“Evaluation of the antibacterial efficacy of EZLASE diode LASER on the infected root canal system:” An in vivo study

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Abstract

Aims and Objectives: The aim of this in vivo study was to evaluate the antibacterial efficacy of a diode LASER on the infected root canal system.

Methodology: A total of forty patients with infected root canals were selected. The root canals were prepared mechanically, and irrigation was done with 3% sodium hypochlorite (NaOCl). Microbiological samples were collected before LASER treatment with the help of the master apical file (MAF) to full working length for subsequent microbiological examination. The procedure was followed by laser treatment with an EZLASE diode LASER (940 nm), at different output powers. Following irradiation, samples were collected for microbiological examination. The samples were transferred to an agar plate and incubated for 48 h at 37°C. The colonies formed were then counted, and the total number of bacteria per ml before and after LASER application was assessed. Colony-forming unit (CFU/ml) was counted for Enterococcus faecalis and other bacterial flora from pretreatment and posttreatment samples that were then analyzed using the Kruskal–Wallis test and Mann–Whitney U-test.

Results: The highest power of laser used (1.95 W) had resulted in maximum reduction of bacterial flora and E. faecalis counts.

Conclusions: Combination therapy consisting of irrigation using NaOCl and LASER irradiation, especially at high output power was an effective treatment option for a reduction in E. faecalis as well as other bacterial flora from the root canal system.

Keywords: Bacterial flora; Enterococcus faecalis; EZLASE diode LASER; in vivo study

INTRODUCTION

It has always been a major goal of endodontic treatment to achieve a bacteria-free environment in the root canal system to prevent any risk for successful treatment.[1] The process of root canal disinfection is by the mechanical action of instruments and chemical action of irrigating solutions.[2] The eradication of persisting microorganisms in distant areas of the tubular system is a major challenge in the present day treatment regimen.[3,4] To improve the efficacy of irrigants, dynamic irrigation techniques such as the use of sonics and ultrasonic devices have been employed.[5] Various LASER systems have been examined as adjuncts to currently used disinfection methods in root canal treatment.[6] LASER technology enhances cleaning ability, removal of debris, and the smear layer from the root canals thus improving the decontamination of the endodontic system.[7] Different wavelengths have been shown to be effective in significantly reducing bacteria in infected canals, and studies have confirmed these results in vitro.[8] Further studies have revealed the efficiency of the LASER in combination with commonly used irrigants, such as 17% Ethylenediaminetetraacetic acid, 10% citric

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acid, and 5.25% sodium hypochlorite (NaOCl). The use of diode LASER in endodontic treatment has been increasing in recent years. Hence, the present study is designed to evaluate the antibacterial effectiveness of a diode LASER (940 nm) at different output powers.

**Aims and objectives**
The aims of this *in vivo* study were to evaluate the antibacterial efficacy of a diode LASER at different output powers on the infected root canal system and to compare the antibacterial efficacy of a diode LASER with 3% NaOCl irrigating solution.

**METHODOLOGY**
The study population included patients (20–50 years) who reported to the Department of Conservative Dentistry and Endodontics requiring a root canal treatment. The sample size was determined using the bio-statistician using G-power software (G-power: Universität Düsseldorf). A total of forty patients with infected root canals and only single-rooted teeth were selected. All the teeth showed radiographic signs of apical inflammation which mainly included widening of the periodontal ligament and periapical radiolucency. Pulpal state of all the teeth was assessed with the help of thermal vitality test (cold test). A detailed medical and dental history were recorded to fulfill the inclusion/exclusion criteria, as well as informed consent, was priorly obtained from all the patients participating in the study. The ethical clearance was obtained from the Institutional Ethical Committee Review Board.

