Comparative Evaluation Of Microbial Eradication In Root Canal By 5.25% Sodium Hypochlorite And 940 Nm Diode Laser: An In Vivo Study

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Citation

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Abstract

Background: It is well known that the causative agent for endodontic disease is microbial growth, and therefore, to prevent or eliminate apical periodontitis, a complete microbial eradication is essential. Many researches have demonstrated that eradication of microbial growth before obturation leads to a more promising outcome for endodontic therapy.

Purpose: This in-vivo study is considered the first study to compare in-vivo the antibacterial efficacy of 5.25% sodium hypochlorite with 940 nm diode laser in the infected root canal in Duhok city.

Method: The study included 200 patients with pulpitis; the diagnosis was based on clinical signs (examination, probing, percussion, palpation) and X-ray investigation. They were divided into 2 groups; group 1 (n = 100) for the irradiation with 940 nm diode laser, and group 2 (n = 100) for the irrigation with 5.25% sodium hypochlorite. In each group, microbiological samples were taken on accessing the canal before treatments, and the second sample was taken after completion of the treatment. All microbiological samples were transferred immediately for microbiological analysis.

Results: Hundred eighty-seven of the 200 patients (root canals) were included in the results since 13 patients showed no growth, and 299 bacterial isolates along with Candida albican were isolated and identified. Laser treatment eradicated the root canal of 70 patients, and 38 patients by the chemomechanical treatment. Significant differences were found in both treatments (One-way ANOVA, P□0.05). The highest prevalence of bacterial isolates belongs to Enterococcus faecalis, followed by Streptococcus, Staphylococcus, and Candida albican. Both treatments eradicated the root canal of 108 (54%) patients and a reduction of the microbial growth in 79 (39%) of the patients.

Conclusion: Both treatments showed a significant difference in eradicating the isolated microorganisms statistically. The 940 nm diode laser was more effective than chemomechanical treatment, as the root canal of more patients were eradicated from root canal bacterial growth. However, combining both treatments will give more effective against the microorganisms present in the root canal.

INTRODUCTION

Root canal therapy aims to preserve the natural teeth by either eradicating or reducing microorganisms, which are the main etiological agents in endodontic diseases. Microorganisms invade pulp chamber and the three-dimensional root canal system, where they form biofilms and reservoir within the dentinal tubules away from the immune system, systemic antibiotics, and even irrigating solutions (Masilionyte and Gutknecht, 2018). Once inside, bacteria and their by-products irritate and damage the surrounding periapical tissues; therefore, root canal treatment aims to clean, sterilize, and sealed tight obturation to prevent reinfection. Conventionally, chemomechanical methods have been used, which consist of a combination of mechanical instrumentation in removing infected pulp tissue and dentine, along with disinfecting solutions for irrigation. Since its introduction by Walker in 1936, sodium hypochlorite (NaOCl) has been widely used in endodontic treatment because of its excellent antimicrobial activity and ability to dissolve organic tissues (Siqueira et al., 1997). To this day, sodium hypochlorite is still the most preferred irrigating solution as it can flush debris from the canal,

dissolves vital and necrotic tissues, low surface tension and lubricating action along with it being inexpensive, long shelf life and readily available (Poggio et al., 2010).

However, around 40-60% of microorganisms still survive after chemomechanical treatment since the irrigant solution are incapable of reaching locations in isthmuses, additional canals, and apical region (Naghavi et al., 2014). Many studies have shown that sodium hypochlorite has a high bactericidal effect only on direct contact in vitro and its effectiveness in vivo is limited to the inability in removing smear layer and penetration to a depth where bacteria is located (Masilionyte and Gutknecht, 2018; Goldman et al., 1982; Haapasalo et al., 2000).

