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COMPARISON OF THE ANTIBACTERIAL POTENTIAL OF TWO DIFFERENT POWERS OF DIODE LASER AND PHOTODYNAMIC THERAPY ALONE AND IN COMBINATION

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ABSTRACT

Introduction: The intent of the present research was to evaluate the antibacterial efficacy of the two different powers of diode laser and photodynamic therapy PDT by utilizing toluidine blue alone and in combination against *Enterococcus faecalis*.

Methods: The research was done on seventy five human extracted incisor teeth. Their crowns were removed at cementoenamel junction CEJ then the roots were cleaned and shaped. After autoclaving of the roots, they were injected and infected with *Enterococcus faecalis* for 72 hours. They were divided randomly to five equal categories according to type of treatment used. Group (I), high power diode laser with wave length 980 nm and output power 2 W for one minute. Group (II), toluidine blue TBO photosensitizer for five minutes. Group (III), low power 635 nm diode laser with output power 220 mw for 30 seconds. Group (IV), toluidine blue was activated by low power diode laser 635 nm and output power 220 mw for 30 seconds. Group (V), toluidine blue was activated by 980 nm diode laser and output power 2 W for one minute.

Results: Antibacterial efficacy was assessed before and after treatment. 980 nm diode laser reduced bacteria by 86.4 %, toluidine blue reduced it by 19.19%, 630 nm diode laser reduced it by 13.95%, toluidine blue plus low power 635 nm diode laser reduced it by 88.13 %, and toluidine blue plus high power 980 nm diode laser reduced it by 97.76 %.

Conclusion: The most bacterial reduction was found in group toluidine blue plus high power 980 nm diode laser (group V). The combined technique could be an effective modality for elimination of *Enterococcus faecalis* from the root canals.

KEYWORDS: Diode laser, Photodynamic therapy, Toluidine blue, *Enterococcus faecalis*, Root canal infection.

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INTRODUCTION

The intent of the root canal treatment procedure is to eradicate microorganisms from the root canals[1]. This intent is commonly carried out through mechanical instrumentation combined with chemical debridement by the most utilized irrigants like sodium hypochlorite NaOCl, hydrogen peroxide and chlorhexidine CHX and also intra canal medicaments such as calcium hydroxide $Ca(OH)_2$ and tetracycline[2,3]. They can only reduce the number of microorganisms in the root canals by 40-60% and could not eradicate it completely[4-6]. There are some limitations caused by anatomical complexities which lead to deep microbial permeation into dentinal tubules and accessory canals[7]. So researches were done to explore effective adjunctive methods able to kill root canals microorganisms completely[8].

Enterococcus faecalis is a facultative anaerobic Gram positive bacterium that usually detected in the cases of endodontic failures and secondary endodontic infections[9,10]. It can be revive as a solitary bacterium or permeate through the tubules of the root canal dentin and construct biofilms[11,12].

Recently laser technology was displayed in dentistry as it has multiple benefits in endodontics. It aims to amend the results gained from traditional methods of mechanical and chemical preparations of the root canals. It uses the light energy to remove the smear layer and decontaminate the root canal system. All lasers have a bactericidal impact at high power by heat generation. Semiconductor diodes are the most popular lasers used in root canal sterilization [1]. Moreover the low-level lasers promote the recovery of periapical tissues and rebate post-treatment annoyance and complications[13]. Nowadays, within low-level lasers, diode laser was choosing as it had low price and portable [14].

Photodynamic therapy PDT is a process of decontamination of a hard and soft tissue by putting a photosensitizing material to the area adhered to the microbial cells, and after that irradiating the area by light of the laser at a wavelength imbibed via photosensitizing material in the presence of oxygen to reproduce single and radical oxygen[15]. This process leads to demolishing of microbes at the selected area by destroying main molecules such as proteins and nucleic acid [16, 17]. As PDT was perfectly reduced the number of bacteria in the root canals, so it is recommended to be used in conjugation with the traditional methods of cleaning and shaping [10,18]. Toluidine blue TBO is a positively charged photosensitizer agent with blue color. It is amphiphilic and its cellular weight is minimal. Amphiphilic characteristic at TBO makes it a perfect choice for root canal decontamination from both Gram-positive and Gram-negative bacteria [19].