The forty patients taken up for the study were randomly allocated to one of the four study groups, each group having ten patients as follows:

- **Group 1**, \( n = 10 \) LASER irradiating samples at 1.05 W along with 3% NaOCl
- **Group 2**, \( n = 10 \) LASER irradiating samples at 1.5 W along with 3% NaOCl
- **Group 3**, \( n = 10 \) LASER irradiating samples at 1.95 W along with 3% NaOCl
- **Group 4**, \( n = 10 \) Control group (without irradiation) 3% NaOCl alone

**Inclusion criteria**
1. Patients willing to be part of the study and ready to give written informed consent
2. All teeth with radiographic evidence of a single-canal completely developed roots without root caries and previous root canal treatment
3. Patients requiring root canal treatment of single rooted teeth and gave an exaggerated, delayed, or negative response to thermal tests.

**Exclusion criteria**
1. Pregnant women and lactating mothers
2. Patients not agreeable and compliant with the terms of the study
3. Patients with systemic conditions
4. Patients with a history of allergy to any of the components of the study materials
5. Patients who have used systemic antibiotics within the past 6 weeks
6. Patients with compromised periodontal status.

After the application of rubber dam (Hygenic, Coltene Whaledent), access was gained, working length was determined, and the root canals were prepared mechanically up-to size 50 H-file (Mani INC) and irrigation with 3% NaOCl (Vensons Mumbai, India) was done. Microbiological samples from the dentinal walls were collected before the LASER treatment with the help of a sterilized master apical file (MAF) size 50 H file to the full working length for subsequent microbiological examination, and hence that bulk of sample could be collected in the grooves. Then, 15 mm of the MAF (apical tip) was aseptically cut off into a vial containing 1 m brain heart infusion (BHI)-broth immediately after being removed from the root canal to prepare the sample for the subsequent microbiological examination. The control group was only irrigated with 3% NaOCl. The root canals were irradiated with a 940 nm EZlase diode LASER (BIOLASE) at the output power 1.05 W, 1.5 W and 1.95 W for 15 s. The optical fiber (BIOLASE) was inserted as far as the working length previously determined. The LASER was then activated, and the root canal was slowly irradiated from apical to coronal in continuous circling movements to treat all dentinal tubules in one cycle for each power [Figure 1]. Following irradiation, microbiological samples were collected using MAF size 50 H-file to full working length to collect dentine shavings from the root canal walls. Then, 15 mm of the MAF (apical tip) was aseptically cut off into a vial containing 1 m BHI-broth. Collected samples in vials-containing transport media were mixed thoroughly using Vortex to achieve a uniform sample solution. Microbiologic analysis began with taking 1 ml of solution sample from each vial using a micropipette and then inoculated in the agar plate (enriched and selective culture medium according to the requirement). To determine the growth of facultative anaerobes, *Enterococcus faecalis* samples were incubated at 37°C for 48–72 h in 5%–10% CO₂ jar. For obligate anaerobes, samples were incubated at 37°C for 3–4 days in an anaerobic jar. After completion of incubation, the colony characters of the required organisms were noted and also the colony count was done for quantification.

These organisms were confirmed by Gram staining and key biochemical tests.
The colonies were then counted, and the total number of mixed bacterial flora and *E. faecalis* (Colony-Forming Units = CFU) per ml were assessed before and after LASER treatment. [Graph 1]

**RESULTS**

According to statistical analysis, maximum reduction in *E. faecalis* count (CFU/ml) was seen in Group D with the highest power of LASER 1.95 watts with 3% NaOCl - 72.3% followed by Group C 48.13%, Group B 25%, and Group A 20% using the Wilcoxon matched pairs test. There was no statistically significant difference between groups with respect to reduction in *E. faecalis* with a value of $P = 0.123$, by Kruskal–Wallis ANOVA. [Table 1]

The statistically significant difference for *E. faecalis* was seen from pretreatment to post-treatment samples in Group C with $P$ value being 0.0431 ($P < 0.05$). In case of bacterial flora count (CFU/ml), maximum reduction was seen in Group D with the highest power of LASER, i.e., 1.95W with 3% NaOCl 77.78% followed by Group C 59.57%, Group A 44.10% and Group B 35.23%. By Wilcoxon matched pairs test. [Table 2].

