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COMPARISON OF ANTIMICROBIAL EFFICACY OF DIODE LASER AND PHOTO ACTIVATED DISINFECTION (PAD) IN *E.FAECALIS* CONTAMINATED ROOT CANALS

Endodontic

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ABSTRACT

Aim: To compare the antimicrobial efficacy of Diode laser and Photo Activated Disinfection(PAD) against E.faecalis.
Methodology: Thirty extracted premolars were instrumented upto Protaper rotary file size F2 and contaminated with a known species of E.faecalis .The samples were Group 1:PAD and Saline; Group 2:Diode laser and Saline; Group 3: NaOCl and PAD; Group 4:NaOCl and Diode laser; Group 5: Positive control. The samples were incubated in 37◦C for 48 h in BHI agar and colony forming units were counted.
Statistical analysis: Descriptive analysis for CFUs as Mean & SD. Kruskal Wallis Test & Mann whitney Post hoc Analysis for comparison between groups.
Results: Diode laser with NaOCl and PAD with NaOCl showed similar reduction in the growth of E.faecalis ($P \leq 0.001$).
Conclusions: A combination of NaOCl irrigant activated with Diode or PAD showed similar outcome. PAD can be considered as an alternative to Diode Laser for root canal disinfection.

KEYWORDS

Diode Laser, Photoactivated disinfection, E.faecalis, Root canal therapy

INTRODUCTION

The key to successful root canal treatment depends on removal of microorganisms present in the root canal system.[1, 2] At present techniques of root canal debridement and disinfection involve mechanical preparation in combination with the use of root canal irrigants. Despite the availability of various conventional mechanical root canal cleaning methods and chemical irrigants, root canals are not completely free of microorganisms.[3]

Enterococcus faecalis is a facultative anaerobic gram-positive bacterium rarely found in cases of primary endodontic infections, but represent 38% to 70% of the microbiota in cases of failed root canal treated teeth. A high prevalence of *E.faecalis* is frequently observed in filled root canals associated with persistent apical period ontitis.[4]*E.faecalis* organism has been widely used in culture and molecular methods based studies. It serves as a valuable microbiological pathogen in studies as it can form biofilm and colonize the root canal.[5] *E.faecalis* penetrates upto 400 µm into the dentinal tubules and this enables it to escape the action of endodontic treatments and irrigants used during chemomechanichal preparation. [6] Its ability to form biofilms in root canals is important for its resistance and persistence after intracanal antimicrobial procedures.[7]

Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution during chemomechanical preparation. It has unique properties of organic tissue dissolution, saponification and antibacterial action.[8] Studies have reported that NaOCl can only penetrate into the dentinal tubules up to a depth of 130 µm.[9]Root canal irrigant act mainly through their direct contact with the root dentin. The microorganisms embedded in deeper layers of dentinal tubules cannot be effectively eliminated with irrigants alone.

Diode laser and Photo Activated Disinfection(PAD) can be used as adjuncts to conventional root canal disinfection in reducing the bacterial load.[10]Laser assisted disinfection of root canals have been successfully used in several studies to for root canal disinfection along with conventional chemomechanical preparation.[11] However, the use of lasers there has caused concerns about heat damage to the periapical tissues and hence low energy output settings are used for disinfection.[12] The high cost of laser devices makes them unaffordable for most clinicians and hampers their widespread use.

PAD is a new antimicrobial strategy that involves the use of a nontoxic photosensitizer and a light source.[13] The photosensitizer, methylene blue has shown to kill wide range of oral bacterial species.[14]The photosensitizer dye is introduced into the canal which then attaches to the bacterial membrane of microorganisms. When a light of an appropriate wavelength is irradiated , it activates the photosensitizer and reacts with molecular oxygen of the bacterial membrane to produce highly reactive oxygen species, leading to injury and death to microorganisms.[15]The successful use of photo activated disinfection in in combination with photosensitizers has been reported in the literature.[10,14,16]

Since endodontic therapy cannot do away with irrigants, in the present study we aimed to compare antimicrobial efficacy of Diode laser and Photo Activated Disinfection (PAD) with Sodium hypochlorite and Saline as irrigant in eradicating *Enterococcus faecalis* contaminated root canals.

MATERIALS AND METHODS

Thirty extracted human single-rooted mandibular premolars extracted for orthodontic purpose was used for the present study.

Inclusion criteria:

Premolar teeth with single root canal

Exclusion criteria

Premolars with more than one root canal or root canal curvature of more than 25°

Teeth with root caries

Teeth with calcified canals.

After access opening, a #10 K-file was introduced into each canal until it appeared at the apical foramen. The working length was established by subtracting 1 mm from this length. The canals were shaped using Protaper system up to F2 size 20; 6% using EDTA as lubricant between each instruments.

Sterilization of teeth samples:

Teeth samples were soaked in 5% hypochlorite solution for 30mins at 65℃ and rinsed in distilled water. The Teeth sample was next soaked in 70% ethanol for 10mins and rinsed with distilled water. At this stage, Five teeth was randomly selected and was kept as negative control.