MATERIALS AND METHODS

Calculated sample size

Sample size calculated depending on a past study of Afkhami [20] as reference. According to this study, the minimally agreeable sample size was 15 per group, as the restraint in every subject category was normally dispensed by standard deviation 119000. Because estimated variation was 125000, so we need to survey 15 subjects in each group when possibility (power) was 0.8. Type I error possibility correlated to the experiment was 0.05. Sample size was calculated by using P.S Power 3.1.6. software.

Description of study sample

Seventy five extracted human maxillary central incisors with closed apex were chosen. They were collected from people extracted their sound teeth due to periodontal disease. The selected teeth were examined for absence of resorption, cracks or fractures [20].

Teeth preparation

Mechanical preparation of the teeth

The teeth were decapitated with fissure bur in high speed hand piece under water coolant to the

cementoenamel junction CEJ where the working root length became 14 +/- 1 mm. Working length was located shorter than the foramen of the root apex by 1mm. Endodontic treatment was done to roots by ProTaper rotary endodontic files.

Irrigation protocol during mechanical preparation

One ml of sodium hypochlorite NaOCl (2.5% concentration) was utilized after every file. At the end of canal preparation; one ml of ethylenediaminetetraacetic acid EDTA (17% concentration), then one ml of saline, and then 2.5% NaOCl were used for three minutes for every irrigant to get rid of smear layer. Every root canal was rinsed with 5ml of sterile saline as a final root canal irrigant.

Closure of the apices of the roots

All the root apices were closed by glass ionomer to restrain any leakage apically. Two layers of nail varnish were painted over the whole root surface to prohibit extrinsic bacterial infection [20].

Sterilization of the roots:

All prepared roots were autoclaved for fifteen minutes at 121 °C. All procedures were completed in a sterile condition and work was done under laminar air flow [20].

Production of inoculation suspension

Bile esculin agar plates were used for culturing *Enterococcus faecalis* (ATCC 4083). The bacterial suspension was adjusted to 1.5 McFarland. Bacteriological loop was used to gather the bacterial colonies from agar plates and emulsify them in one ml of sterilized brain heart infusion BHI broth. The infected broth was vortexed for 35 seconds. Such procedure was reduplicated while the bacterial concentration was equal to 1.5 McFarland.

Infection of roots

Ten ml of *Enterococcus feacalis* suspension were inoculated inside each root canal. After inoculation, each root was immersed with BHI broth then inserted in the incubator at 37ºC in aerobic environment, for 72 hours.

Lasers used in the study

A high power diode laser with 980 nm wavelength and 2 W output power (Denlase, China).

A low power diode laser with 635 nm wavelength and output power 220 mw. (Pioon dental laser, China).

Classification of the roots

Seventy five roots were randomly divided into five groups. Each group consisted of fifteen roots treated as follow**:**

Group I: The root canals were irradiated using high power diode laser with wave length 980 nm and output power 2 W in pulsed mode for 20 seconds repeated three times with 20 seconds interval between irradiated cycles. The total irradiation time was 60 second. After exposure to laser beam, the roots were irrigated with 5 ml saline as a final.

Group II: The root canals were injected with ten ml of 0.1 mg/ml toluidine blue TBO (Blue T, Novateb; Iran) photosensitizer. After that they were stored for 5 minutes at a room temperature in a dark place.

Group III: Root canals were irradiated with low power 635 nm diode laser with output power 220 mw for 30 seconds.

Group IV: Toluidine blue was activated by low power diode laser 635 nm and output power 220 mw for 30 seconds.

Group V: Toluidine blue was activated by 980 nm diode laser and output power 2 W in pulsed mode for 20 seconds repeated three times with 20 seconds interval between irradiated cycles. The total irradiation time was 60 second.

