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ISSN: 2161-1122
Dentistry

Case Report

Comparison of Antimicrobial Efficacy of 0.2% Chitosan, 3% Sodium Hypochlorite, 2% Chlorhexidine against *Enterococcus faecalis*, Alone and in Combination with Diode Laser an *In Vitro* Study

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ABSTRACT

The aim of this study was to compare the antimicrobial efficacy of 0.2% chitosan, 3% sodium hypochlorite, 2% chlorhexidine against *Enterococcus faecalis*, alone and in combination with diode laser.

The root canals of 72 extracted intact human single rooted teeth with single canals were prepared and *E. faecalis* was incubated in the root canals for 7 days. The teeth were then randomly divided into the following 4 experimental groups: Group I: Saline, group II: 0.2% Chitosan, group III: 3% Sodium hypochlorite and group IV: 2% Chlorhexidine. These groups were further subdivided into 3 groups: a) 10 ml irrigant only, b) 10 ml irrigant, dried and irradiation with diode laser, c) diode laser was used for activation of irrigant solution. Samples were obtained from subgroups in each group and checked for turbidity. The effect of each irrigant was evaluated by counting the number of colony forming units observed on inoculation with samples taken from irrigated canal on bile esculin azide agar. The data thus obtained was recorded and put to statistical analysis.

Results: Significant reductions were noted in *E. faecalis* colony counts in all groups ($P<0.05$). The greatest reduction in colony count (0%) was noted in the group IV followed by group II. Also, that samples disinfected with diode laser after root canal irrigation showed less number of colony forming units per ml as compared to the samples irrigated with root canal solutions alone or diode laser alone.

Conclusion: Chitosan has the potential to be used as an adjunct for disinfection of the root canal system. Irradiation with diode laser should be used in conjunction with the irrigant so as to obtain maximum antibacterial effect against *Enterococcus faecalis*.

Keywords: Chitosan; Chlorhexidine; Diode laser; Sodium hypochlorite; Antimicrobial efficacy

INTRODUCTION

Complete debridement and disinfection of the pulpal space is considered to be essential for inevitable long term success in endodontic treatment. even after scrupulous mechanical preparation residual pulpal tissue, bacteria and dentin debris may persist in the irregularities of the root canal system. Therefore, irrigation is considered an integral part of biomechanical preparation.

Sodium hypochlorite solution has three important properties: Organic tissue dissolving capacity, bactericidal effect and absence of clinical toxicity, when properly used. CHX is a hydrophobic and lipophilic molecule which has a positive charge. Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls, thereby altering the cells osmotic equilibrium. It is less caustic, has substantivity, has broad spectrum antibacterial effects and is recommended in retreatment cases.

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Received: 03-Aug-2020, Manuscript No. DCR-24-5816; **Editor assigned:** 06-Aug-2020, PreQC No. DCR-24-5816 (PQ); **Reviewed:** 20-Aug-2020, QC No. DCR-24-5816; **Revised:** 01-Aug-2024, Manuscript No. DCR-24-5816 (R); **Published:** 29-Aug-2024, DOI: 10.35248/2161-1122.23.14.697

Citation: Goel P (2024) Comparison of Antimicrobial Efficacy of 0.2% Chitosan, 3% Sodium Hypochlorite, 2% Chlorhexidine against *Enterococcus faecalis*, Alone and in Combination with Diode Laser an *In Vitro* Study. J Dentistry. 14:697.

These root canal irrigants fulfill many ideal requirements as they have a broad antimicrobial range, are able to dissolve necrotic pulp tissue, inactivate endotoxins and either prevent the formation of smear layers or dissolve them once they have developed. However, these root canal irrigants have some disadvantages. The chelating agents that alter the dentin's structural characteristics resulting in a compromised mechanical integrity and an increased potential for collagen adherence to bacteria. Ultrastructural damage into the dentin has been observed after use of a high concentration of NaOCl solution for long periods of time. However, NaOCl is not able to fully debrid the root canals or remove the bacteria from the biofilms [1].

Because of the recognized limitations of these endodontic irrigants, research continues for better irrigating solutions for endodontic procedures which are capable of cleaning dentinal tubules effectively by removing the smear layer along with the debris and necrotic tissue of the canal.

Chitosan is a non-toxic cationic biopolymer typically obtained from chitin by alkaline deacetylation, which is the primary component of crustacean exoskeletons. The covalent immobilization of chitosan on dentinal collagen was proposed to induce remineralization of the exposed and demineralised dentin structure because its functional phosphate groups could bind to calcium ions to form a favorable surface for crystal nucleation, resulting in the formation of a calcium phosphate layer. Chitosan has captivated a great deal of heed in dental research because of its biocompatibility, lack of toxicity and antimicrobial properties.

