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# Postoperative Pain and Microbiological Evaluation After Using 810nm Diode Laser Disinfection in Single Rooted Teeth with Necrotic Pulp and Periapical Radiolucency: Randomized Control Clinical Trial

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## *ABSTRACT*

**Purpose:** This study aimed to evaluate the treatment outcome after using diode laser disinfection of root canals in necrotic teeth with periapical radiolucency. **Subjects and Methods:** Thirty patients of age range between 20 and 50 years old were involved in this study. They were divided randomly into two groups (15 teeth each). Group I: Access cavity preparation was performed and microbiological sample (SI1) was taken. The root canal was prepared using ProTaper rotary instruments up to #F4 and irrigated with 2ml of 2.6% sodium hypochlorite, this was repeated after each instrument use with total volume 14ml. Smear layer removal with 17% EDTA (5ml) was performed, saline solution (5ml) was used as a final irrigant then, the canal was irradiated with 810 nm diode laser in a continuous wave mode with a power of 1.5 w then, microbiological sample (SI2) was taken. Group II: The same steps were followed as in group I up to the use of saline solution (5ml) as a final irrigant, then microbiological sample (SII2) was taken. The treatment outcome was evaluated clinically (pain recording using Visual Analogue Scale (VAS) at 6,12,24,48 and 72hours) and microbiologically (reduction in number of CFU of microorganisms). **Results:** There was no statistically significant difference between pain scores in the two groups. Group I showed statistically significant higher mean percentage reduction in bacterial and fungal counts than group II. **Conclusion:** 810 nm diode laser can be used as an adjunct to conventional root canal treatment in necrotic teeth with periapical radiolucency.

***KEYWORDS***

*Diode Laser,*
*Disinfection,*
*Postoperative Pain,*
*Necrotic Teeth*

- Paper extracted from Doctor thesis titled "Evaluation of the Treatment Outcome after Using Diode Laser Disinfection of Root Canals in Teeth with Necrotic Pulp and Periapical Radiolucency: A Randomized Control Trial"

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## INTRODUCTION

The main goal of root canal treatment is eradication of the microorganisms which considered the main cause of emergence and evolution of pulp and periapical diseases, thus attaining bacteria-free environment will improve the success rate in the endodontic treatment outcome[1].

Traditionally endodontic treatment uses chemo-mechanical preparation to decontaminate the root canal system; it includes mechanical instrumentation and its complementary irrigation which can result in removal of pulp tissue remnants, bacteria and their byproducts. However microbial free root canal cannot be obtained since nearly half of the dentinal walls of the root canal stay undebrided after instrumentation. Also the complex morphology and unexpected anatomy of the root canal system with deep penetration of microorganism into the dentinal tubules restricts permeation of irrigating solution even those has antimicrobial effect as well as intracanal medicaments to the main root canal and hinder its effect [1, 2]. To overcome limitations of the standard techniques, endodontic inquiries interested to identify alternative adjunctive methods target residual microorganisms that assist destruction and elimination from the root canal system[3].

Today, lasers have been used in different endodontic procedures such as pulpotomy, pulp capping, cleaning and disinfection of the root canal system. It has been suggested to reduce microbial infection in the endodontic treatment [4]. Diode laser is a solid-state semiconductor laser that uses a combination of gallium, arsenide, aluminium and/or indium as the active medium, it emits radiation within the visible (mostly 660 nm) and infrared (810 to 980 nm) range of the electromagnetic spectrum and it's using as an adjunct to the standard endodontic therapy has newly been proposed in root canal treatment [5]. The diode laser was reported to have large water transmission capacities that can affect bacteria in deep area of the dentinal tubules and complex ramifications [6].

Effective root canal disinfection after using diode laser was observed since 980nm and 940nm diode laser wavelengths were reported to have high bactericidal effect on Enterococcus faecalis at 1000 $\mu$m depth in the dentinal tubules [7, 8]. The studies evaluating the antibacterial efficacy of 810nm diode laser against Enterococcus faecalis revealed that 810nm diode laser resulted in bacterial reduction[9,10]. Regarding postoperative pain, a recent study compared the effect of 810 nm diode laser with conventional root canal therapy on postoperative pain and has shown that it significantly reduced postoperative pain [11].

