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RESEARCH ARTICLE

EFFECT OF DIODE LASER ON E.FAECALIS INFECTED ROOT CANALS IN COMPARISON TO SODIUM HYPOCHLORITE- AN IN VITRO STUDY

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ABSTRACT

The aim of the study was to evaluate the effect of Diode Laser on E.Faecalis infected root canals in comparison to sodium hypochlorite. After decoronation and preparation of the root canals, they were sterilised. A liquid culture suspension of 0.5 Mcfarland standard BHI broth was prepared to obtain 1.5×10^8 colony forming units/ml from a subculture of E.faecalis. Sterilised teeth were kept in an Eppendrof tube of the bacterial suspension and was incubated for a week under aerobic and static conditions at 37°C.The medium was confirmed for E.faecalis growth. The cultures were checked for purity by Gram stain and colony morphology on BHI agar with 10% sheep blood. In the Laminar Flow Chamber, Group A samples were irrigated with 2.5% sodium hypochlorite and Group B samples were treated with diode laser. After 10 fold serial dilutions, aliquots of 0.1 ml were plated onto BHI agar plates and incubated at 37°C for 24hr.The colony forming units grown were counted and recorded. Results were calculated by measuring the zones of inhibition. Diode laser and Sodium hypochlorite were proved to be equally efficacious.

INTRODUCTION

The success of a root canal treatment depends on thorough chemomechanical debridement of pulpal tissue, dentin debris, and infective microorganisms. Bacterial infection has long been recognized as the primary etiologic factor in the development of pulp and periapical lesions.[1] The purpose of root canal treatment is to eliminate entirely the infection of the root canal system and prevent reinfection.

Root canals are very complex morphologically as they have intricate configurations and numerous fine canal aberrations because of which irrigating solutions cannot adequately remove all micro-organisms and other noxious agents. Many studies have shown that persistent endodontic infections are frequently caused by Enterococcus faecalis.[2]

Enterococcus faecalis, (E.faecalis) is a facultative anaerobic Gram positive coccus, which is the most commonly isolated species from the root canals of the teeth with failed endodontic treatment [3].It is ecologically tolerant and can servive in water without nutrients. It is resistant to most of the intracanal medicaments and can tolerate a phupto 11.5. (fabricius etal1982).

Sodium hypochlorite (NaOcl) has been widely used in dentistry as an irrigant as reported by Walker in 1936.It has a broad spectrum antimicrobial activity against bacteria, bacteriophages, spores, yeast and viruses. It acts as an organic solvent for pulpal remnants and debris while causing minimum irritation to the vital tissues however, its cytotoxic properties force the researchers to innovate the current irrigation regimen.[4] Lasers have become latest choice to eradicate microorganisms in the root canal, especially in the lateral dentinal tubuli. It has been proved in numerous studies that an emission of laser light directly in the root canal does have bactericidal effect.[5]The antibacterial effect of a laser beam is based on thermal properties of laser tissue interaction.[6]The high-power diode laser has been used in several areas of dentistry, with promising results in relation to dentinal disinfection.[7,8] Diode laser has been proved to be resource worth testing.

The aim of this study was to evaluate the effect ofdiode laser on E. faecalis infected root canals in comparison to sodium hypochlorite.

MATERIALS AND METHOD

This study involved 30 extracted human mandibular incisor teeth. The collected teeth were cleaned of blood, soft tissue tags and calculus and were stored in normal saline solution. Crowns were decoronated using diamond disk mounted on a DFS Mandrel in a straight handpiece at 30,000 rpm. to obtain 14 mm of root length. After decoronation, cleaning and shaping of the root canals was done using rotary protaper file with crown down technique till F2 file. During the entire procedure, 17% EDTA with 3% sodium hypochlorite was used

as an irrigant using an endodontic needle and syringe. Recapitulation was performed with an ISO #15 K-file. All apices were then sealed with nail varnish. Then the roots were autoclaved at 12°C for15 min. at15lbs pressure. Roots were then incubated in BHI broth for 48 hr. at 37°C. to ensure that there is no bacterial contamination.

A liquid culture suspension of 0.5Mcfarland standard BHI broth was prepared to obtain 1.5×10^8colony-forming units/ml from a subculture of E. faecalis. The sterilized tooth was placed in an Eppendorf tube, 1ml of the bacterial suspension was added in to this tube and was incubated for a week under aerobic and static conditions at 37°C.The medium was changed every two days to avoid saturation and confirmed the growth of E.faecalis and the cultures were checked for purity by Gram stain and colony morphplogy on BHI agar with 10% sheep blood.

After the incubation period, roots were assigned to groups A and B.

Group A (n =15) - treated with 2.5 % sodium hypochlorite
Group B (n=15) - - treated with diode laser

In Group A, root canals were irrigated with 5ml of 2.5% sodium hypochlorite for 60sec. using 5ml syringe and 26 G needle for Group B, root canals were treated with a diode laser (ilase, Biolase, California, USA) with an with an endodontic tip (e z Tip Endo,20mm/200µm).Specimens were treated with energy set at 1.5W.The optical fiber was introduced 1mm short of the apex and was recessed in helicoidal movements at a speed of apprx.2mm/s for 5s,repeated 4 times at intervals of 10 s, between each one to avoid temperature change. All procedures were carried out in a laminar flow chamber using sterile instruments. Sterile paper poits were introduced till working length for 15 sec and allowed to saturate. Following each sampling, paper points were transferred to tubes containg 1ml of freshly prepared BHI broth and vortexed for 20 sec. After 10 fold serial dilutions aliquots of 0.1ml were plated onto BHI agar plates and incubated at 37°C for 24 hr. The colony forming units grown were counted and recorded.

