic form but also in biofilms, which are difficult to eradicate leading to endodontic treatment failures. E.faecalis, the most commonly isolated bacteria in such cases is resistant to various irrigants and intracanal medicaments used during cleaning and shaping of root canal system. Apart from the mechanical preparation, irrigating solutions with potent antimicrobial action is crucial in reducing the microorganisms present in the intricacies of root canal system, which are usually left untouched by instrumentation alone. Pathogenic microorganisms thrive in the dentinal tubules, isthmuses, 'fins' and other difficult-to-reach places which accounts to 35 - 50% of the surface area of the canal system.[1]

Sodium hypochlorite irrigant has been routinely used, is shown to have excellent tissue dissolving property but weak antimicrobial property and high cytotoxicity, necessitating the use of CHX alternatively in root canal cases with open apices, resorptions, periapical pathoses and failed cases due to its property of substantivity that is seen for upto 12 weeks. [2&3]

However, neither chlorhexidine (CHX) nor NaOCl can completely eliminate the bacterial population from the root canal. [4] PHMB is a polymer cationic antiseptic with a broad spectrum of antimicrobial, antifungal and antiviral properties widely used in the field of medicine to treat skin infections, wound disinfection etc. PHMB is shown to be effective against E.faecalis. [5] Studies have shown a better antimicrobial property, substantivity of PHMB when compared with CHX. However, the antimicrobial efficacy of 0.2% PHMB and 2% CHX against E.faecalis has not been evaluated. So, this study aimed to evaluate and compare the antimicrobial efficacy of 0.2% PHMB, 2% CHX, 3% sodium hypochlorite against E.faecalis by agar diffusion method.

Materials and Methodology

Enterococcus Faecalis ATCC29212 was obtained from Azyme Biosciences Private Limited, Bangalore, was cultivated in brain heart infusion broth for 24 hours to obtain the organism required for the study. E Faecalis(200 µL) were spread on 10 agar. 4 wells of diameter 0.6 cm were cut using well borer in each agar plate. 50 µL each of Saline, 3% NaOCl, 2% CHX and 0.2 % PHMB were then added to these wells and incubated at 37°C for 24 hrs and the zone of clearing was measured in mm from each well using a metric ruler.

Results

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. The mean value in millimeters of the zone of clearing seen with 0.2 % PHMB was 30.7 mm followed by 2% CHX and 3% NaOCl which were 19.3 mm 24.8 mm respectively (Fig 1-4). The difference in their mean was statistically significant when compared between groups (Table 1).

Discussion

E.faecalis is the most frequently isolated organism in failed root canal cases and is shown to penetrate the root dentine for upto 1000 microns. Kayaoglu et al. [6] and Chivatxaranukul et al. [7] suggested that E. faecalis could form a biofilm and bind to collagen and hydroxyapatite components of the tooth. Also a protein, 19-kDa found in E. faecalis resulted in resistance to usual intracanal medicaments over a period of time.[8]

This necessitates the use of a more potent antimicrobial agent. One such is, PHMB used in medical field against Gram-positive and Gram-negative bacteria, yeasts [9] and viruses. 0.2% PHMB is already in use as a mouthwash formulation and it was shown to be effective against plaque formation and superior substantivity compared to CHX. Hence, PHMB was used to compare with CHX for the antimicrobial property.

Analysing our result, it is seen that the zone of inhibition was maximum wrt 0.2% PHMB with a mean of 30.7 mm, than 2% CHX (mean 19.3 mm) and NaOCl (mean 24.8 mm) which is in accordance with the study done by Khan et al [10] comparing the effectiveness of different intracanal medicaments against polymicrobial biofilm formed by Enterococcus faecalis, Staphylococcus aureus, and Candida albicans using calcium hydroxide (CH), CHX and PHMB Gel. 2% CHX gel has a time-dependent antimicrobial effect, its substantivity is known to last for 12 weeks. However, Chandki et al have shown that PHMB has a superior substantivity. [11] PHMB requires an optimal contact period of 10-15 minutes to be effective. The mechanism of polyhexanide's antimicrobial action, a cationic antiseptic could be based on its affinity to negatively charged phospholipids of the bacterial cell membrane forming polyhexanide–phospholipid complex which disrupts the integrity of bacteria's cytoplasmic membrane decreasing the fluidity of the outer phospholipid layer forming hydrophilic sites in it.PHMB targets are lipopolysaccharidesin the outer cell membrane of Gram-negative bacteria, Teichoic acid, peptidoglycan and cytoplasmic membraneproteins of gram positive bacteria.

