International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 7 Issue 6, June-2018, Impact Factor: 4.059

Evaluation of the bacterial recovery after root canal disinfection with Er,Cr:YSGG laser an in vivo study

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ABSTRACT

Aims: The basis of the study was to demonstrate the effectiveness of Er,Cr:YSGG laser against Enterococcus faecalis in root canals, and to assess its count after seven days of irradiation with different powers of laser.
Methodology: Forty-five single rooted teeth, indicated for endodontic management with intact crowns. Root canals were exposed to instrumentation with normal saline followed by treatment with Er,Cr:YSGG laser radiation then samples taken from the root canals before laser irradiation, immediately after irradiation, and after 7 days.
Results: Microbiological examination detected reduction in bacterial colonies in all cases immediately following irradiation, while after seven days there was growth of E. faecalis.
Conclusions: Heat resistant bacteria need multiple visits for root canal laser application to ensure complete killing of these bacteria.
Keywords: Laser, *E. faecalis*, Root canal disinfection, One visit laser application.

HOW TO CITE THIS ARTICLE

Njwan F. Shehab, "Evaluation of the bacterial recovery after root canal disinfection with Er,Cr:YSGG laser an in vivo study", International Journal of Enhanced Research in Science, Technology & Engineering, ISSN: 2319-7463, Vol. 7 Issue 6, June -2018.

INTRODUCTION

Endodontic treatment in dentistry is very important. Patients ask dentists to save their teeth with no further complications. Then treated teeth crowned or subjected to expensive reconstruction procedures. Therefore, this technique must have long-term prognosis. Dispose of bacteria by the conventional methods of disinfection have many defect which lead to failure [1], due to anatomical complexity of root canals, limitations of instruments, irrigants and intracanal medicaments[2].

*E. faecalis*caused failure in endodontic treatments because it is penetrated easily into the lateral dentinal tubules, which regarded as the ideal condition for its growth and from this reservoir, reinfection occurs[3].The presence of these pathogens inside the root canals may increase the risk of iatrogenic exacerbations (flare ups) when infected dentin debris is transported into the apical region[4].The criteria for success, is to completely eliminate all bacteria within the canals of teeth[3].

Today, root-filled teeth have better prognosis after application of laser in the field of dentistry. laser light, penetrates up to >1000 μminto the dentin. This provides a distinct advantage, since *E.faecalis*could invade dentinal tubules to a depth of more than 1350 μm. Moreover, effectiveness of laser radiation against bacteria cause changes in the bacterial cell wall that lead to killing of bacteria[5].For this reason, the aim of the study was to evaluate in vivo the effectiveness of Er,Cr:YSGG laser against *E. faecalis* infected root canals in one visit laser irradiation and to determine the condition of root canals in term of sterilization after seven days of laser application.

MATERIAL AND METHODS

Patient Selection:
Forty-five patients with the ages ranged between 21-35 years. A medical and dental history was obtained from each patient. Patients were not involved if (1) pregnant and systemically ill patients were on antibiotic therapy for 2 weeks before the treatment, (2) tooth not suitable for isolation by rubber dam, (3) teeth with large per apical lesion and there was sinus tract. Only anterior or lower premolars with single root were enrolled in the study. Canals of root were detected radio-graphically. Patients were divided randomly into three groups, fifteen patients in each.

In group I, root canals disinfected with laser at the output power of 0.75W. In group II, root canals disinfected with laser at the output power of 1W.

In group III, root canals disinfected with laser at the output power of 1.5W.

Microbiological Sample Collection:
Samples were obtained from the canal for each patient before laser application and immediately after laser treatment in the first appointment then after 7 days in the second appointment.