Sampling procedures

Initial sample (before treatment sample): Microbial samples were gathered from infected

canals with sterile paper points before treatment for acting as a positive control.

Final sample (after treatment sample): Microbial samples were taken immediately after application of treatment.

Sterile paper points were placed in each root canal for 1 minute then removed and placed in Eppendorf tubes containing 0.5ml BHI broth. Each tube was vortexed for 30 seconds to distribute microorganisms within BHI broth. Fifty μl was taken from each Eppendorf tube and cultured on bile esculin agar plates smeared by sterile cotton swabs. After incubation of the plates for twenty four hours at 37 °C, the colony forming units CFUs were measured. Numbers of CFUs before and after treatments were counted per ml.

Data analysis

SPSS 16® (statistical package for scientific researches) was used for data analysis. Study of the results was completed by Kolmogorov Smirnov and by Shapiro Wilk test to check the normality which showed; data were founded from normal data. Comparing between various categories was executed by One Way ANOVA test then by Tukey`s Post Hoc test, whereas comparing between before and after groups was done by using Paired t test.

RESULTS

Comparison between before and after treatment

Our findings proved that after treatment was less than before significantly in all categories as P< 0.05 (Table 1) (Figure 1).

Percent of reduction was calculated and revealed that: toluidine blue plus high power diode laser with wave length 980 nm (group V) (97.76%), was the highest, then high power diode laser with wave length 980 nm (group I) (86.4%) and toluidine blue plus low power diode laser 635 nm (group IV) (88.13%), then toluidine blue (group II) (19.19%), while low power diode laser 635 nm (group III) was the lowest (13.95%).

Comparison between different groups:

Before treatment; there was insignificant difference between all groups as P=0.83 (Figure 2).

TABLE (1) Standard deviations and means related to before and after treatment in all categories and difference between them. Also, comparison between different groups:

	Before		After		Difference					
					MD	SD	95% CI		P value	% of reduction
	M	SD	M	SD			L	U		
Group I	3.9	0.4	0.53 a	0.032	-3.37	0.41	-3.6	-3.14	<0.0001*	86.4
Group II	3.96	0.5	3.2 b	0.26	-0.76	0.62	-1.10	-0.42	0.0003*	19.19
Group III	3.87	0.24	3.33 c	0.07	-0.54	0.26	-0.68	-0.39	<0.0001*	13.95
Group IV	3.98	0.34	0.47 a	0.07	-3.51	0.34	-3.70	-3.32	<0.0001*	88.13
Group V	4.02	0.41	0.09 d	0.012	-3.93	0.41	-4.15	-3.70	<0.0001*	97.76
P value	0.83ns		<0.0001*							

M: mean SD: standard deviation MD: mean difference (Ns) difference non significantly as P> 0.05

****difference significantly as P< 0.05. 95% CI: Confidence interval L:lower arm U: upper arm***

Means with the same coordinating letters were insignificantly different as P> 0.05.

Means with different coordinating letters were significantly different as P <0.05.