Postoperative pain (PP) is an unpleasant sensation and one of the annoying endodontic complications with occurrence range of 3%-58%. Pain after endodontic procedures either root canal obturation or intracanal medicament application can be caused by many factors including mechanical, chemical or microbial. However; it was reported that remaining microorganisms in the root canal system are the most causing factor, as these microorganisms and its byproducts can be extruded to periapical area causing damage of surrounding tissue and elevate the inflammatory mediators' levels [12, 13]. Since the fact that eradication of microorganisms present in the root canal system can affect the post treatment pain and success rate in endodontics; The aim of this study was to evaluate postoperative pain and microbiological reduction after using 810nm diode laser disinfection in root canal treatment in mature teeth with necrotic pulp and periapical radiolucency. Null hypothesis, there is no difference between effect of 810nm diode laser and conventional irrigation technique on root canal disinfection and postoperative pain.

## SUBJECTS AND METHODS

### Study design:

The current study was designed as randomized controlled clinical trial with an allocation ratio 1:1 and conducted in the clinic of Endodontic Department,

Faculty of Dental Medicine for Girls, Al-Azhar University. The study was designed, analyzed and interpreted according to the Consolidated Standards of Reporting Trials (CONSORT 2010) checklist of information [14]. Ethical approval for the human research was obtained in accordance with guidelines from Research Ethics Committee (REC) of the institute with the code number: REC-EN-21-10. All patients read and signed an informed consent form with details about the study along with the benefits and risks of the therapy.

The clinical question in this study was addressed in terms of a PICO question which involves 4 elements: [problem (P), intervention (I), comparison(C) and outcome (O)] as following:

P (Problem): Teeth with necrotic pulp and periapical radiolucency.

I (Intervention): Conventional chemomechanical disinfection with final diode laser disinfection.

C (Comparison): Conventional chemomechanical disinfection without final diode laser disinfection.

O (Outcome): Primary outcome is pain recording using Visual Analogue Scale (VAS) at 6, 12, 24, 48 and 72 hours after the treatment and secondary outcome is microbial reduction using bacterial count through colony forming units (CFU).

**Sample size calculation:**

Sample size was calculated at 80% power of the study and 95% confidence level. By the following equation: $n = (Z\alpha/2+Z\beta)\ 2 * (p1\ (1-p1) + p2\ (1-p2)) / (p1-p2)2$.

* P1= proportion of absence of pain in the laser group (100%)
* P2= proportion of absence of pain in the control group (60%)

  From previous research [15].
* Z (1-α) = is the critical value of the normal distribution at α/2 (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96).
* Z (1-β) = Zβ is the critical value of the normal distribution at β (e.g. for a power of 80%, β is 0.2 and the critical value is 0.84) so we selected 12 patients for each group. Accordingly n=11.75. To compensate losses during the follow-up period this number was increased to 15 patients for each group.

**Patients' selection, randomization and blinding:**

A total of thirty patients were selected for this study after clinical and radiographic examinations from cases that were referred to the Endodontic clinic. The patients who met the inclusion criteria to be of age category between 20 and 50 years, noncontributory medical condition and complaining of asymptomatic necrotic single rooted (anterior or premolars) teeth with periapical radiolucency (0-5mm).

The exclusion criteria were patients used antibiotics within the last month or anti-inflammatory analgesic within last few days, pregnant females, patients have traumatic occlusion or other teeth need root canal treatment and teeth with root resorption, calcified canals, sinus tract, periodontal diseases or drastic crown destruction.

The thirty patients were divided randomly into two groups (15 cases each) according to the technique of root canal disinfection either by using 810nm diode laser or conventional irrigation technique. To confirm randomization ahead of the RCT, sealed opaque envelopes containing printed pieces of paper with numbers were chosen by the patients and according to the number in the paper inside the envelope the patient was included in diode laser group or conventional group. Only single endodontic operator performed the treatment procedures over the period of the study.

