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Effect of Ultrasonic and Diode Laser Irrigation Activation on Post-operative Pain and Microbial Reduction in Single Visit Endodontic Treatment of Necrotic Mandibular Molars

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Abstract

AIM: The aim of the study was to clinically evaluate the effect of continuous ultrasonic and diode laser 810 wave length irrigation activation techniques on post-operative pain and bacterial reduction in single visit endodontic treatment of mandibular molars.

MATERIALS AND METHODS: Forty patients requiring root canal therapy for necrotic mandibular molar teeth were included in this study with age ranges between 20 and 45 years. In all cases, single visit endodontic treatment was carried out using Revo-S rotary file system in crown down sequence. NaOCl 2.5% irrigation solution was used during treatment. Patients were randomly and equally assigned into two groups according to the irrigation technique. Group 1: Conventional syringe irrigation and Group 2: Continuous ultrasonic irrigation (CUI). Each group was subdivided into two sub groups (n = 10); Subgroup 1A (*conventional syringe irrigation with no laser*), Subgroup 1B (*conventional syringe irrigation with diode laser*), Subgroup 2A (CUI *with no laser*), and Subgroup 2B (CUI *with diode laser)*. Post-operative pain evaluation was done using visual analog scale at 6-, 12-, 24-, 36-, 48-h and 7-day postoperatively. Microbiological detection of bacterial reduction was done by taking Samples (S1 and S2) for bacterial cultures. S1 after finishing access cavity and before mechanical preparation and S2 after finishing mechanical preparation and irrigation activation and before obturation. Samples were cultured on blood agar and determined as colony forming units per ml (CFU/mL). Microbiological bacterial reduction was calculated accordingly. Statistical analyses were analyzed using the Mann–Whitney U test. The significance level was set at $p < 0.05$.

RESULTS: In all subgroups, post-operative pain decreased by time in all time intervals but pain was significantly lower in Subgroup 2B (CUI with diode laser) than Subgroup 1A (conventional syringe irrigation with no laser) in all time intervals. Microbiological results showed that the highest bacterial reduction was in Subgroup (2B) (CUI with diode laser) and least bacterial reduction was in Subgroup (1A) (conventional syringe irrigation with no laser).

CONCLUSION: Using diode laser and CUI, activation techniques as adjunctive methods showed improvement in post-operative pain records and enhanced bacterial reduction in root canal therapy.

Introduction

Post-operative pain can be defined as a common unwanted pain sensation that occurs after termination of endodontic treatment procedures. Post-endodontic pain etiology is primarily correlated to microbial reason caused by periapical area injury, due to irrigant extrusion beyond the apex during mechanical cleaning and shaping by endodontic instruments. Slight degree of variation in pain perception starts from discomfort and increased to frank pain that could be considered as complications of endodontic treatment procedures. Many researches tried to clarify factors related with those frequently resultant complications and how to overcome and introduce preventive measures that can be used. Unfortunately, variable outcomes have been found [1].

Post-operative pain limitations could be detected following root canal and treatment; however, successful treatment was carried. Several studies revealed that pain ranges from 25% to 40% clinically [2], [3], [4], [5]. To ensure adequate endodontic treatment, proper elimination of soft-tissues debris, smear layer, and microorganisms in root canal system should be achieved. However, it is impossible to fully disinfect and clean debris that build up. That is why, adjunctive aids including using lasers with different wave lengths have been introduced during conventional endodontic treatment in cleaning maneuvers [6], [7], [8], [9]. From the various types of lasers, diode laser is considered the most appropriate commonly used one due to its high depth of penetration through dentinal tubules and highly effective antibacterial action [10], [11].

On the other hand, irrigation that is ultrasonically activated can also offer privilege over conventional irrigation with syringe needle in canal system debridement [12], [13], [14]. Passive ultrasonic irrigation activation by file in root canal includes irrigation solution activation with mechanical instrumentation simultaneously [15], [16]. Ultrasonic file oscillation amplitude increases proportionally with increasing ultrasonic device intensity, leading to irrigation solution moving rapidly around the file [17]. Irrigation replenishment occurs by intermittent flushing using syringe or introducing headpiece through canal orifice [18], [19], [20].

Moreover, irrigation and ultrasonic activation are concomitantly done at the same time by continuous ultrasonic irrigation (CUI) that introduced the irrigant through a needle which is activated ultrasonically after being inserted into the root canal [18]. The purpose of this study was to evaluate the effect of diode laser and CUI on post-operative pain and bacterial reduction in the root canals of necrotic mandibular molars.

Materials and Methods

The protocol of the trial was approved by the Ethics Committee of the National Research Centre (National Research Centre ethical approval certificate registration number 17-076). The study was conducted in accordance with the revised Helsinki declaration and the Local Ethical Committee regulations.