Local anesthesia was administered. Carious lesion was removed, isolation was performed using rubber dam then access cavity was prepared, working length was detected. Instrumentation was done by manual step-back technique using k files with irrigation by normal saline to avoid any disinfecting action. Biomechanical preparation was established until 3 file sizes greater than the initial file, and root canal was dried with sterile paper points. After that the last file that used to full working length was taken to collect dentine shaving from the canal wall and its 15mm of apical tip was cut off aseptically into 1m Brain-Heart Infusion Broth(transport medium) immediately for the microbiological examination[6].This procedure was followed by laser treatment. The infected canals exposed to Chromium: Yttrium-Scandium-Gallium-Garnet (Er,Cr:YSGG) laser irradiation, λ=2.78μm, with radially emitting firing tip type RFT3 that mounted on a 90°-angled hand piece. The laser was adjusted at the following powers 0.75W, 1W and 1.5 W, 20Hz, 10% air flow without water. The laser tip was inserted in canals as far as the apex; the correct insertion depth was ensured by measure. The canal slowly irradiated in a continuous circling movement by hand up and down in a cervical-apical and apical-cervical direction to treat all dentinal tubules. The irradiation procedure was repeated four times of 5second, resting approximately 15 second between each irradiation [7].

Following irradiation, the second microbiologic sample was obtained as mentioned above, then the exposed root canal sealed with sterile cotton pellet (without any medicament) then sealed with zinc phosphate cement. After that transfer samples for microbiological study. Another microbiological sample collected after seven days from the same tooth of patients.

Microbiological Study:
Each screw-capped vial of BHT-broth containing sample was shaken to disperse the sample content evenly, 0.1ml of it was taken using micropipette that cultured on Enterococcus agar and incubated aerobically for 48 hours at 37°C. Plates were examined and the numbers of colonies were counted.

RESULTS

One way analysis of variance (ANOVA) at level ($P \leq 0.05$) performed to evaluate the differences in the colonies number of *E.faecalis* before and following laser application in root canals. This is shown in (Table 1and 2) and (Figure 1, and 2).

Statistical analysis detected that high rate of *E.faecalis* in the canals before laser treatment, and no differences significantly among groups before laser treatment. Analysis demonstrated significant differences in mean colony count between groups of samples before treatment and immediately treated groups at all output powers. Also there were differences in colony count significantly between groups of samples before irradiation and samples collected after 7days at the output powers 1W and 1.5W but at the power of 0.75W the result detected reduction in the bacterial colonies but not different significantly from colonies number before laser treatment.

When comparison was made among the selected powers in the groups of samples which were taken immediately following laser application, the result detected significant difference among them. Furthermore, the result of the statistic showed no significant differences in the colonies number get it immediately after treatment with a power of 0.75W and after 7 days at the output power 1W.

Table (1): One way analysis of variance for the differences on the antibacterial effect at all output powers of laser.

	Sum of Squares	Df	Mean Squares	F	P-value*
Between Groups	75145.659	8	9393.207		
Within Groups	2556.533	126	20.290		
Total	77702.193	134		462.949	0.000

*df=degree of freedom
*P≤0.05 mean significant different exist.

Table (2): Duncan's New Multiple Test for the comparison at all powers of laser inroot canals and comparison between the counts of bacteria immediately and following 7 days of exposure to laser

Groups of laser powers	Microbiological Sample Collection		
	Mean±SD**		
	Before treatment	Immediate after laser treatment	After7 days of laser treatment
0.75W I	**89.66±3.71 EF***	**79.53±4.06 D**	**87.46±3.20 E**
1 W II	**91.86±4.24 F**	**62.20±6.76 B**	**82.00±3.27 D**
1.5W III	**92.73±4.38 F**	**13.33±3.92 A**	**72.66±5.74 C**

*The different letters mean significant difference exist.
**SD= Standard deviation.

