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The bactericidal effect of the Nd: YAG laser on the bacteria of the infected root canals: an in vitro study

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Abstract

It is very important to obtain the maximum sterilization and antibacterial effect during treatment of the infected root canals. This study aimed to explore the bactericidal effect of the Nd:YAG laser. The method basically is based on irradiating a bacterial sample by Nd:YAG laser and calculating the number of colony forming units (that will appear on agar plates) before and after laser application. 30 swabs were taken from infected root canals immediately after tooth extraction and immersed in a tubes containing liquid nutritive media. These swabs undergone a serial dilution and then streaked on a blood agars and incubated. After 24 hours, the colony forming units were calculated. The sample (in the tube) then irradiated by laser, and also streaked on blood agar and incubated. The colony forming units were calculated again. Nd:YAG laser showed a weak bactericidal effect with about 25% of the bacterial cells were eradicated. based on the results of this study, the Nd:YAG laser was merely effective as a bactericidal agent.

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Introduction

Infection of the root canal (Pulpitis) is inflammation of the dental pulp resulting from untreated caries. Its principal symptom is the pain. Diagnosis is based on signs and symptoms and is confirmed by x-ray. Treatment involves removing decay, root canal therapy or extracting the tooth (R. Rajendran, 2009). Endodontic treatment, is a sequence of steps for the infected pulp of a tooth which leads to the elimination of infection and the protection of the decontaminated tooth from future microbial invasion (*Cohen, Stephen 2006)*. Studies have reported different species in the pulp of necrotic teeth and, therefore, one or multiple kinds of bacterial pathogens can be isolated from an infected root canal (Gunnar Bergenholtz 2009, Philip D Marsh, Michael V Martin 2009), for example (*Prevotella spp, Porphyromonas spp, Fusobacterium spp, Actinomyces spp, Streptococcus* spp, *Enterococcus faecalis*) (Narayanan, L Lakshmi, and C Vaishnavi 2010). The use of irrigating solutions is an essential part of effective chemomechanical preparation. It enhances bacterial elimination and facilitates removal of necrotic tissue and dentine chips from the root canal; thus irrigants prevent packing of infected tissue apically in the root canal and into the periapical area. Besides, many irrigating solutions have other beneficial effects. The most popular types of root canal irrigants are (ethylene–diamine–tetra-acetic acid (EDTA), Sodium hypochlorite (NaOCl), Chlorhexidine gluconate (CHX).

This study is done to test the ability of Nd:YAG laser to kill the bacteria of infected root canals, since the laser has many advantages over the conventional root canal sterilizers, such as bending around corners, and more penetration depth. Laser systems were widely involved in dental treatments. Many applications of lasers in treatment protocols for dental hard and soft tissues are in use now or being developed. Also, new wavelengths and different methods, are being applied in the dental field. In various laser systems used in dentistry, the emitted energy can be delivered into the root canal system by a thin optical fiber (Nd:YAG, ErCr:YSGG, and diode). Thus, the potential bactericidal effect of laser irradiation can be efficiently used for the additional cleaning of the root canal cavity after biomechanical instrumentation. This effect was studied extensively using lasers such as Nd: YAG, diode, and Er: YAG (Layla Mohammed Hassan Al – Aamirry 2003). Previous endodontic studies using different wavelengths of high-intensity laser light have shown the ability of their thermal impacts in the sterilization procedure of dental hard tissues, either in the reduction of bacterial counts in contaminated root canals as well as in the apicectomies at the surgical site. This has been considered a significant advantage over the traditional root canal disinfection procedures (Eduardo Sheila 2001). Nd:YAG laser 1064 nm is one of the laser systems that have many applications in the dental field. Irradiation with Nd: YAG laser is absorbed by protein (high affinity to melanin) and mineral structures, such as phosphates and carbonate hydroxyapatite, as well as water (low affinity). Utilizing thermal stresses created inside the tissue, Nd: YAG disorganizes the skeleton of bacterial cells. In this process, protein denaturation takes place and the bacterial cells die (Adam Stabholz *et al.* 2004) . This experiment aims to determine the effectiveness of Nd: YAG laser and NaOCl in killing bacterial cells, and clarify if the Nd:YAG laser is powerful enough (as the NaOCl) in root canal disinfection.