However, since pathogenic microorganisms penetrate the root dentine up to the depth of more than 1mm, the conventional chemomechanical treatment does not result in complete bacteria removal as irrigant solution only reach a depth of approximately 100 m (Bhatia and Kohli, 2013). The incomplete eradication of microorganisms in the root canal system by the chemomechanical treatment encouraged many researchers to pursue more consistent and safer novel methods and technologies to advance the root canal treatment and look for methods that can prevent reinfections (Masilionyte and Gutknecht, 2018).

Lasers have caught the attention of many researchers in the past couple decades, as their thermal effect which has antimicrobial abilities at different wavelengths and most of them have been proven to be safe and effective when used properly (Masilionyte and Gutknecht, 2018; Schulte-Lünzum et al., 2017; Gutknecht et al, 2005; Hmud, 2010; Ramsköld et al., 1997; Haidary, 2016). The laser has been known to activate in a continuous wave, pulse mode or by the use of the optical fiber conductor, and research are still ongoing for the most appropriate, efficient, safe, and accessible wavelength (Asnaashari et al., 2016). The diode laser has recently caught more attention, due to their affordability, spectra of indications, and also due to its high absorption in pigments (melanin, Haemoglobin) both of which are prevalent in soft tissues. Diode laser wavelengths (810, 940, and 980 nm) when transmitted in water show deep penetration in dentine (1000 m) and have a similar selective bactericidal effect to that of Nd: YAG 1064 nm laser (Masilionyte and Gutknecht, 2018; Schulte-Lünzum et al., 2017; Al-Karadaghi et al., 2015; Al-Karadaghi et al., 2015).

This study aims to comparatively evaluate the effect of 940 nm diode laser with the effect of 5.25% sodium hypochlorite in root canal treatment.

MATERIAL AND METHOD

Subject selection

Two hundred patients with pulpitis showing symptoms of continuous pain, swelling, tenderness to pressure were included in this in vivo study. The study was performed on patients in the Duhok Dental Polyclinic, Duhok City, Iraq, and the ethical committee of Duhok Directorate General Health approved the research. All the patients had good general health with no significant systemic conditions, age ranging between 18-70 years old. Pregnant and nursing females were excluded, and written consent was obtained from each patient before the treatment.

The patients were put into two groups:

- Group 1. Conventional chemomechanical treatment with 5.25% of sodium hypochlorite (De Net, Turkey) as an irrigating solution (100 patients).
- Group 2. Mechanical instrumentation with normal saline (Mipharm, UK) as an irrigant solution followed by 940 nm epic 10 diode laser (epic 10, BIOLASE tech, Irvine, USA), (100 patients).

Sample collection

The aseptic setting was carried out throughout the root canal procedure. All patients had local anesthetic administered, and the teeth was correctly cleaned, and rubber dam used. Using a sterile high-speed air turbine handpiece (NSK Pana Max-Japan) an access opening was made into the canal. For each group of patients, working length was estimated using a sterile #15 K-file introduced into the root canal about 1-mm short of the apex, with the K-file in root canal x-ray was taken to determine the total canal length and this was further confirmed by using an electronic apex locator. Samples were taken from patients at this stage before any of the two treatment (laser and sodium hypochlorite) using sterile paper point and transferred aseptically into thioglycolate broth for microbiological investigation.

NaOCl treatment

Next was to obtain the samples for group 1 (5.25% sodium hypochlorite), the chemomechanical treatment was carried out in one appointment. Working length was achieved with hand endo nickel-titanium files (D-prefect, China) in a step-back technique. The canal was irrigated with 5.25% sodium

hypochlorite using an endo irrigation needle tip side-vent (YBB, China) and depending on the canal anatomy the apical files ranged from #45-70 each file followed by sodium hypochlorite irrigation. After the final irrigation, a sterile paper point of the corresponding sizes was taken as the second sample for the chemomechanical treatment and transferred into thioglycolate broth for microbiological investigation.