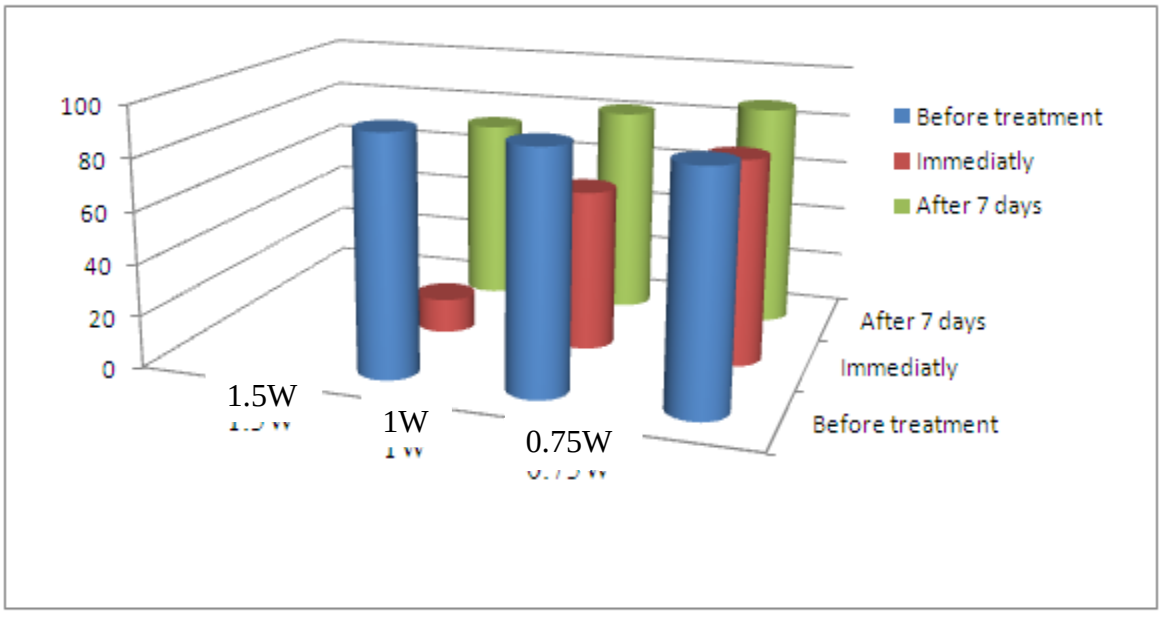

Figure (1): A histogram showing the performance of laser on the *E. faecalis* immediately after irradiation and after 7 days of laser application.