Roots incubated in sterile BHI broth Roots placed in E. faecalis suspension in eppendorf tubes

Sodium Hypochlorite

Diode Laser

CFUs seen in NaOCl group

CFUs seen in Laser group

Statistical Analysis

Descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements were presented on Mean ± SD. Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant.

Analysis of variance (ANOVA) was used to find the significance of study parameters between three groups followed by Tukey's post hoc analysis.

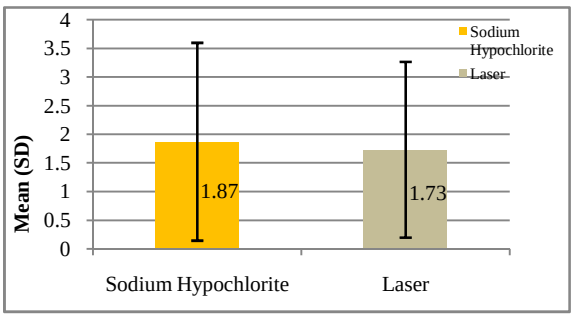
The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft Word and Excel were used to generate graphs, tables etc.

RESULTS

The total microbial count was estimated using a digital colony counter. Count per milliliter of diluted broth was calculated and multiplied by dilution factor.

Table 1 Comparison of the colony forming units in terms of {Mean (SD)} among sodium hypochlorite & laser group

Treatment Group	N	Mean	Std. Deviation	P value (Tukeys post hoc analysis)
Sodium Hypochlorite	15	1.87	1.727	0.995
Laser	15	1.73	1.534	



DISCUSSION

For successful endodontic therapy, the elimination of microorganisms from the root canal system is mandatory. According to studies, microorganisms penetrate deep into the lateral canal, apical ramifications, is thumuses, dentinal tubules and also present in smear layer.[9,10] Mechanical debridement alone cannot totally eliminate microorganisms from the root canalsystems.[10] So, antimicrobial agents have been recommended as an adjunct to instruments to achieve complete debridement.[11,12]

E.faecalis was chosen as the test organism in this study, as it is the most commonly isolated microorganism from the root canals of teeth in failed endodontic treatment. This microorganism can grow in a wide temperature range, high salt conc., can tolerate broad pH range as well as can resist the intracanal procedures. It also has the ability to invade deep into the dentinal tubules and form biofilms in root canals. Many studies have been directed towards finding an effective way to eradicate and/or prevent E. faecalis from gaining access to the root canal space.[13,14,15] This property can enable this species to escape from the action of endodontic instruments and irrigants used during chemo-mechanical preparation.

In our study, 2.5% sodium hypochlorite was used as the irrigant which successfully eradicates E.faecalis. It is a good tissue solvent, has broad spectrum antimicrobial activity, acts as a lubricant for instrumentation and can flush debris from the root canals. However, it has cytotoxic effect on periapical tissues and has potential to cause allergic reactions. Its 2.5% concentration was used as it is found to be more effective and less toxic than 5.25%. 2.5% of sodium hypochlorite is capable to inhibit 100% of E.faecalis in 5 min.[16]

Studies show that penetration of irrigants is limited to 100µm where as E.faecalis is known to penetrate to a depth of 600-1000µm[17,18] , making their eradication difficult. So, besides conventional irrigants, other adjuncts like lasers were proved to be valuable. Diode laser was used in this study for its bactericidal effect and high penetration depth into the dentinal tubules. [8,19] The laser radiation may be transmitted through quartz optical fibers, a property that could facilitate introducing laser light around canal curvatures and irregularities.[20] The fine diameters of optic fibers (200-320 µm) enable effective delivery of laser light to the root canal to help with reduction of bacterial contamination. The antibacterial effect observed reaches over 1mm deep into the dentin, surpassing the effective range of chemical disinfectants, such as NaOCl and displaying moderate effectiveness against E. faecalis even in the deeper layers of dentin.[21]The diode laser used in this study was iLase (Biolase, California, USA) with an endodontic tip (ezTipEndo, 20mm/200µm).

On comparing the antimicrobial efficacy of the diode laser against 2.5% sodium hypochlorite, diode laser proved to be slightly better than the later. However, difference was statistically insignificant (p value=0.995), leading to the conclusion that laser proved to be equally efficacious as a root canal disinfectant as sodium hypochlorite. The superior bactericidal effect of diode laser irradiation could be attributed to its greater depth of penetration (upto 1000µm into dentinal tubules) when compared to the penetration power of chemical disinfectant which is limited to 100µm.[17,18]

A study by S. Kumar *et al* also showed similar results and concluded that diode laser alone and diode laser with sodium hypochlorite showed complete elimination of E. faecalisfrom the root canal.[22] This can be explained by the broad antimicrobial spectrum of sodium hypochlorite, demonstrated by earlier studies[23] as well as to the protein denaturation and photothermal action provided by high-power diode laser over the bacterial cell.[24]

Microbial communities in vivo are quite resistant to and difficult to eradicate and none of the regimens are able to completely eradicate the microorganisms from the root canals. Further, the anti-microbial methods needs to be more standardized and no in vitro method accurately reflects the condition under which microorganisms grow in vivo.

CONCLUSION

Within the limitations of this in vitro study, the following conclusions were made.

1. Significant bacterial reduction was observed in both the groups.
2. Diode laser and sodium hypochlorite proved to be equally efficacious in their anti-microbial properties against E.faecalis.
3. Statistically insignificant difference was observed among two groups.

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