The study was conducted on patients attending National Research Centre clinic where diode laser devices and single compartment safety are available. Informed consents from all patients were obtained before being enrolled in this study.

Inclusion criteria

Medically free patients with mature permanent mandibular molars, asymptomatic non-vital pulps requiring one visit root canal treatment, age range between (20 and 45) years, and having a diagnosis of pulp necrosis (negative response to pulp tests) with or without apical periodontitis were included in the study.

Exclusion criteria

Periapical abscess, previous endodontic treatment and previously accessed teeth, patients having calcified teeth, deep periodontal pockets, persistent exudates or incomplete root formation, failure to achieve apical patency, and teeth having subgingival caries or difficult to isolate or roots with any type of resorption were included in the study. The study sample included 40 patients (Figure 1). To minimize allocation bias, the patients were randomly assigned to two equal groups according to the irrigation activation:

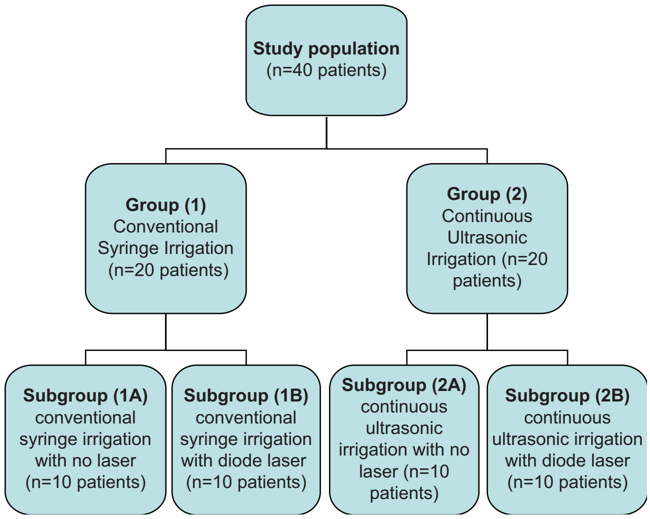

Figure 1: The study design

Group 1

Conventional syringe irrigation was used after mechanical preparation and further divided into two subgroups:

Subgroup 1A

Conventional syringe irrigation with no laser.

Subgroup 1B

Conventional syringe irrigation with diode laser.

Group 2

CUI was used after mechanical preparation and further divided into two subgroups:

Subgroup 2A

CUI with no laser.

Subgroup 2B

CUI with diode laser.

Microbiological samples collection

Samples were taken from the root canals before starting the mechanical preparation sample (S1) was collected using sterile paper point[1] # 15 that was placed in the canal about 1 mm short of the radiographic apex to absorb the fluids in the canal. Each paper point remained in the canal for at least 1 min. Paper points were then transferred to sterile Eppendorf tubes and another sample was taken after finishing mechanical preparation and irrigation activation Sample S2 was collected from all patients after finishing mechanical preparation by paper point size #35 inserted in each root canal for 1 min then transferred to Eppendorf, both samples were sent under aseptic condition for microbiological examination. Samples were placed in sterile Eppendorf tubes, containing 2 ml of thioglycollate broth under complete aseptic precautions and delivered to the microbiology laboratory.

Endodontic protocol (clinical procedure)

Strict aseptic technique was used. Patients were randomly allocated to one of the four subgroups previously described. Selected patients were locally anesthetized by nerve block anesthesia. Teeth were isolated with rubber dam, caries was removed and access cavity was prepared. Saline solution 0.9%[2] was used for irrigation. ISO size 10 or 15 stainless steel K-type file[3] was used for canal negotiation apically. Working length (WL) was determined using electronic apex locator[4] and confirmed radiographically.

Then, the canals were mechanically prepared using Revo-S[5] system files according to the manufacture instruction at rpm 250 and torque 1.6 N\cm^2 file starting by SC1, SC2, and SU till size AS 35#.

Subgroup 1A: Conventional syringe irrigation with no laser

2 ml of NaOCl 2.5% were used between every subsequent file using 30-gauge side vented needle at a rate of 0.1 ml/3 s. After complete cleaning and shaping, 3 ml of sterile saline were used as a final rinse.

Subgroup (1B): Conventional syringe irrigation with diode laser

Same as Subgroup 1A, however, at the end of cleaning and shaping, diode laser[6] was used for activation of 2.5% NaOCl in the root canals. Device adjusted at power of 0.8 Watts, interval and duration of 20 s in a continuous mode using fiber core diameter 200 μm and length 20 mm without tip initiation. After adjustment of WL, fiber optic tip was kept 1 mm short of WL and was activated [10]. Tip was removed from the canal in helicoidal movements (speed of 2 mm/s) and was repeated 4 times at intervals of 20 s, then 3 ml of sterile saline were used as final rinse [21].