Laser Treatment

As for group 2 (diode laser treatment), the mechanical instrumentation was carried out, and again working length obtained the same as in the sodium hypochlorite treatment. The root canal was filed in a step-back technique in a back, and forth rotation and the irrigating solution used was normal saline (Mipharm, UK), and the apical files ranged between #45-70 with normal saline irrigation in between. The use of normal saline as an irrigant in the laser treatment was only to give a mean of lubricating effect and help flush out the debris made by the mechanical preparation, as it does not enhance or synergistically help the antibacterial effect of laser. After complete biomechanical preparation with normal saline, the canal was irradiated with a 940-nm diode laser (epic 10, BIOLASE tech, Irvine, USA). Endodontic fiber tip (200 m diameter and 22 mm length) was inserted into the canal approximately 1 mm short of the apex at an output power of 1.5W. The root canals were then irradiated for 30 s with 10 s resting time, then another 30 s irradiation (1 m laser irradiation). After laser irradiation, a sterile paper point of the corresponding sizes was taken as the second sample and transferred into thioglycolate broth for microbiological investigation.

Microbiological investigation

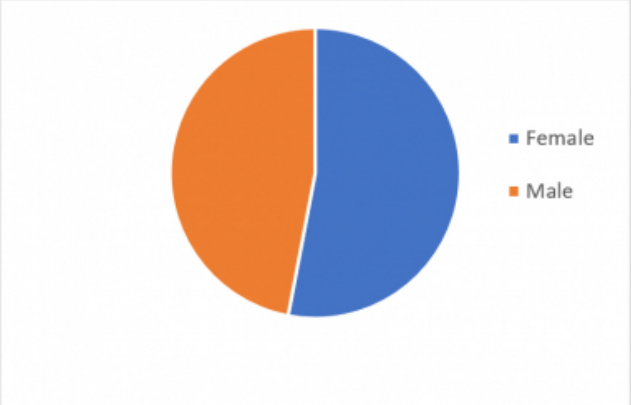
All the microbiological samples were sent off to the Microbiology laboratory in the College of Health Science, Duhok University and incubated at 37C for 24 hours. They then streaked on three types of media (blood agar, chocolate agar, and MacConkey agar) and incubated aerobically and anaerobically utilizing anaerobic jars at 37C for 24, 28 and 72 hours. All the isolated colonies have catalase and oxidase test carried on, and further identified employing cultural, morphological, physiological, and biochemical characteristics by the methods described by Cheesbrough (Cheesbrough, 2006).

RESULTS

Two hundred endodontic patients (200 teeth) participated in this study, with an age range of 18-60 years old. According to gender, 53.5% of the patients were female, and 46.5% were male patients (figure 1). Culture depending methods were used to identify the microorganisms that were isolated from the root canal samples of the 200 patients in chemomechanical and diode laser treatments. The microorganisms (298 microbial growth) were isolated and identified from 187 (93.5%) of the patients, since 13 (6.5%) patients showed no microbial growth.

Figure 1

Gender distribution of the patients.



Using IBM SPSS statistic data editor software, the statistical analysis was obtained for all the data in this study. For all the microorganisms isolated before and after chemomechanical and laser treatment, the descriptive statistic was done to find the mean, standard deviation, maximum, and minimum values (table 1).

Table 1

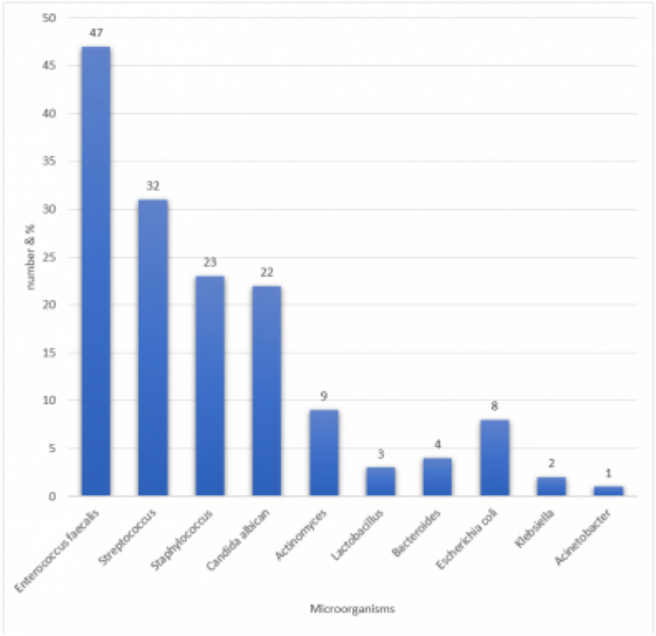
Descriptive statistic of pre and post chemomechanical and laser treatment.