Laser light penetrates upto >1000 micrometer into the dentin and thus has scope for complete canal sterilization. In the recent years the use of lasers for endodontic applications has been investigated and proven for their efficacy in root canal shaping and sterilization, removal of debris and sealing of dentinal tubules in the root canal wall. These devices have been shown to be effective against microbial agents, however there is some controversy regarding the antibacterial effects of different types of lasers within the root canal. A combination of smear layer removal, bacterial reduction and less apical leakage brings importance to this system and makes it viable for endodontic treatment. The principal laser action is photothermal.

Enterococcus faecalis makes up a small proportion of the flora in untreated canals but is a persistent organism that plays a major role in the etiology of periradicular lesions. *E. faecalis* has an ability to survive harsh environments including extreme alkaline pH, salt concentrations. It resists bile salts, detergents, heavy metals, ethanol, azide and desiccation. The prevalence of *E. faecalis* is 40 percent in primary endodontic infection and 24 to 77 percent in chronic endodontic infection. It binds to dentin and proficiently invades dentinal tubules.

Till date none of the available irrigants have the ideal requisites for attaining successful treatment outcome. Studies have tried to evaluate different permutations and combinations of irrigating solutions to achieve better results but so far there are limited studies on concomitant use of chitosan and diode laser. Thus, the present study intends to compare antimicrobial efficacy of 0.2% chitosan, 3% sodium hypochlorite, 2% chlorhexidine against *Enterococcus faecalis*, alone and in combination with diode laser [2].

CASE PRESENTATION

This study was conducted on 72 single-canal freshly extracted human teeth at Swami Devi Dyal dental college after obtaining approval from the ethical committee of the institute. The teeth were confirmed to have single canal by taking radiographs of both views that is mesiodistal and labiolingual of each tooth. The working length was considered 1 mm short of the apical foramen. Root canals were instrumented using K3 rotary files and apical preparation was done till #30 file. In between the use of rotary files, canals were rinsed with distilled water. The teeth were then transferred into microtubes and autoclaved twice at 121°C for 20 minutes. Sterility of the sample was checked by dipping each sample in a test tube containing 5 ml of freshly prepared sterile Brain Heart Infusion broth (BHI) which was incubated at 37°C for 48 hrs. If no turbidity appeared the samples were considered as sterile however in case of turbidity, the samples were resterilized.

Bacterial inoculation of root canals

Enterococcus faecalis (MTCC 2729 equivalent to ATCC10100) in the freeze dried form was first inoculated in tryptone soy broth. For confirmation, the culture was inoculated on bile esculin azide agar at 37°C for 24 hrs. *Enterococcus faecalis* showed black color colonies on bile esculin azide agar. Each sample was dipped into a test tube containing 5 ml of sterile brain heart infusion broth. These test tubes containing the samples were contaminated. *Enterococcus faecalis* colonies were picked up with an inoculating loop from plates of bile esculin azide agar, dissolved in test tubes containing the samples and then incubated at 37°C for a minimum period of 7 days [3].

Checking for turbidity

The infected samples were taken out from the turbid brain heart infusion broth and rinsed with freshly prepared distilled water. The external surface of the sample was wiped with gauze dipped in alcohol. The samples were then dipped into fresh sterile BHI broth and incubated at 37°C. Appearance of turbidity indicated that the samples were infected with *Enterococcus faecalis*. In cases where no turbidity appeared they were re infected and the whole procedure was repeated again till the samples gave positive results.

Experimental procedures

The samples (n=72) thus prepared were randomly divided into 4 equal groups as follows.

Group I at the prepared infected canal was irrigated passively with 2 ml of saline using side vented needle inserted until slightly short of middle third of the canal. The solution was left in the canal for 2 minutes and this procedure was repeated for 4 more times for a total period of 10 minutes of treatment and 10 ml solution. Then the sample was dabbed in dry sterile gauze

and the canals were dried with the sterile paper points which were then dipped in sterile brain heart infusion broth and incubated for 72 hrs at 37°C. Group I b canal was dried using paper points after irrigation with 10 ml saline. A diode laser with a wavelength of 810 nm was used to irradiate the dried root canals by using 200 µm endodontic tip. The fibre tip was placed into the canal 1 mm short of the apex for 5 seconds and withdrawn coronally with helical movement at 1 or 2 mm/sec and this procedure was repeated for 4 more times, following which sample was taken from dried canals using wet paper points which were then dipped in sterile brain heart infusion broth and incubated for 72 hrs at 37°C. Group I c a diode laser with a wavelength of 810 nm was used to irradiate the canals using 200 µm endodontic tip. 2 ml of saline was applied and activated for 5 seconds using an endodontic fibre tip placed into the canal 1 mm short of the apex and withdrawn coronally with helical movement at 1 or 2 mm/sec. This procedure was repeated 4 more time to a total of 10 ml solution. Then the sample was dabbed in dry sterile gauze and the canals were dried with the sterile paper points which were then dipped in sterile brain heart infusion broth and incubated for 72 hrs at 37°C. Similarly, procedure was done in group II, III, IV using 0.2% chitosan, 3% sodium hypochlorite and 2% chlorhexidine respectively [4].