Subgroup 2A

Root canals were cleaned, shaped, and irrigated using the ProUltra PiezoFlow[7] that was used for activation of the irrigating solution according to the manufacturer's recommendations. The needle was operated using Satelec P5 Piezoelectric Ultrasonic Unit[8] at power setting of 5. The stopper on the PiezoFlow needle was set 1 mm short of binding in the canals, but not more than 75% of the WL. A syringe containing 5 mL of 2.5% NaOCl was attached to the Piezoflow activation needle and the inactive needle was inserted in the canal, and irrigant flow was started before activation. During activation, the needle was moved up and down passively in the canal.

Subgroup 2B

Same as Subgroup 2A, then diode laser was used for irrigant activation as described in Subgroup 1B. After that, 3 ml of sterile saline were used.

Then, 2 ml 17% EDTA was used and followed by 3 ml of sterile saline were used as finial rinse.

For all groups, master cone Gutta Percha AS35 Revo-S (#35, 6%) was checked for WL accuracy by digital radiographic X-ray[9] using Ez-Sensor classic[10]. Obturation was done using lateral compaction technique and ADseal[11] resin sealer. A final post-operative X-ray was taken to check obturation quality.

Instructions were given to the patients after finishing root canal treatment not to take any analgesics unless there is a severe pain (Ibuprofen 400 mg were prescribed) after informing the operator. Degree of post-operative pain was assessed for patients using a visual analog scale (VAS). Patients recorded the degree of pain at 6-, 12-, 24-, 36-, 48-h and 7-day postoperatively according to the instructions provided previously. One week later, the patient returned the questionnaire back to the clinic after signing the chart.

Microbiological assessment (Bacterial detection and identification)

Bacterial count was measured according to the number of microorganisms that could be recovered per plate. Identification of microorganisms using API 20 system for full identification of microorganisms was used.

1 META BIOMED CO., LTD. Chungbuuk, Korea.
2 1 Al mottahedoon Pharma, Egypt.
3 Mani Inc, Japan.
4 Root ZX II J.Morita, Tokyo, Japan.
5 MICRO-MEGA®+, BESANCON cedex, France.
6 PicassoLite, AMD, LASERS® LLC, USA
7 Dentsply Tulsa Dental Specialties, Tulsa, OK, USA.
8 Acteon, Mount Laurel, NJ, USA.
9 ViVi, S.r.l, Italy.
10 Vatech, Samsung, Hwaseong-Si, Gyeonggi-Do, Korea.
11 META BIOMED CO., LTD. Chungbuuk, Korea.

For the growth of aerobic and anaerobic microorganisms and according to Eyad *et al.*, [22] two samples were taken before and after irrigation and were diluted 1:10 and 1:100. Aerobic plates were incubated overnight at 37°C. Transported samples were plated on blood agar by semi-quantitative technique under both aerobic and anaerobic conditions. Each agar plate was divided into four quadrants and using a calibration loop of 6 mm diameter holding 0.01 ml of the transport media. After streaking, the plates were incubated at 37°C for 72 h in an anaerobic jar (Oxoid) for anaerobic plates. Anaerobiosis was maintained and growth of the ACCC strain of Bacteroides fragilis 28285 was test-grown with every jar holder to detect efficiency of the anaerobic jar. The number of viable organisms was counted and expressed as colony-forming units per 1 mL (CFU/mL).

Statistical analysis

The mean and standard deviation values were calculated for each group in each test. Viable counts of antibacterial activity were transformed to their log10 values. Data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests, pain data showed non-parametric (not-normal) distribution (scores). Rest of data showed parametric (normal) distribution.

For non-parametric data, Friedman was used to compare between more than two groups in related samples. Wilcoxon was used to compare between two groups in related samples. Mann–Whitney test was used to compare between two groups in non-related samples. For parametric data, paired sample t-test was used to compare between two groups in related samples. Independent sample t-test was used to compare between two groups in non-related samples. Two-way analysis of variance test was used to test the interactions between different variables.

The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Sample size calculation

To a total sample size of 40 samples was sufficient to detect the effect size of 0.40, a power ($1-\beta$) of 80%, and at a significant level of 5% ($p < 0.05$), each experimental group would be represented by 10 samples. The sample size was calculated according to the G*Power software version 3.1.9.4. Where;

fS the effect size

A = 0.05

B = 0.2

Power = $1- \beta = 0.8$

Results

Post-operative pain results

Effect of irrigation activation techniques

No-laser subgroups

After 6 h, 12 h, 24 h, 36 h, and 48 h, Subgroup 1A (conventional syringe irrigation with no laser) recorded a statistically significant higher mean value of post-operative pain than Subgroup 2A (CUI with no laser), where ($p < 0.001$). After 7 days, Subgroup 1A (conventional syringe irrigation with no laser) recorded a statistically significant higher mean value than Subgroup 2A (CUI with no laser), where ($p = 0.003$) (Table 1 and Figure 2).