Treatments	Minimum	Maximum	Mean	Std. Deviation
Pre	5	100	29.70	29.922
Post	2	45	16.60	13.810

In group 1, before the chemomechanical treatment, a total of 151 cultivated isolates belonging to 10 different bacterial species along with Candida albican were isolated and identified from 93 patients, since 7 patients showed no growth (figure 2).

Figure 2

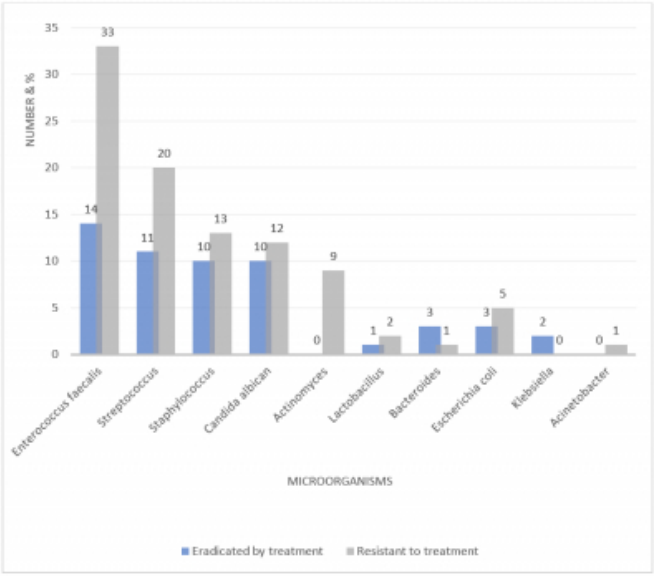
Isolated microorganisms before 5.25% sodium hypochlorite treatment.



In this treatment, 54 (36%) microbial isolates were eradicated out of the 151 isolates, whereas 96 (63.6%) isolates were resistance to treatment as illustrated in figure 3. Utilizing one-way ANOVA, a significant difference (P 0.011) was found in the isolated microorganisms before and after chemomechanical treatment. In term of patients, the treatment completely eradicated microbial growth from the root canals of 38 (41%) patients out of the 93 patients undergoing the chemomechanical treatment.

Figure 3

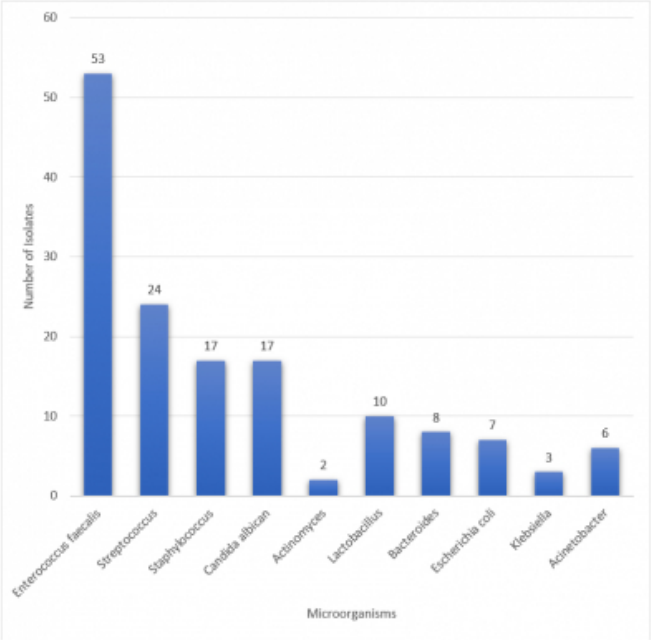
Isolated microorganisms that were eradicated and resistant to 5.25% NaOCl treatment.