Materials and methods

30 Swabs were taken from infected root canals immediately after tooth extraction and immersed in tubes containing liquid nutritive media. These swabs undergone a serial dilution to decrease the concentration of the sample. one ml from the bacterial sample was placed in an Eppendorf tube and irradiated by laser. Swabs were taken from the sample (before and after laser exposure) and streaked on agar plates to record the number of colony forming units.

Laser-sample setup

The sample held tightly by an adjustable clamp which is carried by a carrier and stabilized on the ground by a stand. It was placed at 5 cm from the laser beam (because of manufacturer setup) just at the metal

opening of the handpiece. The laser handpiece also held by the same way as for the sample as shown in Fig.1.

Laser parameters and irradiation method

The Eppendorf tubes were irradiated by Nd:YAG laser for two minutes, and a swab was taken after irradiation to count the number of CFU. The laser-sample set up was done in clinics of laser institutes for postgraduate studies/ Baghdad University. The laser device used in this study (Cynergy Changchun, Korea) is long pulsed solid state (delivery system: free running pulses) with 1064 nm wavelength and maximum energy 110 joule. The pulse duration ranging from 5-40 ms and the frequency from 0.5-2 Hertz. The laser parameters used in this study were 110-joule energy, 2 Hertz frequency, 40 ms pulse duration, treatment time for each sample were 120 seconds. 6 mm spot size (the same diameter of the Eppendorf tube opening), energy density $3.8924*10^4$ j/ cm^2. All parameters are listed in table 1.

Statistical analysis

Done by using SPSS (student version).

Results

After laser exposure, the number of CFU on the blood agars were calculated. The long pulsed Nd:YAG laser reveals weak level of antibacterial activity where only about 25% of bacterial cells died as listed in table 2 , Fig.2, Fig.3.

Table (2) shows the numbers of CFUs/ml before and after laser irradiation, and the numbers are not really decreased after laser exposure.

Table 1. Shows the parameter of the laser device used in the study.

No.	The parameter	Value of parameter	unit
1	Wavelength	1064	nm
2	Energy	110	J
3	energy density	$3.8924*10^4$	j/cm^2
4	Pulse duration	40	Ms
5	Frequency	2	Hz
6	Spot size	6	mm
7	Time of treatment session	120	Second.

Fig(2) shows colony forming units grew on blood agar, with no big difference in their numbers after laser irradiation.

Fig. (3) is a statistical analysis by using stem-leaf plot method, shows that the numbers of CFUs/ml is not highly diminished.

Discussion

One of the most important goals of root canal treatment is removing of these inflamed tissues from the root canal cavity and eradication of bacteria living inside. In this study, the work achieved on a Biomass, which means all the living micro-organisms were included (all species were targeted). Metal files are used to clean and shape the canal walls and chemical solutions with bactericidal effects such as (NaOCl, CHX, EDTA...etc) (Bettina Ruth Basrani 2015, Nisha Garg and Amish Garg 2013) are used to kill bacterial cells and also to facilitate the movement of the files inside the canal and washing of debris to the outside. In last years, the laser has been exercised widely in different branches of dentistry, either in soft tissue treatment, or in the cutting of bone and dental hard tissues. Lasers in recent time are frequently used dealing with oral lesions and infections or in the eradication of bacteria (especially in root canal treatment). Many types of lasers have been used in endodontic treatment.

The most popular one is the diode laser (because of its availability and low device cost).

Table 2. Shows the number of cfu/ml before and after laser exposure.

No.	Before laser exposure	After laser exposure
1	1220	840
2	1540	1160
3	1160	920
4	1070	790
5	1450	1200
6	1270	980
7	1380	1080
8	840	560
9	670	410
10	1420	1170
11	1260	1010
12	1600	1320
13	790	490
14	1050	800
15	1120	870
16	1210	940
17	710	460
18	920	620
19	1000	740
20	1140	920
21	1080	810
22	970	690
23	1110	810
24	890	570
25	950	670
26	1000	680
27	780	520
28	1040	760
29	880	600
30	910	650
Total	32430	24040
Avg.	1081	801.3

The other types of laser devices also have been practiced in endodontic treatment such as (Er:YAG, ErCr:YSGG, Nd:YAG) in many institutions that provide dental care services. The main uses of lasers in root canal therapy are killing of bacteria, shaping of the canal, and activation of chemical irrigation solutions. The action of the laser in such treatments is explained by the thermal effects that generated by the laser beam. The Nd:YAG laser classified as solid-state laser because it contains a solid lasing medium (Neodymium-doped yttrium aluminum garnet). The wavelength of this type is 1064 nm and it is also available as a second harmonic generation with 532 nm. The laser beam in this wavelength could be delivered optically by using optical fibers. The Nd:YAG laser considered as soft tissue laser, since the laser beam has a peak absorption in pigmented tissues that contain dark pigments such as melanin and hemoglobin. There are many types of bacteria that produce pigments (e.g., black pigmented bacteria), and these types can absorb the Nd:YAG beam easily (Schoop U, Kluger W *et al.* 2004) . The Nd:YAG laser has a good penetration depth through the dentinal tubules.