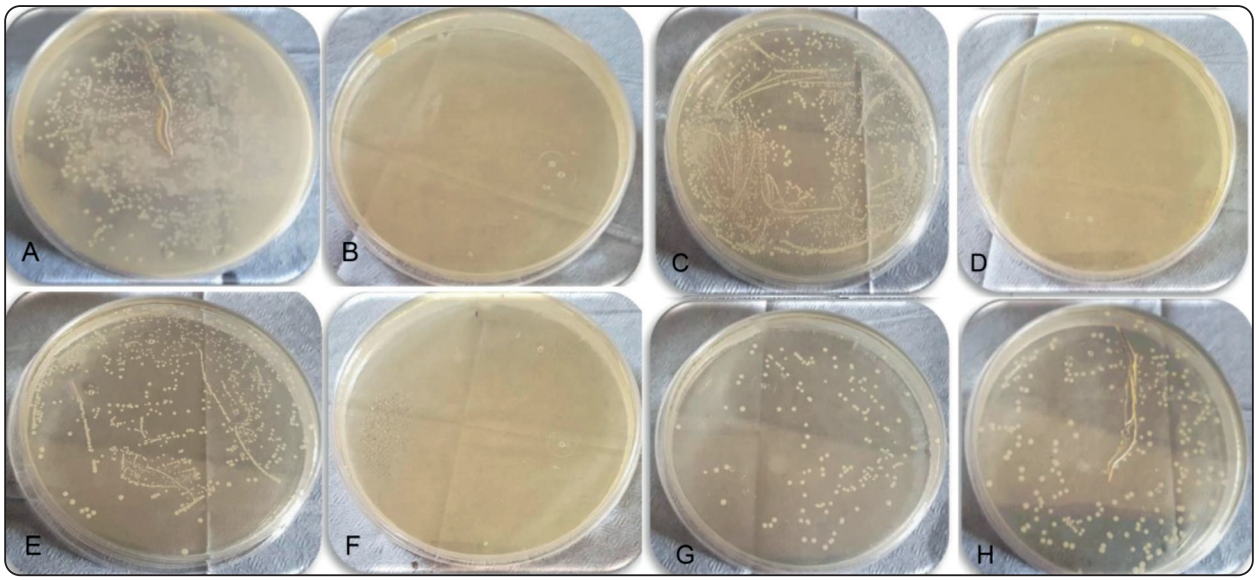

Fig. (1) (A) Before and, (B) after treatment with diode 980 nm plus TBO (group V). (C) Before and, (D) after treatment with diode 980 nm (group I). (E) Before and, (F) after treatment with TBO plus diode 635 nm (group IV). (G) After treatment with TBO (group II). (H) After treatment with diode 635 nm (group III).

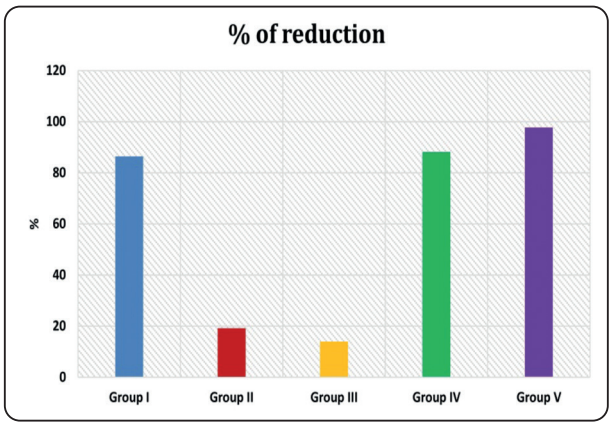

Fig. (2) Bar chart representing % of bacterial reduction in all groups.

After treatment; there was a significant difference among all categories as P< 0.0001*. The toluidine blue plus high power diode laser with wave length 980 nm (group V) (0.09 ±0.012) was significantly the lowest bacterial count, then high power diode laser with wave length 980 nm (group I) (0.53 ±0 .03) and toluidine blue plus low power diode laser 635nm (group IV) (0.47±0.07) with insignificant difference between them, then toluidine blue (group II) (3.2 ± 0.26), while low power diode laser 635 nm (group III) (3.33±0.07) was significantly the highest bacterial count.

DISCUSSION

The mechanical and chemical preparation of infected root canals cannot entirely disinfect the root canals and additional methods are needed for total eradication of microorganisms [21, 22]. The intent of the present research was to evaluate the antibacterial potential of the two different powers of diode laser and photodynamic therapy PDT utilizing toluidine blue alone and in combination against *Enterococcus faecalis*. Which chosen as study microorganism as it is the most common resistant microorganism isolated from cases with secondary infection and cases of endodontic treatment failures [23, 24]. It has the ability to permeate deeply into dentinal tubules, withstand high PH, tolerating starvation and formation of bacterial biofilm [25].

The Toluidine blue was chosen as it is much functional than methylene blue in eradication of microorganisms from infected roots especially *Enterococcus faecalis* because it has a strong ligation to it [15, 26]. All bacteriological procedures in our research were achieved in laminar air flow to repress infections [27]. Antimicrobial assessment was achieved by the CFUs, as it leads to detecting numbers of living microorganisms in root canals [28].