Before the diode laser treatment, 147 cultivated isolates belonging to 9 different bacterial species with Candida albican, were identified in the root canal of 94 patients since 6 patients showed no growth (figure 3).

Figure 4

Number of isolated microorganisms before 940 nm diode laser treatment.

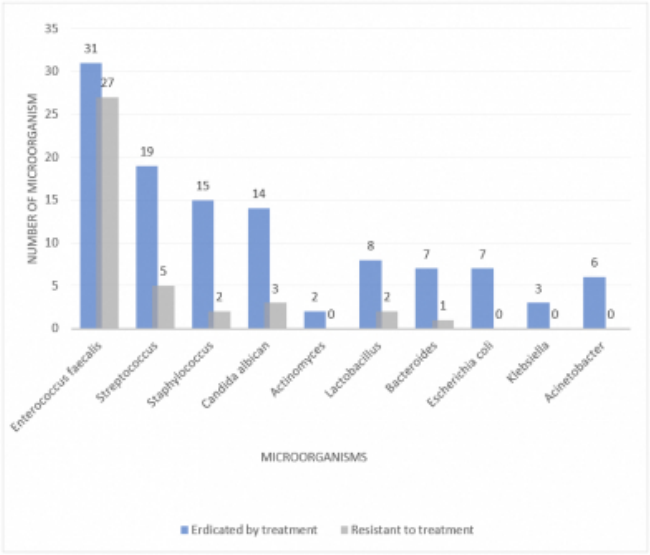


After irradiation with 940nm diode laser 78 (53%) isolates were eradicated out of the 147 identified isolates, whereas,

40 (27%) isolates were resistant to the laser irradiation (figure 4). The one-way ANOVA test showed a significant difference (P 0.034) in 940 nm diode laser treatment. After the irradiation with 940 nm diode laser, 70 (74.5%) patients from the 94 patients that the treatment was carried on, were found entirely free of cultivated microbial growth in their root canals after.

Figure 5

Number of microorganisms that were eradicated and resistant to 940nm diode laser treatment.



Using the pair T-test, when combining the isolated microorganisms before 5.25% NaOCl and 940 nm diode laser together which were 298 microbial isolates and comparing them to the combined results of the eradicated microorganisms in these two treatment, there is a significant difference (P 0.035) in the reduction of isolated and identified microorganisms (table 2).

Table 2

Microorganisms isolates before and after both combined in both treatments

Microorganisms	Isolated before both treatment	Eradicated after both treatment
Enterococcus faecalis	100	45
Streptococcus	55	30
Staphylococcus	40	25
Candida albican	39	24
Actinomyces	11	2
Lactobacillus	13	9
Bacteroides	12	10
Escherichia coli	15	10
Klebsiella	5	5
Acinetobacter	7	6

After the treatment with both 5.25% sodium hypochlorite and diode laser, it was found that Enterococcus faecalis were not eradicated but were reduced to very low levels followed by Streptococcus, Staphylococcus and candida albican, however, the treatment that caused the highest reduction was the diode laser for the isolated microorganisms. Escherichia coli and Actinomyces were completely eradicated by diode treatment and all the other microbial isolates were drastically reduced by this treatment too in comparison to 5.25% sodium hypochlorite. In total, gram positive microorganisms which were isolated are the most followed by gram negative microorganisms which were very low in comparison (table 3).

Table 3

Gram stain frequency of the isolated and eradicated microorganisms in both treatments.