Inoculum Preparation

A standard strains of *E.faecalis* (ATCC 29212) was obtained for the study. The bacterium was sub-cultured in nutrient broth at 37°C for 48 hours before inoculating in the root canals.

STUDY MODEL

Inoculation of teeth samples:

Enterococcus faecalis was inoculated into freshly prepared Brain heart infusion broth and was incubated at 37℃ for 24hrs.20µl of cultured Enterococcus faecalis broth was added to the teeth sample on 1st, 3rd, 5th and 7th day respectively using 1ml of sterile insulin syringes without overflowing and placed in upright position and was incubated at 37℃.

Diode laser

A Diode laser with wavelength of 940nm (Photon Dental Diode Laser, Zolar Technology,Canada) was used in the present study.

Photo Activated Disinfection (PAD)

A light-emitting diode (LED) lamp emitting light in the red spectrum with a power peak at 628 nm. (FotoSan;CMS Dental, Denmark) was used in the present study

Experimental Groups

Group 1: Photo activated disinfection and Saline

Five samples were the filled with methylene blue dye 15µg/mL. The tip of the lamp was inserted into the canal orifice and irradiated for 30 seconds by LED lamp 630 nm (Fotosan 630, CMS Dental) with an output power of 2- 4 mw/cm 2 according to manufacturer instructions . The specimen was then irrigated with 5 ml saline.

Group 2: Diode laser and Saline

Five samples were filled with methylene blue dye 15µg/mL. 5ml of Saline solution was agitated with a #15 K file and left undisturbed in the canal for 2 minutes as a pre-irradiation time. This was followed by irradiation with 1.5 W diode laser. The optical fiber was introduced 1 mm short of the apex and recessed in helicoidal movements at a speed of approximately 2 mm/s for 5 seconds. This was repeated 4 times with a 10 second rest period between irradiation to avoid heat build-up.

Group 3: Sodium hypochlorite and photo activated disinfection

Five samples were first irrigated with 5 mL 3% NaOCl for 30 seconds and then treated as the Group 1.

Group 4: Sodium hypochlorite and Diode laser

Five samples were first irrigated with 5mL of 3% NaOCl followed by diode laser irradiation at 1.5 W as the Group 2.

Group 5: Positive control

No experiment was done in this group. The positive control served to determine the total bacterial number and was used to determine the percentage killing by the various experiment treatments.

Sample culturing

The teeth were placed in vials, which contained 2 mL of the nutrient broth. Dilution of the samples was maintained at 10^{-3} . 6 mL of broth, in duplicate, from all the samples together was collected and seeded on a Petri dish containing BHI agar in order to count the CFUs. It was incubated in 37◦C for 48 h in BHI agar. The colony forming units CFUs grown were counted with the aid of a magnifying lens.

Statistical analysis

Statistical Package for Social Sciences [SPSS] was used to perform statistical analyses. The Descriptive analysis included expression of CFUs in terms of Mean & SD. Kruskal Wallis Test followed by Mann whitney Post hoc Analysis was used to compare mean CFUs between the study groups. The level of significance was set at P<0.05.

RESULTS

The results showed a maximum reduction in microbial load in the Group 4: NaoCl and Diode laser followed by Group 3: NaOCl and PAD.The two experimental Group 3 and Group 4 showed comparable results in reduction of *E.faecalis* microorganisms .(p<0.01) .

Table 1: Represents the number of colony forming units (CFUs) in each group

Sl.no	Positive control	Group 1	Group 2	Group 3	Group 4
1	66	43	44	1	0
2	51	25	46	0	0
3	24	48	48	0	0
4	135	60	43	1	1
5	120	74	51	2	1
Mean	79.2	50.0	46.4	0.8	0.4

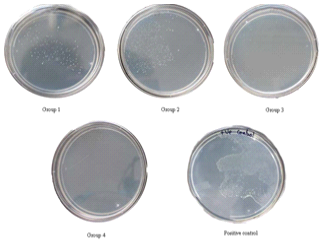

Fig 1: Colony forming units (CFUs) on BHI agar plates of various groups

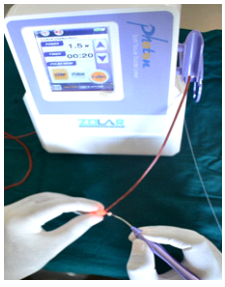

Fig 2: Diode laser (Photon Dental Diode Laser, Zolar Technology, Canada)

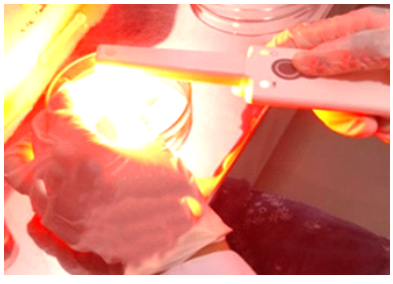

Fig 3: PAD (FotoSan;CMS Dental, Denmark)