The returns of our research revealed that the percent of reduction in *Enterococcus faecalis* count before and after treatment with 2w 980 nm diode laser in group (I) was 86.4 %. This may be attributed to the ability of 980 nm high-power diode laser to penetrate into areas which cannot be reached by traditional techniques of instrumentation and disinfection of the root canals [29]. It was consisted of two coats of semi-conductor substances tangled to a nonconductive coat. These results were in accordance with the results of Roshdy [25] and results of Shaktawat[30] who proved that irrigation followed by application of diode laser was an efficient treatment format for eradication of *Enterococcus faecalis* from the canals. Furthermore the results of our research was in agreement to results of Kushwah[31] who stated that 980nm diode laser was able to reduce the numbers of *Enterococcus faecalis* from the infected root canals. Also results of Kadour[32] who proved that diode laser had a significant capability of reducing the numbers of *Enterococcus faecalis* from root canals. This results was in disagreement with the results of Ghorbanzadeh [33] and Goel [34] who stated that diode laser alone is not effective in reducing the numbers of *Enterococcus faecalis*. This may be attributed to the using of diode laser with a different wavelength; 810nm in their studies. And with the results of Hendi[35] who stated that diode laser alone was not sufficient to decrease the number of bacteria in the root canal. This may be related to the using of different technique for laser application.

In our study the highest percentage reduction of numbers of *Enterococcus faecalis* before and after treatment was found in group (V) treated with combination of toluidine blue and 2 watts 980nm diode laser. The percentage reduction was 97.76%. This may be attributed to combination of the antibacterial benefits of both high power diode laser 2w of 980 nm and photodynamic therapy by toluidine blue. As the PDT has high antimicrobial efficacy. Photodynamic therapy was done in two steps; the first one was photosensitization of infected tissues in the root canals followed by irradiation of the photosensitized tissue. This procedure leads to microbial cell disintegration [29]. These results were in agreement with the results of Afkhami [20] who stated that the LED and diode laser with toluidine blue can be used with conventional root canal treatment to eliminate microorganisms. Also Masuda [12] who stated that diode laser in combination with photodynamic therapy is effective in elimination of *Enterococcus faecalis* without any toxic belongings to human fibroblasts.

However the least percentage of reduction of numbers of *Enterococcus faecalis* before and after treatment was found in group (II) treated with toluidine blue and group (III) treated with 635nm diode laser. The percentage of reductions were 19.19% and 13.95% respectively. This may be related to the inability of toluidine blue to penetrate deeply into dentinal tubules of the root canals leading to incomplete eradication of bacteria. In addition to the low percentage of oxygen in root canals, which was responsible for photodynamic reaction. Because of this situation, the formulation of oxygen formatives that will have cytotoxic impact on bacteria may not be happened or kept scanty [3, 36]. Furthermore the antibacterial capability of diode laser changes with the variation in its parameters as length of pulses and fluency. So the low power diode laser 635 nm in our study had limited antibacterial properties [29]. But when low power diode laser combined with toluidine blue as in group (IV) the percentage of reduction increased to 88.13%. These results were in agreement with the results of Sin [37] and results of López-Jiménez [18] who stated that photo activated disinfection is less efficient in reducing the numbers of *Enterococcus faecalis*. This result was in disagreement with the findings of Sarda[38] who stated that Photodynamic therapy PDT was an influential method in elimination of root canal microorganisms. This may be attributed to using of a different type of dye as they used methylene blue dye in their study. Furthermore these results were in disagreement with the results of Pourhajibagher[29] who proved that there was a reduction in the

numbers of microorganisms from infected root canal by using photodynamic therapy. This finding probably related to the using of a different test.

CONCLUSIONS

The combined technique (toluidine blue plus high power diode laser with wave length 980 nm) could be effective modality for elimination of *Enterococcus faecalis* from the root canals.

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