Gram stain	Isolated before 5.25% NaOCl	Eradicated after 5.25% NaOCl	Isolated before 940nm laser	Eradicated after 940 nm laser
Gram positive microorganisms	113	77	123	89
Gram negative microorganisms	16	7	25	23

DISCUSSION

The main aim of root canal treatment is to effectively control bacterial infection within the root canal system by eradicating all the pathogenic microflora, toxins and tissue debris. When a negative microbiological culture is obtained at the time of obturation, there will be a 94% success rate, however, the success rate is reduced to 68% if the culture is positive at the time of filling (Narayan, 2013). Regardless of the large number of studies carried out on root canal treatment, complete in-vivo disinfection of the root canal system is still not achieved (Narayan, 2013). This incomplete eradication of pathogenic bacteria could be due to the formation of biofilms on the root canal system, presences of resistant bacteria (e.g. Enterococcus faecalis), and the chemical environment of the root canal that may alter the antimicrobial efficacy of disinfectants (Narayan, 2013).

Although a number of in-vitro studies evaluating the antimicrobial efficacy of diode laser and conventional chemomechanical treatment are available, yet there are limited studies of their usefulness in vivo. Hence the present study was planned as an in-vivo study to evaluate the effectiveness of 940 nm diode laser in comparison to the 5.25% sodium hypochlorite treatment. The conventional chemomechanical root canal therapy is the routine procedure carried out in Duhok Dental Polyclinic which uses 5.25% sodium hypochlorite as an irrigant, where a syringe is used to flow sodium hypochlorite into the root canal is widely used as it is very convenient and cheap. The irrigant works mechanically, through the flow and the backflow of the

solution, which causes the debris and bacteria to loosen (Narayan, 2013). These are then flushed out of the root canal and the bacterial reduction is further enhanced by the antibacterial effect of the irrigant solution in area which are inaccessible to mechanical debridement (Narayan, 2013). Sodium hypochlorite was used as the irrigant of choice, due to its widespread use in endodontic practice, broad spectrum of antimicrobial action, its ability to hydrolyse necrotic pulp tissue and dissolves organic matter in the root canal. Sodium hypochlorite effectiveness is dependent on the concentration, volume, contact time increasing temperature and flow and surface tension of NaOCl (Nio, 2017).

However, one important factor to consider in regard to sodium hypochlorite irrigation is the depth of penetration, which is around 130 µm into the dentinal tubules, whereas, the infection (bacteria) may occur around 1000 µm (Parhar, 2012). Similarly, many other studies have shown that regardless of the technique, instrument or irrigants used in chemomechanical preparation, some parts of the root canal space remain untouched (Parhar, 2012). As a result, complete bacterial sterilization of the root canal was difficult to achieve by the conventional chemomechanical treatments. Overcoming this, 940 nm diode laser (epic 10, BIOLASE tech, Irvine, USA) was used in this study, since other studies have shown that the use of diode laser was the only method able to penetrate dentine to its full length and have high antibacterial efficacy (Zhang, 2017). Nowadays, the diode laser has become more popular in dentistry mostly due to its relatively small size and low cost.

In this study, sterile paper points were the mode of sample collection, since paper point cultures of the root canal detect bacteria more frequently than using dentine filling cultures on the reamers (Zhang, 2017). Both aerobic and anaerobic isolates were identified from infected root canals in chemomechanical and diode laser treatments. Among aerobic isolates were Staphylococcus, E.coli, Neisseria, Klebsiella, Bacteroides and Acinetobacter. Whereas, among the anaerobic isolates were the most resistant endodontic Enterococcus faecalis, Streptococcus, Actinomyces and Lactobacillus. Candida albican was also among the microbial isolates from the infected root canals in the 940 nm diode laser and 5.25% sodium hypochlorite treatments. The highest isolates identified in both treatments were Enterococcus faecalis, followed by Streptococcus, Staphylococcus and then candida albican.