The statistically significant difference for bacterial flora was seen from pre- to post-treatment in all the groups with $P$ value being 0.0117 for Group A, 0.0180 for Group B and 0.0176 for Group C and 0.0431 for Group D ($P < 0.05$).

The statistically significant difference for bacterial flora was seen between Group A and Group C with $P = 0.0176$ and between Group A and Group D with $P = 0.0440$ ($P < 0.05$) [Table 3].

**DISCUSSION**

This study was conducted to evaluate the antibacterial efficacy of diode LASER in combination with 3% NaOCl, at different output powers to disinfect contaminated root canals. NaOCl in varying concentrations is the most commonly used root canal irrigant, owing to its ability to kill bacteria, destroy biofilms and dissolve vital and necrotic tissues.  

LASER has shown to possess good antimicrobial properties. Different studies have evaluated various types of wavelength for disinfection of root canals. In a study in assessing the bacterial effect of diode LASER (810 nm) in deep layers of infected root canal wall, reduction in their count was achieved to 74%, Furthermore, when...
diode LASER with wavelength of 980 nm in continuous mode was used, it could increase the success rate of treatment by the elimination of bacteria in deep dentin.\[^{[13]}\]

The diode LASER of 940 nm wavelength was used in the present study since its clinical application in endodontics has been scarcely studied and also because of its low cost, greater versatility, and portability due to its compact size. The thin flexible fiber of 200 μm tip provides better access to the apex. The penetration of diode LASER energy into the dentinal tubules is better than erbium, chromium: yttrium-scandium-gallium-garnet laser due to less absorption of the LASER beam by both hydroxyapatite and water. It also works efficiently at lower power with less heat production with power output ranging from 0.5 W to 7 W.\[^{[14]}\]

Using different outputs, all the wavelengths destroy the cell wall due to their photothermal effect. Due to the structural characteristics of the different cell walls, Gram-negative bacteria are more easily destroyed with less energy and radiation than Gram-positive bacteria.\[^{[15]}\] The superior bactericidal effect of diode LASER irradiation could be attributed to its greater depth of penetration (more than 1000 μm into dentinal tubules). In addition, the diode LASER causes a thermal photo disruptive action in the unreachable parts of dentin, resulting in an enhanced bactericidal effect in the root canal dentin.\[^{[16,17]}\]

The high-power diode LASER reduces dentine permeability.\[^{[18]}\] Its light presents a spectrum that allows for greater absorption by water than dental tissues when compared to neodymium-doped yttrium aluminum garnet (Nd: YAG) LASER.\[^{[19]}\] As the antibacterial property is an important feature of irrigating solution, this study was conducted with an aim to measure the antibacterial efficacy of diode LASER with or without conventional irrigation. To evaluate the antibacterial property, bacterial load was analyzed using bacterial culture method.

In the present study, the maximum reduction in bacterial load is seen in the group with the highest power of diode LASER along with NaOCl. The results of this experiment are comparable with the results obtained by Hendy and Nagwan who showed significant reduction of *E. faecalis* quantity in root canal following irradiation by Nd: YAG and diode (1064 nm) LASER. In the present study, the maximum reduction in *E. faecalis* is seen in Group D, in which highest power of the laser, i.e., 1.95 W and 3% NaOCl was used in combination. Results supporting the present study were obtained when Kreisler *et al*. investigated the bactericidal effect of a semiconductor LASER used in combination with NaOCl/hydrogen peroxide irradiation.\[^{[20]}\]

Recent studies indicate that in addition to the bactericidal effect, diode laser has a biostimulative effect which is of great importance in regard to the healing of periapical tissues.\[^{[21]}\]

### CONCLUSIONS

Within the limitations of the present study, it is concluded that

1. The highest power of LASER (1.95 W) has resulted in a maximum reduction of bacterial flora and *E. faecalis* counts
2. Combination therapy consisting of irrigation using NaOCl and LASER irradiation is an effective treatment option for reduction in *E. faecalis* as well as other bacterial flora from the root canal system.

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### Conflicts of interest

There are no conflicts of interest.

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