Double blinding was considered in this study by the outcome assessor and the statistician.

**Treatment protocol:**

***Group I (810nm diode laser group):***

Aseptic technique was used throughout the root canal treatment and sample acquisition. After application of local anesthesia (4% articaine with 1:100,000 epinephrine) and rubber-dam isolation, removal of old coronal restoration and caries, the operating field was disinfected with 30% H2O2 for 30 seconds, followed by 5.25% NaOCl for an additional 30 seconds. Proper access cavity was prepared using sterile high speed round and tapered diamond bur (Dentsply, Maillfer, Switzerland).

***Initial Microbiological Sample (S1):***

After confirming canal patency with sterile # 10 K file (Kerr UK, Peterborough, UK) and irrigation of the canal with 1 ml saline, the first sample (SI1) was taken using three sterile paper points size 25 which were sustained in the root canal for 1 minute, then inserted in sterile test tube containing brain heart infusion broth as transport media to the microbiological laboratory in the Regional Center for Mycology and Biotechnology (RCMB), Al Azhar University.

The working length was determined using an electronic apex locator (DentaPort ZX: Morita Co., Tokyo, Japan) and confirmed using periapical radiograph. The root canal was prepared using ProTaper Universal rotary files (Dentsply, Maillfer, Switzerland) up to #F4 in crown-down technique, speed and torque were adjusted according to manufacturers' instruction. Irrigation with 2ml of 2.6% sodium hypochlorite (NaOCl) solution (Alex. Deteregents and Chemical Co., Egypt) for 1 minute after each instrument dispensed through a 31-gauge Navi-Tip flexible irrigating needle (Navi-Tip, Ultradent product, South Jourdan, UT) was done ,followed by smear layer removal with 17% EDTA (5 ml) for 1min and then saline solution (5 ml) was used as a final irrigant [15].

Laser activated disinfection was performed using 810 nm diode laser (Elexxion clarosdental laser, Singen Deutschland, Germany) in a continuous wave mode with a power of 1.5 Watts in 4 cycles of 5 seconds with 20 seconds intervals in between each. After all the individuals in the room have worn protective laser safety eye glasses, the fiber optic tip 200 μm (Lite medics, Italy) was inserted 1 mm from WL, activated and moved in slow helical motion from the apex to the cervical third with alternating between clockwise and counterclockwise direction at speed of about 2 mm/s. 5 ml saline solution was used for each application and finally before taking second microbiological sample.

Second microbiological sample (SI2) was taken after laser application using 3 sterile paper point size 40 which taken, preserved and transferred as the same as (SI1). Finally the root canal system was obturated with # F4 gutta-percha cone (Dentsply-Maillefer, Ballaigues, Switzerland) and ADSEAL resin-based sealer (Meta Biomed Co, Cheongju, Korea) using cold lateral condensation method after completely dried using paper points of comparable size to the master cone and final coronal restoration direct composite filling (Filtek Bulk Fill,3M ESPE, USA) was performed in the same visit.

**Group II** (Conventional group):

The same technique and instruments were applied as mentioned in group I of taking first microbial sample (SII1) and root canal cleaning and shaping. However; the second microbial sample (SII2) was taken directly after finishing the mechanical preparation and irrigating the canal with 5 ml saline solution. The obturation of the root canal and the coronal restoration was performed as in group I.

**Methods of evaluation:**

Assessment of postoperative pain which was performed using Visual Analogue Scale (VAS) [16]; it utilizes a line of 10 cm length anchored at the 2 extremes with descriptors representing the absolute minimum and the absolute maximum of pain. A home questionnaire form of VAS was given for the patient to score the level of pain at 6 and 12 hours after the treatment and was recalled after 24, 48, and 72 hours for examination and assign

VAS. No medication was prescribed immediately after the root canal treatment and if any; the patient was asked to mention its type and frequency in the questionnaire.