The results of this research have showed a weak bactericidal effect of the Nd:YAG laser. The numbers of the colony forming units were 25% reduced after laser irradiation, which means that the laser wavelength is not well absorbed.

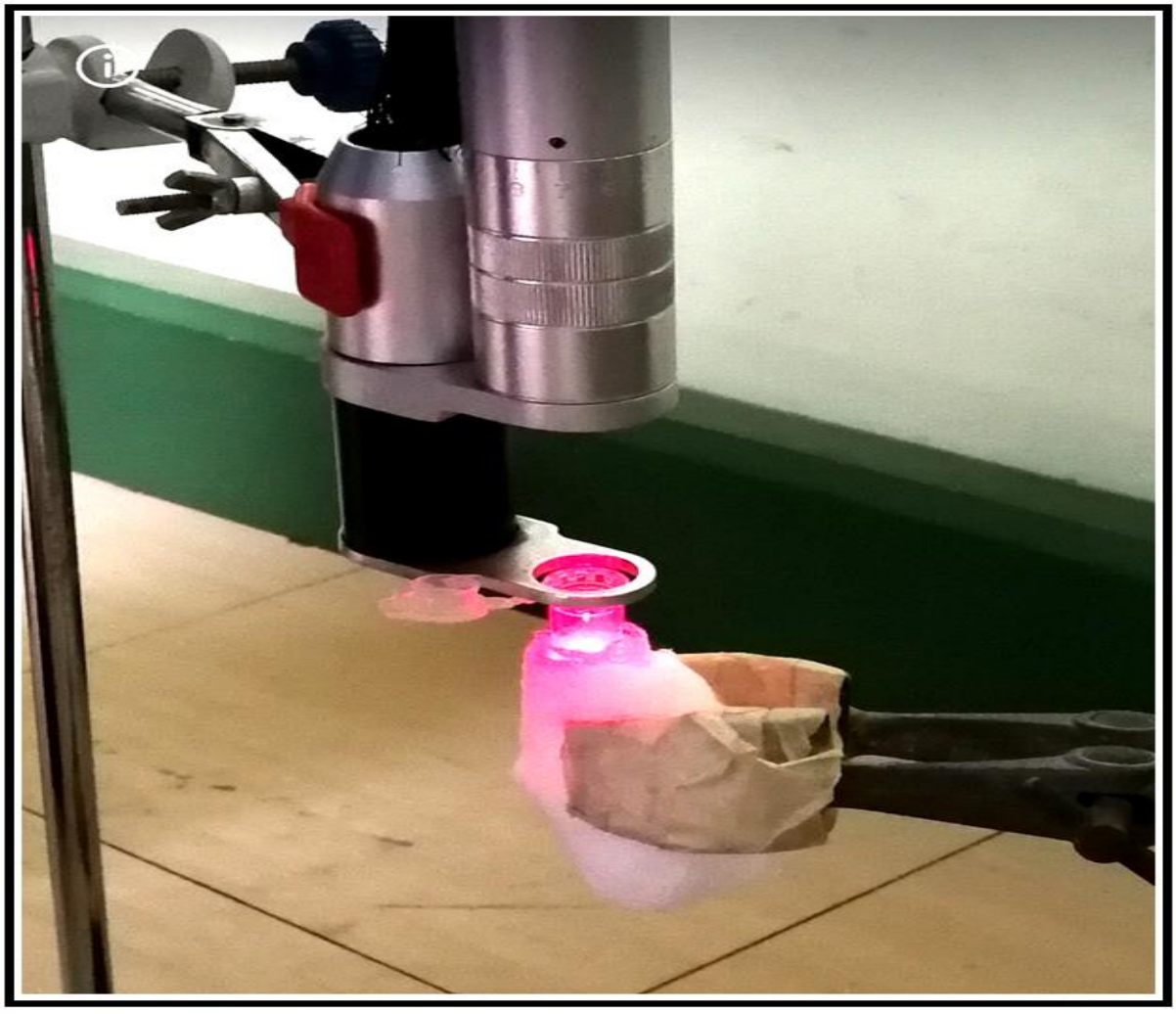

Fig. 1. Laser-sample setup.

only few bacterial cells absorp the laser light and affected by it. That explains why only 25% of the cells died.