Checking for disinfection of samples

Sample paper points were placed in a test tube containing BHI broth, incubated at 37°C for 48 hrs and turbidity was checked by comparing with a test tube containing the uninfected samples dipped in BHI broth.

Antimicrobial assessment after irrigation (quantitative analysis of Enterococcus faecalis)

All the samples whether it showed turbidity or not were serially diluted 5 times to obtain the final suspension. 1 ml of suspension from the last dilution with a micropipette was inoculated on bile esculin azide agar and incubated at 37°C for 24 hrs. The plates were then observed for growth or no growth. Colony forming units were obtained from the plates in which growth was observed by dilution plate method [5].

Statistical analysis

The effect of each irrigant was evaluated by counting the number of colony forming units observed on inoculation with samples taken from irrigated canal on bile esculin azide agar. The data thus obtained was recorded and put to statistical analysis using non-parametric Kruskal Walis test followed by Mann-Whitney test for comparison of subgroups a, b, c within each group.

RESULTS

The results of the present study showed that number of bacterial colonies varied from irrigant to irrigant and no growth was observed in samples irrigated with 2% chlorhexidine [6]. Growth was observed in 40 samples and there was absence of growth in 32 samples. In case of saline CFU/ml varied between 1-125, with sodium hypochlorite CFU/ml 1-70, 0.2% chitosan between 1-2 and for chlorhexidine 0. When the results were put to statistical analysis, it was found out that there was a significant difference when group I (saline) was compared to group II (0.2% chitosan), group III (3% sodium hypochlorite) and group IV (2% chlorhexidine). It was observed that the maximum number of colony forming units were observed in the group I (106.83 CFU/ml), followed by group III (54.33), group II (0.50 CFU/ml) and group IV (0 CFU/ml). As our data was skewed, on applying nonparametric Kruskal Walis test followed by Mann-Whitney test it was found that there was a significant difference among the groups tested. The mean colony forming units of all the groups in descending order is group Ia-106.83>group Ic-56.00>group IIIa-54.33>group Ib-6.83>group IIIb-2.33=group IIIc-2.33>group IIa-0.50>group IIb-0.17>group IIc-0.17>group IV a-0.000=group IV b-0.000=group IVc-0.000.

DISCUSSION

The long term success of endodontic treatment depends on the eradication of microbes from the root-canal system and prevention of reinfection. Residual pulpal tissue, bacteria and dentin debris may persist in the irregularities of the root canal system even after meticulous mechanical preparation. Therefore, irrigation is considered an integral part of biomechanical preparation.

Considering the importance of efficient cleaning and elimination of microorganisms from the root canal system, this study aimed to assess the efficacy of 3 different irrigating solutions used alone or in combination with diode laser, which could be used as an adjunct to mechanical debridement [6].

Enterococcus faecalis was selected for the purpose of the present study because it is able to successfully colonize the root canal in a biofilm like style, invade dentinal tubules and resist endodontic treatment procedure. It is believed to be one of the intracanal bacteria which are most resistant to elimination by disinfecting agents.

Barber, et al., showed that 5.25% concentration of NaOCl is the most potent amongst three different concentrations of 0.5%, 2.5%, 5.25%. But higher concentration may cause ultrastructural damage into the dentin and have more infuriating effects on apical and periapical tissues. Therefore; we had chosen 3% concentration for our study as it is less toxic and commercially available.

Chlorhexidine has been suggested as an efficient alternative to NaOCl. It has a broad-spectrum antimicrobial activity, targeting both gram positive and gram-negative microbes. Despite its usefulness as a final irrigant, chlorhexidine can't be used as the key irrigant in standard endodontic cases, because it cannot dissolve the necrotic tissue remains, it may discolor the teeth and also lacks lacks the ability to dissolve the tissue. Other side effects include loss of taste, burning sensation of the oral mucosa and subjective dryness of the oral cavity and discoloration of the tongue.