Laser subgroups

After 6 h, 12 h, 24 h, 36 h, and 48 h, Subgroup 1B (conventional syringe irrigation with diode laser) recorded a statistically significant higher mean value of post-operative pain than Subgroup 2B (CUI with diode laser), where ($p < 0.001$). After 7 days, Subgroup 1B (conventional syringe irrigation with diode laser) recorded a statistically significant higher mean value than Subgroup 2B (CUI with diode laser), where ($p < 0.05$) (Table 2 and Figure 2).

Effect of laser

Conventional syringe groups

After 6 h, 12 h, 24 h, 36 h, and 48 h, Subgroup 1A (conventional syringe irrigation with no laser) recorded a statistically significant higher mean value than Subgroup 1B (conventional syringe irrigation with diode laser) where p-value was ($p = 0.007$), ($p = 0.014$), ($p = 0.022$), ($p = 0.006$), and ($p = 0.016$), respectively (Table 3 and Figure 3). While after 7 days, there was no statistically significant difference between Subgroup 1A (conventional syringe irrigation with no laser) which recorded a higher mean value than

Table 1: The mean and standard deviation (SD) values of post-operative pain in no laser subgroups

Variables				After 6 h	After 12 h	After 24 h	After 36 h	After 48 h	After 7 days
Post-operative Pain scores	No laser	Conventional syringe irrigation (1A)	Mean	74.5	52.4	41.4	32.2	28.6	1.7
			SD	5.15	4.35	3.37	3.46	2.67	0.67
		Continuous Ultrasonic irrigation (2A)	Mean	58.8	39	27.8	19.4	10.1	0.8
			SD	3.82	4.03	5.03	3.47	2.02	0.42
	p-value			<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.003*

*Significant ($p < 0.05$) ns: Non-significant ($p > 0.05$).

Table 2: The mean and standard deviation (SD) values of post-operative pain in laser subgroups

Variables				After 6 h	After 12 h	After 24 h	After 36 h	After 48 h	After 7 days
Post-operative Pain scores	Laser	Conventional syringe irrigation with diode laser (1B)	Mean	58.9	39.7	25.7	17.6	9.3	1.2
			SD	3.11	5.81	4.62	3.5	1.83	0.42
		Continuous Ultrasonic irrigation with diode laser (2B)	Mean	47.4	27.3	17.5	10.4	5.1	0.6
			SD	4.7	4.76	4.33	0.7	0.88	0.52
	p-value			<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.015*

*Significant (p < 0.05) ns: Non-significant (p > 0.05).

Subgroup 1B (conventional syringe irrigation with diode laser) where (p = 0.192) (Table 3 and Figure 3).

CUI groups

After 6 h, 12 h, 24 h, 36 h, and 48 h, Subgroup 2A (CUI with no laser) recorded a statistically significant higher mean value than Subgroup 2B (CUI with diode laser) where (p < 0.001).

While after 7 days, there was no statistically significant difference between Subgroup 2A (CUI with no laser) which recorded a higher mean value than Subgroup 2B (CUI with diode laser) where (p = 0.066) (Table 4 and Figure 3).

Microbiological results

No statistically significant difference was found between Samples S1 in all sub groups.

Colony forming unit (CFU) results (Table 5)

CFU results of conventional syringe irrigation groups and of continuous ultrasonic groups showed that in no laser and laser sub groups, there was a statistically significant difference between Sample S1 which recorded a higher mean value than Sample S2 in all subgroups where (p < 0.001).

Percentage of bacterial reduction results

Effect of laser

In conventional syringe irrigation groups, Subgroup 1A (Conventional syringe irrigation with no laser) recorded a statistically significant lower mean value of bacterial reduction than Subgroup 1B (conventional syringe irrigation with diode laser) where (p < 0.001) (Table 6 and Figure 4).

In CUI groups, Subgroup 1B (conventional syringe irrigation with diode laser) recorded the statistically significant lower mean value of bacterial reduction than Subgroup 2B (CUI with diode laser) which recorded the highest mean value where (p < 0.001) (Table 6 and Figure 4).

Effect of irrigation activation techniques

In no laser subgroups, Subgroup 1A (Conventional syringe irrigation) recorded the statistically significant lower mean value of bacterial reduction than Subgroup 2A (CUI) which recorded the highest mean where (*p*<0.001) (Table 6 and Figure 4). In laser subgroups, Subgroup 1B (conventional syringe irrigation with diode laser) recorded the statistically significant lower mean value than Subgroup 2B (CUI with diode laser) which recorded the highest mean where (p < 0.001) (Table 6 and Figure 4).