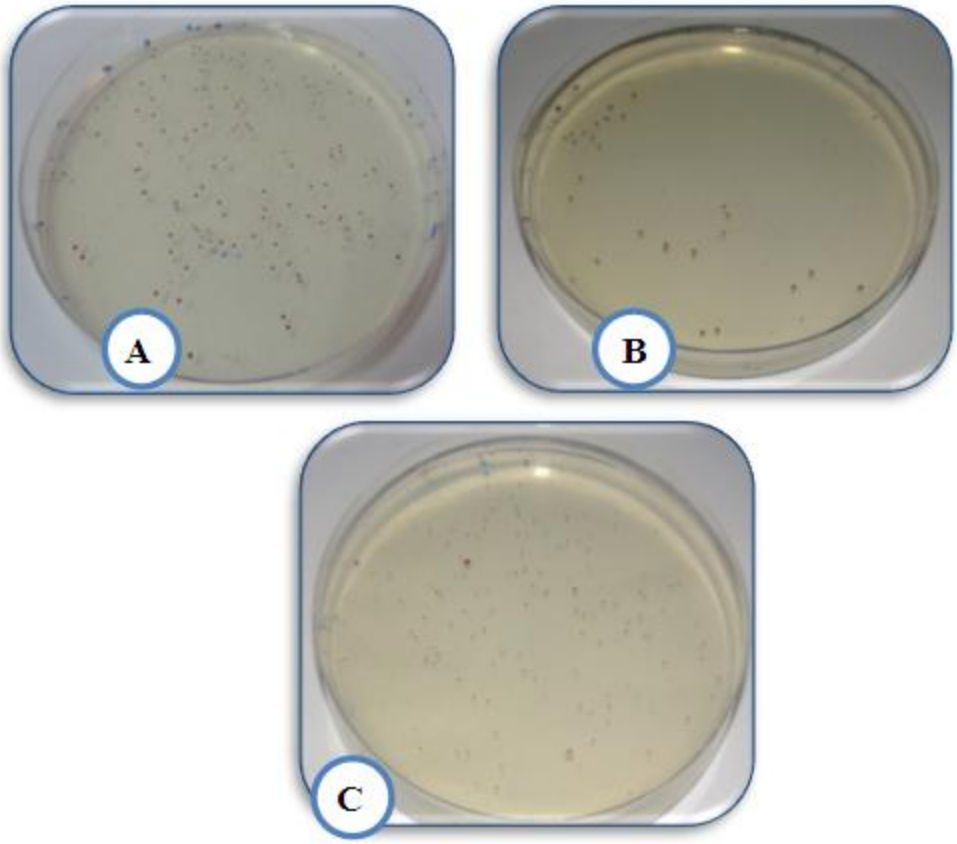

Figure (2): Number of bacterial colonies: (A) before treatment. (B) After laser treatment in first appointment. (C) After 7 days of laser treatment.

DISCUSSION

Bacterial free root-canals still the mainaims of endodontic therapy, which concentrated on disinfection to remove the remaining bacteria from narrow areas of root-canal system which cannot be accessed by irrigating solution[8].

*E. faecalis*isa persistent micro-organism probably live within the root canal as a single organism and it has a role in the persistent of periradicular infection[9]. This species exist in primary endodontic lesions and in secondary endodontic apical lesions [10].

One of the factors affecting the use of laser in canals is the exiting of laser beam from the tips of apex used to transfer radiation on account of the lateral surfaces of root canals cannot be affected by laser radiation. Therefore, Stabholz et al., 2003[11] discover a new endodontic tip called "Side firing" by which laser light exited laterally from the tip sides. By elimination of these obstacles, laser in root canal disinfection getting more accurate. Er,Cr:YSGG laser was used in this study because of its used with emitting firing tip. The results detected *E. faecalis* in canals before treatment which is agree with the results that obtained by Chiara et al., 2008[10] who showed that *E. faecalis* was reported in primary endodontic infections.

The results proved the ability of laser radiation to kill bacteria in the infected canals when the samples were immediately taken from root canals. This explained the capacity of laser to achieve expansion and collapse of intratubular water. This micro-pulse-induced absorption was able to produce waves efficiently strong to kill intratubular bacteria[12].

The study detected significant differences against the bacteria at all powers against this bacteria also found that bacterial recovery decreased when irradiation duration or power increased. Kolnick 2011[12] showed that reduction of 99.7% of *E. faecalis* in canals.

The mean value of *E.faecalis* growth in samples that taken after 7 days of irradiation in the same patients at all selected powers is very high with no difference significantly between these samples and before irradiation. This may explained that persistent ability of *E.faecalis* for a long time and multiply rapidly in dentinal tubules. Furthermore, dentinal tubules act as a great reservoir of bacteria completely outside immunological control[10].

Due to persistent of *E. faecalis* in the root canals after laser therapy. Therefore, greater than one visit of irradiation must be considered. Additionally, more effective clinical methodologies for disinfection of root canals must be established to eradicate this pathogen in the course of endodontic treatment[13]. *E. faecalis* may survive in the smear layer and in debris inside the root canal, laser has the ability to get rid the smear layer, which is difficult to be removed in the apical third of root canal by irrigation and instrumentation[14].

Finally, it is very important in endodontic therapy to achieve total elimination of all bacteria from the root canal.

CONCLUSION

*E.faecalis*required multiple visits irradiation with Er,Cr:YSGG laserduring the disinfection.

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