Microbiological count: Microbiological count of samples through determination of colony forming units (CFU) / milliliter of the culture plate was performed in order to evaluate the reduction in the number of CFU of microbiological count. Nutrient agar was chosen for bacterial culture and sabouraud dextrose agar was used for cultivation of candida species.

The agar plates were incubated at 37°C for 24 hours. Bacterial colonies were counted and reported as colony forming units per milliliter (CFU/ml). On the other hand, for fungal culture: chloramphenicol was added to sabouraud dextrose agar (SDA) plate to prevent bacterial growth. The inoculated SDA agar plates were incubated at 37°C for 24hours. Colonies of candida species were reported as colony forming units per milliliter (CFU/ml).

**Statistical Analysis:**

Logarithmic transformation of bacterial count data was performed due to the high range of bacterial counts. Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Log10 Colony Forming Unit (Log10 CFU) as well as percentage reduction of bacterial and fungal counts data showed normal (parametric) distribution while pain scores showed non-parametric distribution. Data were presented as mean, standard deviation (SD), median and range values. For parametric data; repeated measures ANOVA test was used to compare between the groups as well as to study the changes within each group. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. Student's t-test was used to compare between percentage reduction of bacterial and fungal counts in the two groups. For non-parametric data, Mann-Whitney U test was used to compare between the two groups. Friedman's test was used to study the changes by time within each group. Dunn's test was used for pair-wise comparisons when Friedman's test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

## RESULTS

### I- Pain evaluation: Comparison of pain scores among the tested groups at 6, 12, 24, 48 and 72 hours: (Fig. 1)

The median and range values of pain scores in group I (diode laser group) pre-operatively, after 6, 12, 24, 48 and 72 hours were 0 (0-0), 0 (0-7), 0 (0-7), 0 (0-5), 0 (0-2) and 0 (0-0), respectively. In group II (conventional group), the median and range values of pain scores pre-operatively, after 6, 12, 24, 48 and 72 hours were 0 (0-0), 0 (0-9), 0 (0-10), 0 (0-8), 0 (0-4), 0 (0-2), respectively.

The results showed that 810nm diode laser disinfection had lower pain scores than conventional irrigation technique. However, there was no statistically significant difference between pain scores in the two groups pre-operatively, after 6, 12, 24, 48 as well as 72 hours (P-value = 1), (P-value = 0.769), (P-value = 0.311), (P-value = 0.379), (P-value = 0.544) and (P-value = 0.317), respectively.

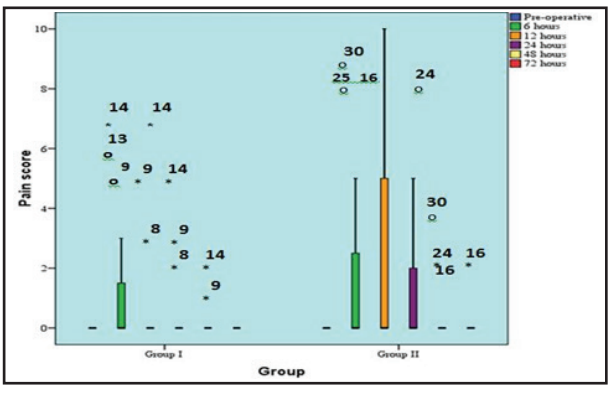


Figure (1) Box plot representing median and range values for pain scores in the two groups (Stars and circles represent outliers)

**II. Microbiological results:**

**II.1 Comparison of bacterial reduction among the tested groups after root canal disinfection:** ***(Table 1)***

The mean values and SD for Log10 CFU of total bacterial count of SI1 and SII1 were 7.23±1.17 and 7.27±1.28 respectively. There was no statistical significant difference among the tested group (P-value = 0.943, Effect size = 0.0002).