Discussion

Endodontic success is a result of successful removal of vital pulp and pulp remnants, necrotic tissues, microorganisms, and microbial toxins from the root canal system. Although this might be accomplished through chemomechanical debridement, it is unachievable to shape and clean completely the root canal due to its complex anatomy [18].

Great importance was given toward single visit endodontic treatment for non-vital pulp along through disinfection enhancement using different techniques for irrigation activation resulting in better prognosis. Moreover, it helps getting less post-operative pain after the treatment achieving better endodontic outcome [23]. Pain postoperatively is a relatively concomitant phenomenon which frequently happens after cleaning and shaping of root canals. Chemical, mechanical, or bacterial injuries to periradicular tissues occurred during root canal treatment can be attributed to be the etiology of post-operative pain [5], [24], [25].

The present study evaluated the use of CUI and diode laser 810 nm on post-operative pain and microbial

Table 3: The mean and standard deviation (SD) values of post-operative pain of conventional syringe irrigation groups

Variables				After 6 h	After 12 h	After 24 h	After 36 h	After 48 h	After 7 days
Post-operative Pain scores	Conventional syringe irrigation	No Laser	Mean	74.5	52.4	41.4	32.2	28.6	1.7
			SD	5.15	4.35	3.37	3.46	2.67	0.67
		Laser	Mean	58.9	39.7	25.7	17.6	9.3	1.2
			SD	3.11	5.81	4.62	3.5	1.83	0.42
	p-value			0.007*	0.014*	0.022*	0.006*	0.016*	0.192ns

*Significant (p<0.05) ns: Non-significant (p>0.05).

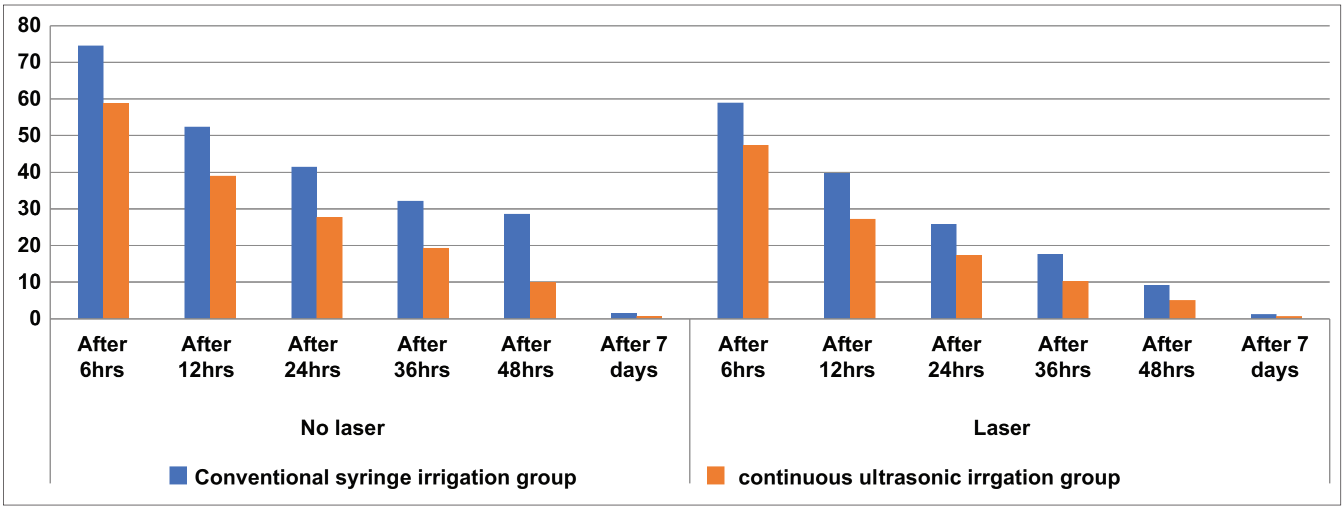

Figure 2: Bar chart representing the effect of irrigation activation techniques for different groups at different examination periods

reduction in endodontic treatment. Apart from ultrasonic irrigation techniques, since laser was introduced in dentistry, its applications in endodontics have been a major field of research. The near-infrared (NIR) wavelengths (810–1064 nm) interaction is predominately through photothermal effect with host tissue. Considering their great penetration depth into dental tissue that may reach >1000 μm through scattering and transmission along the dentinal tubules, which sequentially act as "light guides" [26]. Photonic energy of NIR-laser can directly inactivate only pigmented microorganisms and chromophores such as melanin [27]. Moreover, photothermal damage indirectly occurs, resulting in microbicidal effect [28].