Zone of clearing seen with CHX was next. Chlorhexidine is a synthetic cationic bis-guanide that consists of two symmetric 4-cholorophenyl rings and two biguanide groups, connected by a central hexamethylene chain.[12] CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism. [13] It's efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls [14]thereby altering the cells' osmotic equilibrium. This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacteria.

Zone of inhibition seen with NaOCl was least. The antimicrobial effectiveness of sodium hypochlorite, based in its high pH (hydroxyl ions action), is similar to the mechanism of action of calcium hydroxide. The high pH of sodium hypochlorite interferes with the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism and phospholipid degradation. The amino acid chloramination reaction forming chloramines interferes with cellular metabolism. Oxidation promotes irreversible bacterial enzymatic inhibition replacing hydrogen with chlorine. This enzyme inactivation can be observed in the reaction of chlorine with amino groups and an irreversible oxidation of sulphydryl groups of bacterial enzymes (cystein). Thus, sodium hypochlorite presents antimicrobial activity with action on bacterial essential enzymatic sites promoting irreversible inactivation originated by hydroxyl ions and chloramination action. Dissolution of organic tissue can be verified in the saponification reaction when sodium hypochlorite degrades fatty acids and lipids resulting in soap and glycerol. [15]

Figure 1: Zone Of Inhibition ForSodium Hypochlorite Against E. Faecalis

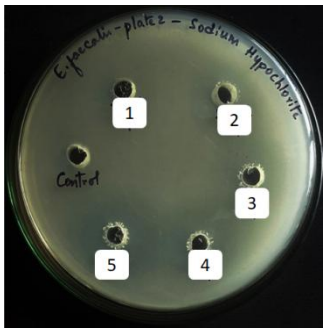


Figure 2: Zone of inhibition for CHX against E.faecalis

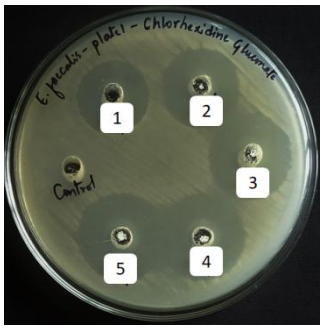


Figure 3: Zone of inhibition for PHMB against E.faecalis

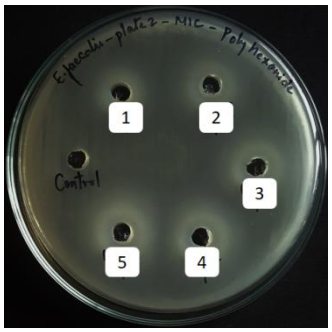
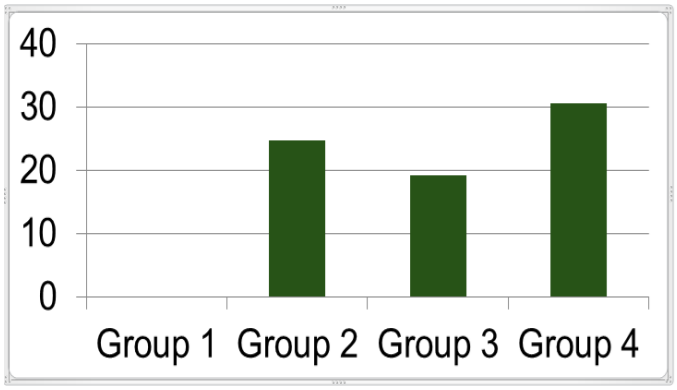


Table 1: Mean values for zones of inhibition for NaOCl, CHX, PHMB

Group	Minimum	Maximum	Mean	Standard deviation	P value
Saline	0	0	0		
3% NaOCl	23	27	24.8	1.096	0.048*
2% CHX	16	22	19.3	2.41	
0.2% PHMB	19	32	30.7	0.81	

*Significant

Graph 1: Zones of inhibition



Conclusion

Within the limitations of the present invitro study, it can be concluded that 0.2% PHMB has better antimicrobial property and substantivity against E.faecalis as compared to 2% CHX and 3% NaOCl and can be safely used in retreatment of failed root canal cases.

Abbreviations

1. NaOCl- Sodium hypochlorite
2. CHX- Chlorhexidine
3. PHMB- Polyhexanide (polyhexamethylene biguanide)

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