There was a significant difference between the microbial isolates before and after the 5.25% sodium hypochlorite treatment in this study. The results of this study showed the highest number of eradicated isolates (36%), was in agreement with many other studies that showed high eradication of microbial isolates after the sodium hypochlorite treatment (Ashofteh et al., 2014; Sohrabi et al., 2016). Enterococcus faecalis is considered the most resistant bacteria in root canal infection, as seen in our study, 5.25% NaOCl managed to eradicate 14 (29%) isolates out of the 47 Enterococcus faecalis isolates. This was also reported by many other studies showing effectiveness of 5.25% NaOCl in eliminating or decreasing the number of Enterococcus faecalis isolates (Nio, 2017; Kho and Baumgartner, 2006). A study by Kho and Baumgartner, which compared the antimicrobial efficacy of 1.3% NaOCl with 5.25% NaOCl, showed that 5.25% NaOCl was significantly more efficient in eliminating microbial isolates and in particular the resistant Enterococcus faecalis (Kho and Baumgartner, 2006). Ashofteh et al., and Sohrabi et al., showed that sodium hypochlorite had the highest number of isolates with no microbial growth (CFU = 0) which also agrees with Baumgartner et al (Ashofteh et al., 2014; Sohrabi et al., 2016). These results agree with the results of our study, which shows the effectiveness of 5.25% sodium hypochlorite in the reduction/eradication of microbial growth in infected root canal.

Candida albican were also isolates identified in this study from the root canals of both treatments. Sodium hypochlorite at 5.25% concentration was effective in eradicating 10 (45%) isolates of Candid albican out of the 22 identified isolates. These results are in consistent with other studies that concluded 5.25% NaOCl was sufficient enough in eradicating Candida albican in 30 seconds as reported by Waltimo, Kudva et al., and Marcia et al (Ghogre, 2014). It is considered the most popular irrigant for its capacity to kill bacteria when they are in intimate contact for enough time (Gołębek et al., 2019; Battista and Pantera, 2019; Gazzaneo et al., 2019; Mohmmed, 2017).

Many scientists try to assess the influence of different concentrations of NaOCl on many aspects such as tissue dissolution, antimicrobial efficacy, deep penetration into the dentine, been able to remove smear layer, decalcification of dentine, microhardness, viability of stem cells in the apical region, viscosity, amount of debris extrusion, tooth surface strain, and postoperative pain (Verma et al., 2019). However, the scientific field is divided into some believing

that the use of higher concentration of NaOCl is most efficient in eliminating microbial growth from root canal infections. Others have shown a negative impact of an increase in concentration due to high toxicity levels and yet another group of studies reported that there is no significant difference between low or high concentrations of NaOCl when it comes to antimicrobial efficacy, tissue dissolution and smear layer removal (Verma et al., 2019).

This is consistent with other ex-vivo and in-vivo studies, which were carried out using culture dependent and independent methods, stating that chemomechanical treatment is very effective in reducing microbial growth in most of the cases (Gazzaneo et al., 2019). However, in spite of the instrument used, sodium hypochlorite concentration, exposure time, and volume it is not possible to render the root canal bacteria free (Gazzaneo et al., 2019). The variation in the results of these studies is mostly due to different microbial species/strains used, different planktonic or biofilms growth, and the detection methods used (culture dependent or culture independent) (Gazzaneo et al., 2019).

The 940 nm wavelength diode laser was used in this in-vivo study since its clinical application in endodontics has not been sufficiently studied and also due to its low cost, greater versatility and portability because of its compact size. For the 940 nm diode laser treatment, sterile saline solution was used as an irrigant solution. Saline will not enhance or synergistically help the antimicrobial effect and will only act as a lubricating agent and help in the flushing out of debris during the biomechanical preparation. The results 940 nm diode laser treatment were very effective in eradicating a high number of the isolated microorganisms 78 (53%) and reducing the number of colonies in the resistant isolates. The treatment showed a significant difference in the number of isolates before and after laser irradiation.