Berkiten *et al.* has tested the antibacterial efficiency of Nd:YAG laser and measure the penetration depth of the laser beam under SEM. He used two powers: 1.8 watts and 2.4 watts.

The 1.8 W laser beam shows a penetration depth ranged from 400 to 800 µm. while for the 2.4 W laser the depth was 600 to about 750 µm (Berkiten *et.al* 2000). In this study, The Nd:YAG laser shows a weak bactericidal effect when it is used alone.

This indicates weak absorption of bacterial cells to Nd:YAG laser. This result agrees with Meire M *et al.* grew E.faecalis on dentin disks and expose it to ND:YAG laser. The number of the surviving bacteria was counted by using plate count. He found that the Nd:YAG laser has the least effective bactericidal effect if compared to Er:YAG and NaOCl (Meire M *et al 2012*). The weak bactericidal effect of the laser (when used alone) coincides with Pirnat *et al.* who used a sapphire substrate, loaded with *E.faecalis* and irradiate it with Nd:YAG laser. He found that the Nd:YAG has a weak direct bactericidal effect on the pigmented and non-pigmented bacterial cells (as a biomass) (Pirnat *et al* 2010*)*. Asnaashari M *et al* compare between different laser wavelengths including Diode (810nm), Er:YAG (2940nm), Er,Cr:YSGG (2780nm) and Nd:YAG (1064nm) in killing bacterial cells and conclude that all " all lasers were efficacious in reducing bacterial population without damaging thermal effects, particularly the Erbium family" (Asnaashari M *et al.*2013).