On comparing the mean values and SD for Log10 CFU of total bacterial count of S2; SI2 was 3.02 ± 0.89 and SII2 was 4.98 ± 1.09. Diode laser group showed statistically significant lower mean Log10 CFU of bacterial counts than conventional group (P <0.001, Effect size = 0.511).

The percentage reduction in bacterial counts in diode laser group and conventional group were 99.99% and 98.78 % respectively. Diode laser group showed statistically significant higher mean percentage reduction in bacterial counts than conventional group (P = 0.006, Effect size = 1.075**).**

**II.2 Comparison of fungal reduction among the tested groups after root canal disinfection:** ***(Table 2).***

The mean values and SD for Log10 CFU of total fungal count of SI1 and SII1 were 7.12 ± 0.88 and 6.95 ± 1.09 respectively. There was no statistically significant difference between Log10 CFU of fungal counts in the two groups (P = 0.652, Effect size = 0.007).

On comparing the mean values and SD for Log10 CFU of total fungal count of S2, SI2 was 3.13 ± 0.91 and SII2 was 5.09 ± 0.84. Diode laser group showed statistically significant lower mean Log10 CFU of fungal counts than conventional group (P<0.001, Effect size = 0.574)

The percentage reduction in fungal counts in diode laser group and conventional group were 99.96% and 95.81 % respectively. Diode laser group showed statistically significant higher mean percentage reduction in fungal counts than conventional group (P = 0.040, Effect size = 0.786).

**Table (1)** *Descriptive statistics and results of repeated measures ANOVA test for comparison between Log10 CFU of bacterial counts in the two groups, changes within group and Student's t-test for comparison between percentage reduction in bacterial counts.*

| | Group I (n = 15) | | Group II (n = 15) | | P-value | Effect size |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| **S1** | 7.23 | 1.17 | 7.27 | 1.28 | 0.943 | Partial Eta squared = 0.0002 |
| **S2** | 3.02 | 0.89 | 4.98 | 1.09 | <0.001* | Partial Eta squared = 0.511 |
| **P-value** | <0.001* | | <0.001* | | | |
| **Effect size (Partial Eta squared)** | 0.959 | | 0.873 | | | |
| **Percentage reduction** | 99.99 | 0.02 | 98.78 | 1.59 | 0.006* | d = 1.075 |

** Significant at P ≤ 0.05.*

**Table (2)** *Descriptive statistics and results of repeated measures ANOVA test for comparison between Log10 CFU of fungal counts in the two groups, changes within group and Student's t-test for comparison between percentage reduction in fungal counts*

| | Group I (n = 15) | | Group II (n = 15) | | P-value | Effect size |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| **S1** | 7.12 | 0.88 | 6.95 | 1.09 | 0.652 | Partial Eta squared = 0.007 |
| **S2** | 3.13 | 0.91 | 5.09 | 0.84 | <0.001* | Partial Eta squared = 0.574 |
| **P-value** | <0.001* | | <0.001* | | | |
| **Effect size (Partial Eta squared)** | 0.948 | | 0.8 | | | |
| **Percentage reduction** | 99.96 | 0.11 | 95.81 | 7.46 | 0.040* | d = 0.786 |

**Significant at P ≤ 0.05*

## DISCUSSION

The application of lasers in the endodontic field give rise to increase in efficiency and success rate, it is an essential instrument makes various endodontic treatments easy, reduce time and help in achieving better care for the patients and promote immediate disinfection of the root canal system[5]. Diode laser of wavelength 810 nm within the infrared range was recommended for endodontic procedures, it was reported that it is effective in removing the organic tissue and smear layer [17]. The laser was applied using a flexible optic fiber tip (200 $\mu$m) that target deep areas thus effective delivery and distribution of laser light expected to improve root canal disinfection and microbial reduction [15]

The teeth which were the target in this study those have necrotic pulp with periapical radiolucency, high presence of different types of microorganisms as bacteria, fungi and viruses were reported to be isolated form such teeth type which significantly affect success rate of the root canal treatment [18]. Therefore, microorganism play crucial role in causing postoperative pain and adversely affect the outcome of endodontic treatment thus the aim of this study was to evaluate effect of 810nm diode laser on postoperative pain perception and microbial reduction of necrotic teeth with periapical radiolucency.