Laboratory studies that run-in root canals both in wet and dry conditions showed antimicrobial promising results. Unfortunately, one drawback is using laser in non-wet environment resulting in root canal walls melting and overheating [29]. On the other hand, in wet canals laser mechanism based on cavitation; in irrigants, sub-ablative setting of laser activation may cause large elliptical vapor bubbles formation, which expand, swell and implode. Bubble implodes rapidly after 100–200 μs, an underpressure develops and sucks fluid back into the canal, inducing secondary cavitation effects [30]. In the diode laser activation method, irrigants presence in the canals during lasing and lessen thermal damage that might be an unwanted side effect to the radicular dentine and periodontium.

Table 5: The mean and standard deviation (SD) values of CFU of different groups

Variables	CFU							
	Conventional syringe irrigation group				Continuous ultrasonic irrigation group			
	No Laser		Laser		No Laser		Laser	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sample (S1)	5.034	0.475	5.207	0.500	5.110	0.500	5.216	0.500
Sample (S2)	5.004	0.525	4.809	0.461	4.675	0.426	3.861	0.239
p-value	<0.001*		<0.001*		<0.001*		<0.001*	

*Significant (p < 0.05) ns: Non-significant (p > 0.05).

Among the numerous lasers, diode laser is the most commonly used one. Diode laser has various advantages including; compact device, affordability, ease of use, setting-up in simple way, versatility, and small in size. Diode laser acting selectively and accurately [31], according to its specific wavelength

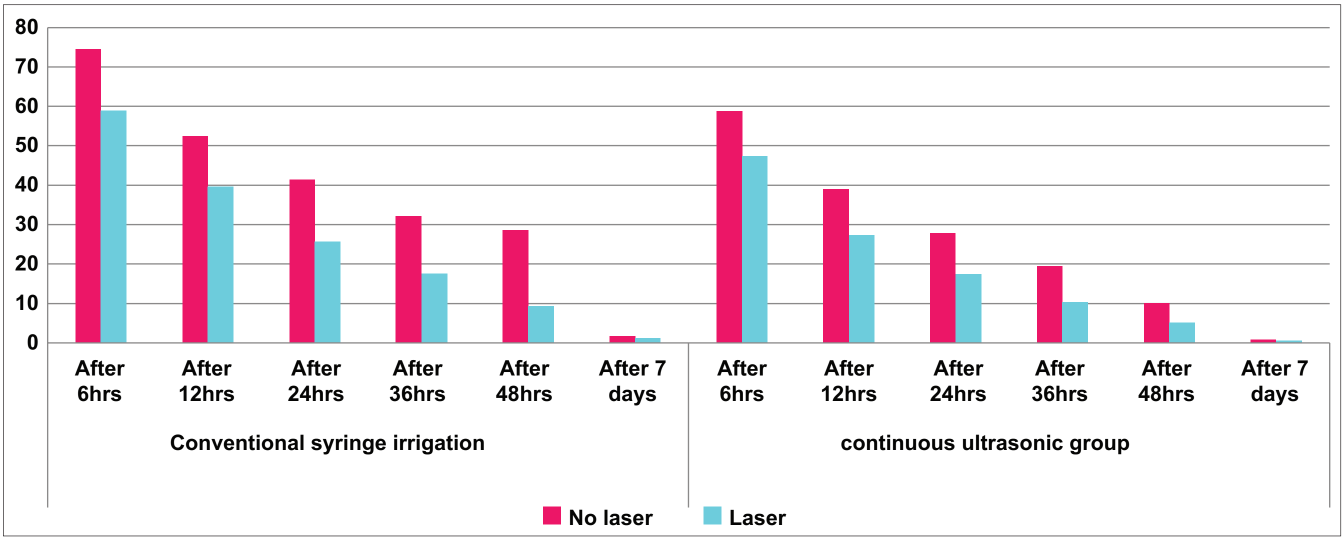

Figure 3: Bar chart representing the effect of laser on post-operative pain at different examination periods

Table 4: The mean and standard deviation (SD) values of post-operative pain in continuous ultrasonic irrigation groups

Variables				After 6 h	After 12 h	After 24 h	After 36 h	After 48 h	After 7 days
Post-operative Pain scores	Continuous ultrasonic irrigation	No Laser	Mean	58.8	39	27.8	19.4	10.1	0.8
			SD	3.82	4.03	5.03	3.47	2.02	0.42
		Laser	Mean	47.4	27.3	17.5	10.4	5.1	0.6
			SD	4.7	4.76	4.33	0.7	0.88	0.52
	p-value			<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.066ns

*Significant (p < 0.05) ns: Non-significant (p > 0.05).

which is highly absorbed in pigmented hemoglobin and melanin and have diminutive absorption in dental hard tissue. They are also greatly absorbed by H_2O [32].

Numerous studies proposed that post-operative pain may be affected by instrumentation technique. Crown-down technique using multiple rotary systems diminishes pain postoperatively in comparison with manual instrumentation and also reciprocation systems [33].