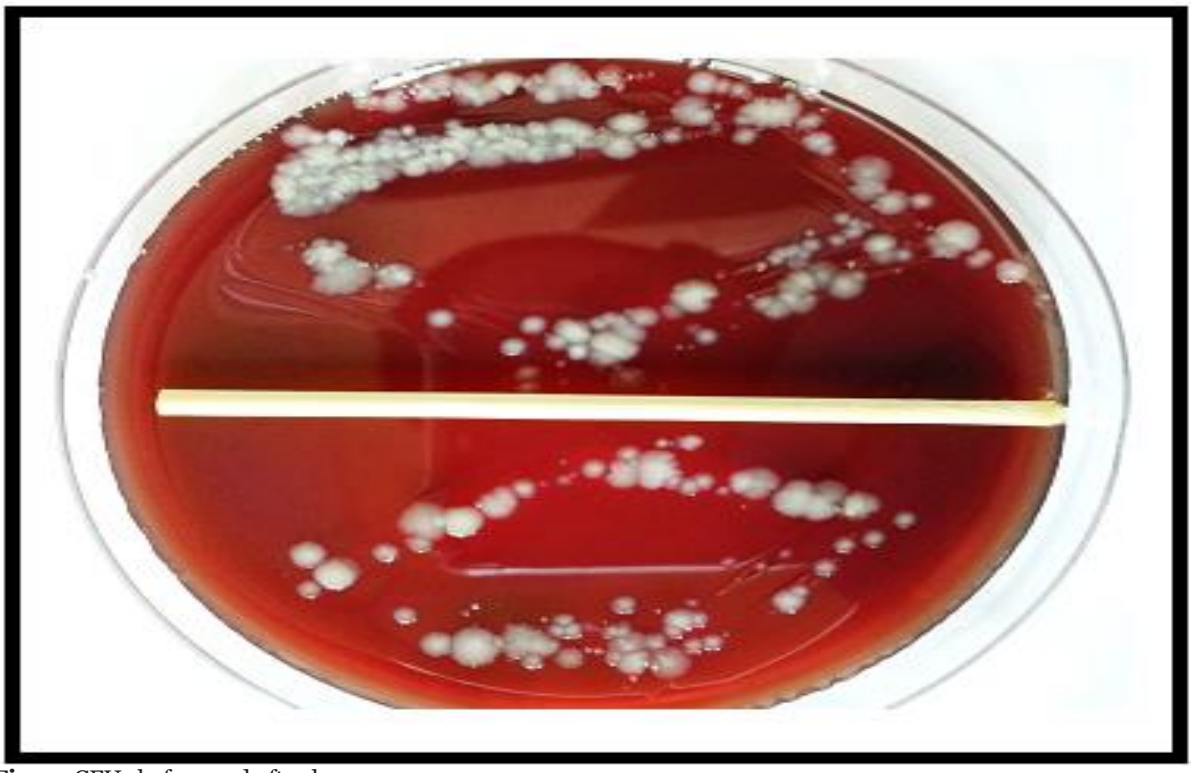

Fig. 2. CFUs before and after laser exposure.

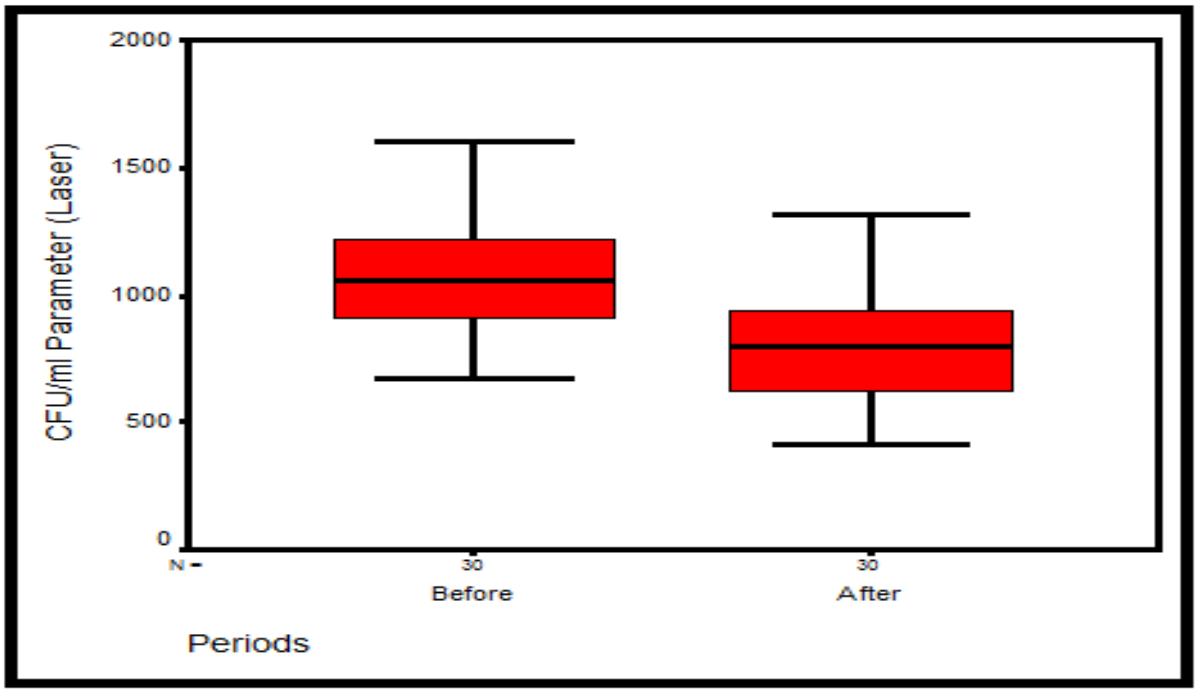

Fig. 3. Stem-Leaf plot for exploring the behavior of CFU/ml. Parameter before and after treated with Laser technique.

The Erbium family has more bactericidal action because the 2940, 2780 nm wavelengths have very strong absorption in water (which forms the most component of any living cell). The erbium laser has an ablative action which damages the walls of the root canals during the treatment. This is considered the main disadvantage of such type of laser (HOSSAIN M *et al* 1999). Asnaashari M *et al* measures the penetration depth of diode laser through the dentin under SEM and he found it about 500 µm

(Asnaashari M *et al.*2013), this result indicates that the Nd:YAG laser has a preference of greater penetration depth through canal walls (as explored by Berkiten *et al.* 2000) which means better sterilization ability.

Conclusion

Long pulse Nd:YAG laser 1064 nm has a weak antibacterial activity when used alone as a bactericidal agent. It is not absorbed by all species of bacteria.

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