Because of the recognized limitations of these endodontic irrigants, developing new and better irrigating solutions for

endodontic procedures which are capable of cleaning dentinal tubules effectively by removing the smear layer along with the debris and necrotic tissue of the canal remains an area of great interest [7].

Chitosan is a natural polysaccharide which is obtained from the acetylation of chitin, derived from the shells of prawns and crabs. It has properties of biocompatibility, biodegradability, bioadhesion and is not toxic to the human body.

Whether antimicrobial effect of various irrigants is because of their chemical nature or just the pure physical flushing action, saline was used as the fourth irrigant which is totally chemically inert and is devoid of antibacterial action.

Most currently use of irrigants and intra canal medicaments have limited anti-bacterial spectrum and a limited ability to diffuse into the dentinal tubules (100 μm) therefore newer treatment strategies should be considered to remove microbes from the root canal system which penetrate upto 1,110 μm. Laser light which penetrates up to >1000 μm into the dentin thus has scope for complete canal sterilization [8].

Limitations in laser applications can be the increase of temperature and the fact that it is not possible for laser to reach some surfaces. The purpose of using the laser in wet canal was to warm the irrigating solution to increase its disinfecting effect, in addition laser induces cavitation, which enhance the removal of the smear layer.

Till date none of the available irrigants have the ideal requisites for attaining successful treatment outcome. Studies have tried to evaluate different permutations and combinations of irrigating solutions to achieve better results but so far there are limited studies on concomitant use of chitosan and diode laser. The current study focused on this issue [9].

After 2% chlorhexidine, samples irrigated with 0.2% chitosan (group II) showed statistically least number of CFU/ml as compared to 3% sodium hypochlorite and saline. The better performance of 0.2% chitosan in the present study may be attributed to its ability to interfere with bacterial adhesion thereby hindering the biofilm formation. Also, its polycationic nature interacts with the negatively charged surface of bacteria, altering the cell permeability and resulting in the leakage of intracellular components. In addition, chitosan is able to inhibit bacterial enzymatic degradation reducing the possibility of bacterial penetration and dentinal micro fractures.

The mean colony forming units per ml were less in subgroup b as compared to subgroup a. This may be attributed to the fact that chemical irrigants are limited to the most superficial layers of the root dentin and have insufficient penetration depth whereas laser light penetrates upto >1000 micrometer into the dentin and thus has scope for complete canal sterilization [10].

From the results of the present study, it has been found that samples disinfected with diode laser after root canal irrigation with chemical solutions showed less number of CFU/ml as compared to the samples irrigated with root canal solutions alone or diode laser alone.

Although group I (saline) subgroup c showed lesser number of CFU/mL as compared to the group I subgroup a, colony forming units were more as compared to subgroup b. This may be attributed to the high temperature generated in dried canals which killed the bacteria and charring effect which sealed the dentinal tubules but this was not possible when diode laser was used in wet canals. Also, because warming the solution did not help as saline itself has no antimicrobial properties. But use of diode laser in wet canals in group II (0.2% chitosan) and group III (sodium hypochlorite) potentiated the effect of irrigating solutions, thereby resulting in lesser number of colony forming units as compared to subgroup a and b [11].

From the results of the present study it can be deduced that amongst the tested irrigants 2% chlorhexidine and 0.2% chitosan have best results with least number of bacterial colonies of Enterococcus faecalis and should be the preferred irrigants. Diode laser increases the effectiveness of these irrigants against Enterococcus faecalis. It should be used in conjunction with the irrigant so as to obtain maximum antibacterial effect against Enterococcus faecalis. However, this is an *in-vitro* study, further *in vivo* studies are required before these laboratory results may be extrapolated to the clinical scenario (Table 1) [12].

Table 1: Comparison of mean of all the groups.

Group	Subgroup	n	Mean	Std. deviation
Saline	a	6	106.83	16.75
	b	6	6.83	5.845
	c	6	56	10.139
2% Chitosan	a	6	0.5	0.837
	b	6	0.17	0.408
	c	6	0.17	0.408
3% Sodium hypochlorite	a	6	54.33	16.955

	b	6	2.33	1.751
	c	6	2.33	1.506
2% Chlorhexidine	a	6	0	0
	b	6	0	0
	c	6	0	0

CONCLUSION

Chitosan has the potential to be used as an adjunct for disinfection of the root canal system. Application of an 810 nm diode laser alone did not have adequate antimicrobial activity for use as an adjunct in endodontic treatments. Irradiation with diode laser should be used in conjunction with the irrigant so as to obtain maximum antibacterial effect against Enterococcus faecalis.

ACKNOWLEDGEMENT

The authors deny any conflicts of interest related to this study.

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