Table 2: Comparison of mean CFUs between groups using Kruskal Wallis Test

Groups	N	Mean	SD	Min	Max	P-Value
Group 1	5	50.0	18.4	25	74	<0.001*
Group 2	5	46.4	3.2	43	51	
Group 3	5	0.8	0.8	0	2	
Group 4	5	0.4	0.5	0	1	
Group 5	5	79.2	46.9	24	135	

*- Statistically Significant
Note: Group 1 - Saline/PAD, Group 2 - Saline/Diode Laser, Group 3 -NaoCl/PAD , Group 4 - NaoCl/Diode Laser, Group 5 - Positive Control

Table 3: Multiple comparison of mean difference in CFU levels b/w groups using Mann Whitney Post hoc Test

I Groups	J Groups	Mean Diff. I-J	95% CI of the Diff		P-Value
			Lower	Upper	
Group 1	Group 2	3.6	-39.1	46.3	1.00
	Group 3	49.2	6.5	91.9	0.02*
	Group 4	49.6	6.9	92.3	0.02*
	Group 5	-29.2	-71.9	13.5	0.28
Group 2	Group 3	45.6	2.9	88.3	0.03*
	Group 4	46	3.3	88.7	0.03*
	Group 5	-32.8	-75.5	9.9	0.19
Group 3	Group 4	0.4	-42.3	43.1	1.00
	Group 5	-78.4	-121.1	-35.7	<0.001*
Group 4	Group 5	-78.8	-121.5	-36.1	<0.001*

*- Statistically Significant
Note: Group 1 - Saline/PAD, Group 2 - Saline/Diode Laser, Group 3 -NaoCl/PAD , Group 4 - NaoCl/Diode Laser

DISCUSSION

Many investigators have explored elimination of *E. faecalis* in the root

canal, and different irrigation techniques and devices have been developed to enhance the efficacy and distribution of irrigants. [17]The present study compared the antimicrobial efficacy of Diode laser and Photo Activated Disinfection along with Sodium hypochlorite and Saline in *E.faecalis* contaminated root canals .The rationale for combining Diode laser and PAD with conventional irrigants, was to simulate clinical root canal cleaning and shaping procedures.

Photo activated disinfection uses low level laser as a light source which produces a monochromatic beam of specific wave- length to activate the photosensitizer. [18]PAD acts by activating nontoxic photoactive dye when a light of a specific wavelength is irradiated in the presence of oxygen. The photoactivated dye interacts with the biological substrate to the produce highly reactive oxygen species, such as singlet oxygen and free radicals. These molecules kill microorganisms by damaging the cell membrane, proteins, membrane lipids, and nucleic acid. [19] The photosensitizer dye increases the bactericidal effects and also effectively decreases the thermal energy required for killing of microorganisms.[20] Lasers along with conventional chemomechanical preparation are being used as an effective approach to cleaning and disinfection of infected root canal.[11] Diode lasers with wavelengths ranging between 625 and 805nm have been used in previous studies to eliminate *E. faecalis* from infected root canals. [21]The bactericidal effect of Diode laser is by heat and thermal photodisruptive action on microorganisms present deep within the dentinal tubules.[22]The use of diode laser in for disinfection of root canals has proven to be effective in other studies.[14,20]

In a systematic literature review Siddiqui et al. reported that photodynamic therapy with lasers effectively reduced *E. faecalis* counts in infected root canals compared with traditional endodontic instrumentation/ irrigation treatment protocols. [21]Mehrvarzfar et al. suggested that a combination therapy of chemical irrigation and laser irradiation could totally eliminate all root canal pathogens including *E. faecalis*.[23]A study by Gutknecht et al. used diode laser with 980 nm wavelength for disinfection of root canals contaminated by *E. faecalis*, eliminated the microorganism up to 500 µ depth. [24]In a study by Rios et al showed that Photo Activated Disinfection using a dye and a LED lamp has the potential to be used as an adjunctive antimicrobial procedure in conventional endodontic therapy.[25]

Our study combined chemomechanical irrigation and photodynamic therapy which effectively eliminated E. faecalis microorganism further validating previous studies .[26,27]The results obtained in our study showed that Diode laser and NaOCl and PAD and NaOCl were equally effective for disinfecting the root canals containing *E.faecalis*.($P<0.01$)While lasers generate heat that may be harmful and can potentially injure periapical tissues. LED light used in Photo Activated Disinfection serves as a safer alternative because it does not generate significant heat as compared to laser.[25]LED lamps have lower thermal productivity and minimal injuring of tissue. LED lamps are also more cost effective since, the energy consumption is lower. Comparing to laser, they are cheaper and also portable and easy to use. [7] The LED lamp used in the present study provides a considerably higher energy output, emitting 1 J/s. The temperature rise at the root surface was not measured in this study, but it is unlikely that any heat damage. [18]

CONCLUSION

From our study, a combination of sodium hypochlorite irrigation activated with Diode or PAD showed similar outcome. PAD can be considered as an alternative to Diode Laser in routine dental practice for canal disinfection. Additional studies should be conducted to determine the optimal settings for parameters, such as dose, power, photosensitizer molecule and concentration, pre-irradiation time, and exposure.

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