The cases included in this study were with asymptomatic teeth to avoid bias, since preoperative pain may influence the indicator of postoperative pain, it was reported that postoperative pain correlated with symptomatic teeth was significantly higher than that of asymptomatic [19]. The study was designed as randomized clinical trial in which patients' allocation was randomly assigned into two groups according to the disinfection protocol to avoid bias and all cases were treated by single operator to avoid operator dependent variation which may affect accuracy of the results [20].

The postoperative pain record which is primary indication for the treatment prognosis was selected as an outcome of this study and measured using VAS scale which utilizes scale from 0-10 to measure intensity of pain. Although many pain scales used for pain assessment; VAS is a valid, provides clear reliable records, is easily understandable by patients and appropriate use was observed after explanation for patients [21]**.** The microbiological reduction after root canal disinfection is considered an immediate outcome and the elimination of cultivable microorganisms remains the main objective and can be used as surrogate outcome of the clinical trials [22].

Pain results in the present study showed that 810 nm diode laser group had lower pain scores than conventional group. However, there was no statistically significant difference between the two groups. Authors discussed the mechanism by which diode lasers resulting in decreased postoperative pain and they attributed it to the anti-inflammatory effect of diode laser by lowering in the levels prostaglandin E2, histamine, acetyl choline interleukin1-beta and tumor necrosis factor-alpha [23, 24]. The results of the present study were consistent with another study which reported that, there was no significant difference in pain relief among laser and conventional groups at 24 and 72 h [23]. On the contrary, previous studies showed that diode laser has the ability to significantly relieve postoperative pain compared to conventional root canal treatment [11, 15]. However; the results might be attributed to the applied two visits protocol and pulsed mode in this study compared to single visit and continuous wave mode performed in the present study.

The microbiological results showed that there was statistically significant difference in mean percentage reduction in bacterial counts in 810 nm diode laser group when compared to conventional technique. This may be related to thermal effects of laser which produce alterations in the bacterial cell wall leading to cell death, It can interact with melanin pigments of the root canal microorganisms directly causing a great antimicrobial effect and has high transmission in water and hydroxyapatite. It is among the near infrared range that is absorbed by dentin to a small extent, this property is very effective in root canal disinfection as laser light penetrates into the intertubular dentin. Diode laser penetrates more than1000 $\mu$m in dentin which provides high bactericidal effect since bacteria can reside deep up to 1000 $\mu$m into the dentinal tubules [25, 26].

In corroboration with the results of present study, previous studies reported that diode laser application resulted in significant reduction in bacterial count[8,9,15]. On the other hand, the results of the present study were inconsistent with a study showed that 810nm diode laser was not as effective as NaOCl irrigation. This discrepancy might be attributed to the resistance of E. faecalis to the thermal effect of laser [27].

This study showed statistically significant difference in mean percentage reduction in fungal counts in 810nm diode laser group when compared to conventional group. This may be explained by the very complex root canal system, promoting areas that cannot be reached by the conventional chemomechanical preparation. In addition to biofilm formation by candida albicans which are the most common fungus found in endodontic infection and resistant to conventional technique [28]. The results of the present study were inconsistent with another study which reported that, there was no significant difference between photodynamic therapy with 810nm diode laser and conventional group on C. albicans and E. faecalis. This could be attributed to absence of chelating agents which are important to remove the smear layer and increase the depth of laser penetration into dentin compared to its usage in the present study [29].

## CONCLUSION

Within the limitations of the present study it could be concluded that 810 nm diode laser can be used as an adjunct to conventional root canal treatment in teeth with necrotic pulp and periapical radiolucency.

## RECOMMENDATION

Further studies can be performed using 810nm diode laser on the disinfection of retreated root canals in case of failed root canal treatment.

**NO CONFLICT OF INTEREST** related to the present study.

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