Table 6: The mean and standard deviation (SD) values of percentage of bacterial reduction of different groups

Variables	Percentage of bacterial reduction				p-value
	No-Laser		Laser		
	Mean (%)	SD	Mean (%)	SD	
Conventional	55.92	5.65	79.96	4.59	<0.001*
Ultrasonic	69.81	2.91	96.51	3.86	<0.001*
p-value	<0.001*		<0.001*		

*Significant (p < 0.05), ns: Non-significant (p > 0.05).

Fiber optic diode laser cone tip was inserted 1 mm short of the WL, after that laser was activated. To ensure balanced diffusion of laser beam in lumen of root canal, the fiber tip was moved on the canal walls in slow circular motion, leading to spirals formation from the apical to the coronal third of the canal (helicoidal motion) [34].

Laser therapy has tissue penetration capability that locally affects vascularity resulting in inflammation reduction by its effect on lymphokines, immunoglobulins, substance p, histamine, and enhancing lymphatic drainage. It has marked effect on neurons causing rise in neuronal action potential leading to reduction of post-operative pain [35].

As previously recognized, the microorganisms present in the root canals are considered as principal cause of post-operative pain which are extremely difficult to reach and eradicate by conventional techniques. In the existing study, the decrease in post-operative pain in the experimental groups is due to the lethal potency of laser irradiation on microorganisms that present in the root canal. It is also proposed that intracanal laser

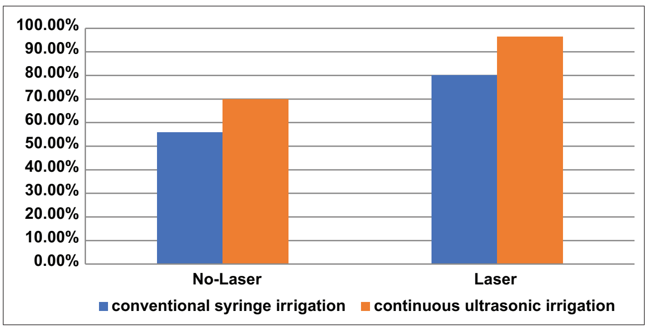

Figure 4: Bar chart representing percentage of bacterial reduction for different groups

activation may eradicate microorganisms present past the root apex [36].

In this present study, continuous ultrasonic activation was applied as a typical technique for irrigant activation as it possesses a strong action on canal cleanliness [37], [38]. It might diminish the number of microbes and diminish the prevalence of positive bacterial cultures after chemomechanical preparation of non-rounded shaped root canals [39]. Furthermore, efficient debris reduction regarding the apical one-third, irregular simulated root canal, and artificially located plugs of dentin debris could be realized [30], [40]. Furthermore, it was stated to diminish post-operative pain [41].

There is a lack of *in-vivo* studies clarifying the diode laser clinical efficacy on removal of debris, elimination of smear layer, and great disinfection reached in irrigation activation. The existing study was planned to measure the post-operative pain after irrigation activation either by ultrasonic or laser on necrotic teeth in one visit treatment. The study was a randomized and clinical trial. The patients' ages ranged from 20 to 45 years old, which allowed for better uniformity of data due to the limited age range. To allow for a more general extrapolation of study results, both males and females were included in the study.

The mandibular molars were chosen because they have the uppermost reported incidence of post-operative pain [42]. In this study, treatment was performed in a one visit root canal treatment as mentioned in two systematic reviews reported and concluded that the prevalence of post-obturation pain was significantly inferior in the single-visit than in the multiple-visit treatment [43], [44].

NaOCl irrigation was used in the present study throughout instrumentation and for final activation, due to its great antimicrobial influence over great number of resistant bacteria on direct exposure. It also dissolves pulpal remnants, collagen, and the dentin's major organic components [45]. It was utilized at a concentration of 2.5% according to numerous researches [40], [46]. The activation of NaOCl has been found to help reach places that are unreachable [47], [48].

In the present experiment, conventional syringe irrigation was performed in three cycles of 20 seconds each, allowing for additional irrigant replenishment [30], [40], [49]. The irrigant was able to reach the end of the root canal with limited apical extrusion using a side vented needle that was 1 mm shorter than the WL.

As mentioned before, 2 ml 17% EDTA was used as final flush [41], [50]. It has the ability to eliminate the smear layer's inorganic portion. Irrigation with both EDTA and NaOCl guaranteed complete eradication of the smear layer [45].

Smear layer comprises bacteria, their by-products, and necrotic tissue. Bacteria can survive, reproduce, and proliferate deep in the dentinal tubules and the smear layer can prevent disinfectants from reaching this area. It may act as a substrate for bacteria, helping them to penetrate further into the dentinal tubules. Pain scores were assessed by VAS. Several studies used a 10 cm VAS due to its applicability for pain severity assessment in variable setting, it was chosen in the present study to record pain. It has great sensitivity and simply explained for the patient to notice the felt pain level [35], [51], [52], [53], [54], [55], [56], [57], [58].