Our results are comparable to other results that showed diode laser treatment has a high efficacy in reducing or eradicating cultivated microbial growth in infected root canals. Gutknecht et al., demonstrated that diode laser can eradicate microbes that have migrated deep into the dentine and more specifically Enterococcus faecalis (Gutknecht et al.,2004). An early study by Gutknecht et al., reported that 83% of infected cases were treated successfully, after been unsuccessfully treated by conventional chemomechanical method (Gutknecht et al.,1996). Based on Gutknecht et al, clinical expertise and patient follow up results, they concluded that microorganism reduction, which is a must for successful endodontic therapy must be due to laser (Gutknecht et al.,1996). Another study showed that the spreading of laser energy and the penetration into the dentinal wall, made diode laser to be physically more efficient than conventional chemical irrigant in reducing dentinal wall bacteria (Olivi, 2013). Diode laser at 810 nm and 1064nm, achieved a microbial reduction of 74%, whereas, 980 nm achieved a maximum microbial reduction (Olivi, 2013). A more recent study suggested that 940 nm diode laser was a more reliable alternative to conventional root canal treatment. They stated by using 940nm diode laser, it will allow a decrease in irrigating solutions, intracanal medication and antibiotics and a faster healing time (Masilionyte and Gutknecht, 2018).

Diode lasers have deep dentine penetration, high absorption in the melanin and haemoglobin which allows for selective killing of pigmented and pigment producing microbes which make up most endodontic infections (Sheima'a et al., 2018). A study by Vatkar et al., 2016 showed that diode laser had the ability to reach dentinal tubule depth beyond 1000□m, proposing that once the laser light is emitted it loses the characteristic of a concentrated beam (Vatkar et al., 2016). And light fog is created inside the dentine. This fog light spreads to the peripheral root dentine, where it can successfully reach and eradicate microbial growth especially the resistant Enterococcus faecalis (Vatkar et al., 2016). There is agreement that 940 nm diode laser irradiation has the potential to kill microorganisms and remove debris and smear layer from root canals when used at appropriate laser setting safely and effectively without any collateral damage to the periodontal tissues (Tilakchand et al., 2018; Bansode et al., 2017; Jhingan et al., 2015).

Many other researchers have done studies on the combining effect of two different diode laser or a diode laser as adjunct to conventional root canal irrigant solution to increase the efficacy of root canal therapy. A study by Erben et al., 2019, demonstrated that combining Er,Cr:YSGG and 940 nm diode laser at safe wavelength, was most effective in decreasing the number of microorganisms in the infected root canal (Erben et al., 2019).170 Another study showed that the combination of sodium hypochlorite and 940 nm diode laser has a synergistic effect and increasing the bactericidal action (Castelo-Baz et al., 2012).171

Conversely, there are some studies which have demonstrated that diode laser has poor bactericidal effect when used in root canal therapy. A study by Bago & Anic in 2014,

showed that high power diode laser demonstrated greater difficulties in eliminating microorganisms in specific Gram-positive Enterococcus faecalis. The author attributed this to the fact that laser induces bacteria killing by thermal heating and Enterococcus faecalis is known for its resistance to heat because of its cell wall structure (Bago & Anic, 2014).159 A study by Schoop et al., 2004 in which root canals where infected with Escherichia coli and Enterococcus faecalis failed to show bacteria eradication when diode laser was used (Schoop et al., 2004).168 Another study showed that diode lasers are less effective in eradicating microorganisms from the infected root canals (Meire et al., 2009).173

CONCLUSION

This is the first in-vivo study that compare the effectiveness of 940 nm diode laser and 5.25% sodium hypochlorite in eliminating microorganisms from infected root canals in Duhok city.

Instrumentation of the root canal system aided by irrigation with 5.25% results in a significant reduction and in some cases a complete eradication of the cultivable microorganisms in the sample.

Treatment with 940 nm diode laser have a statistically significant antimicrobial action on the infected root canals.

Both treatments have managed to eradicate a high number of the most resistant root canal bacterium (Enterococcus faecalis).

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