The time intervals chosen for post-operative pain measurement were 6, 12, 24, 36, and 48 h and 1 week; as post-operative pain was commonly predictable among the first 3 days and probably last up to 1 week after endodontic treatment [59], [60].

Results of this present study showed that post-operative pain decreased by time till 1 week. Post-operative pain was significantly lower at Subgroup 2B (continuous ultrasonic with diode laser) and the highest pain score was in Subgroup 1A (conventional syringe irrigation with no diode laser).

Results of laser groups came in agreement with Morsy *et al.* [59], Berk *et al.* [61], Pawar *et al.* [62], Omar *et al.*, [63] and Sen *et al.* [64]. The exact mechanism through which the use of laser reduces post-operative pain remains a mystery. Some publications claim that the diode laser diminishes pain through a variety of processes. Pawar *et al.* [61] and Bjordal *et al.* [65]. discovered that the diode laser reduces chronic pain and has anti-inflammatory potential by subsiding the formation of PGE2, bradykinin, histamine, acetyl choline, and serotonin, as well as weaken substance P production. This explains the promising results obtained for laser groups concerning post-operative pain.

More pain incidence with syringe irrigation may be explained as a result of apical irrigant extrusion. Special care was taken in the current investigation to avoid irrigant apical extrusion, including initial suction of any canal exudates by paper point, crown down sequence of preparation, use of a side vented needle in irrigant delivery, and a continuous slow rate of irrigant delivery into the canals.

Moreover, CUI group showed less post-operative pain than conventional syringe irrigation group in all time intervals. Results came in agreement with Middha *et al.* [41] results who found that at all time intervals, the continuous irrigation group had lower mean pain scores than the syringe irrigation group. Results of microbial reduction values were higher in the CUI group compared to conventional syringe irrigation group, laser subgroups compared with no laser subgroups, in which the best bacterial reduction was in Subgroup 2B (continuous ultrasonic with diode laser) followed by Subgroup 1B (conventional with diode laser group) followed by Subgroup 2A (continuous ultrasonic with no diode laser) and the least bacterial reduction was Subgroup 1A (conventional with no diode laser).

Furthermore, the remarkable link found between the treatment protocol and post-operative pain suggests that the CUI group could be effective during chemomechanical preparation in terms of decreasing post-operative pain. The proposed benefit of the CUI group over the standard syringe irrigation group could be explained by a variety of causes. CUI may have resulted in less acute post-operative pain due to better microbiological control and lower irrigant extrusion. The presence of bacteria in the root canal has been suggested to be the primary cause of post-operative pain [1].

By prompting acoustic streaming and cavitation of the irrigant, CUI has been shown to improve irrigant delivery to uninstrumented zones of the root canal system and aid in the elimination of residual debris and bacteria [19]. According to many studies, 1 min of CUI follows hand or rotary instrumentation improves canal and isthmuses cleanliness [14], [38] and lowers microbial load [66]. CUI according to the another study [67] improves irrigant infiltration into the main and lateral canals. CUI was found to be more successful in removing debris from the apical third [68] [69] and constricted isthmuses [12]. CUI causes significant shear stress in the apical portion of the canal, resulting in superior reduction of firmly adhering bacterial biofilm [70].

Irrigation extrusion may cause chemical irritation of the periradicular tissues, resulting in post-operative pain [1]. In the existing investigation, the syringe needle was 2 mm short of WL, while the CUI needle was limited to 75% of its WL. With an average WL of 19–20 mm in the mandibular molar, the needle tip should be inserted 4–5 mm distant from the canal terminus. It is probable that in CUI, the needle's position distant from the canal termination resulted in less irrigant extrusion into the periapical region.

While this hypothesis contradicts two previous studies on irrigant extrusion, both of which showed similar [71] or greater extrusion [72] of the irrigant with CUI than conventional irrigation method. These differences in results may be because the conditions of these studies may not precisely match the *in vivo* conditions. These studies were conducted in the laboratory in an open-ended experimental paradigm that does not accurately represent the periapical tissues' resistance to apical extrusion power in a clinical setting.

Furthermore, immediate post-operative pain has been observed to be maximum on the first 48 h after treatment, then it normally diminishes significantly [73].

The impact of any intracanal intervention on pain may be most noticeable in the first 1- or 2-day post treatment. It is possible that the non-significant difference between the groups on days after the 1st day is due to the fact that the reported pain had already decreased substantially to such low levels by the 1st day that any further reduction was irrelevant.

Conclusion

Using diode laser and CUI activation techniques as adjunctive methods showed improvement in post-operative pain records and enhanced bacterial reduction in root canal therapy.

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