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Comparison between the Bactericidal Effect of Nd: YAG Laser and Sodium Hypochlorite: An *In vitro* Study

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ABSTRACT

Objective: *This study aimed to compare the bactericidal effect of Nd: YAG laser and sodium hypochlorite solution.* ***Background:*** *It is very important to obtain the best sterilization and antibacterial effect during treatment of the infected root canals.* ***Materials and methods:*** *Bacterial samples were taken from infected root canals immediately after the tooth extraction. The samples were divided into 3 groups, each group contained 30 individuals. In the first group, sodium hypochlorite was used alone as a bactericidal agent. In the second group, Nd: YAG laser was used as a bactericidal agent. In the third group, both of them were used. Bacterial swabs were taken from these samples before and after treatment by each bactericidal agent, these swabs were streaked on blood agar and incubated, and the number of the colony forming units was recorded. The diameter of the non-growth zone was measured after exposure to each bactericidal agent.* ***Results:*** *The results showed an excellent bactericidal effect for the sodium hypochlorite group. The number of colony forming units was reduced to about 89% from the original number before the treatment. While the Nd: YAG laser exhibited a weak bactericidal effect with about 25% of the bacterial cells eradicated. The best antibacterial activity obtained was when both sodium hypochlorite and Nd: YAG laser were used together, with a percentage of reduction of about 96%.* ***Conclusion:*** *The sodium hypochlorite has a very good bactericidal action if compared to Nd: YAG laser. The Nd: YAG laser was merely effective as a bactericidal agent. The best bactericidal effect was observed when both sodium hypochlorite and Nd: YAG laser were used together. Nd: YAG could be used as a co-adjunctive agent to agitate the sodium hypochlorite solution and to increase its activity.*

Keywords: Pulpitis, Sodium hypochlorite, Chronic pulpitis, Bactericidal agent

INTRODUCTION

Infection of the root canal (pulpitis) is an inflammation of the dental pulp resulting from untreated caries. Its principal symptom is the pain. Diagnosis is based on signs and symptoms and is confirmed by X-ray. Treatment involves removing decay, root canal therapy or extracting the tooth [1]. Root canal therapy, or sometimes called endodontic treatment, is a sequence of steps for the infected pulp of a tooth which leads to the elimination of infection and the protection of the decontaminated tooth from future microbial invasion [2]. Studies have reported different species in the pulp of necrotic teeth and, therefore, one or multiple kinds of bacterial pathogens can be isolated from an infected root canal [3,4], for example (*Prevotella spp, Porphyromonas spp, Fusobacterium spp, Actinomyces spp, Streptococcus spp, Enterococcus faecalis* [5]). The use of irrigating solutions is an essential part of effective chemomechanical preparation. It enhances bacterial elimination and facilitates the removal of necrotic tissue and dentine chips from the root canal; thus irrigants prevent packing of infected tissue apically in the root canal and into the periapical area. Besides, many irrigating solutions have other beneficial effects. The most popular types of root canal irrigants are (ethylene-diamine-tetra-acetic acid (EDTA), sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX). In recent years, laser systems were widely involved in dental treatments. Many applications of lasers in treatment protocols

for dental hard and soft tissues are in use now or being developed. Also, new wavelengths and different methods are being applied in the dental field. In various laser systems used in dentistry, the emitted energy can be delivered into the root canal system by a thin optical fiber (Nd: YAG, Er, Cr: YSGG], argon, and diode) or by a hollow tube (CO_2 and Er: YAG). Thus, the potential bactericidal effect of laser irradiation can be efficiently used for the additional cleaning of the root canal cavity after biomechanical instrumentation. This effect was studied extensively using lasers such as CO_2, Nd: YAG, excimer, diode, and Er: YAG [6]. Previous endodontic studies using different wavelengths of high-intensity laser light have shown the ability of their thermal impacts in the sterilization procedure of dental hard tissues, either in the reduction of bacterial counts in contaminated root canals or in the apicectomies at the surgical site. This has been considered as a significant advantage over the traditional root canal disinfection procedures [7]. Nd: YAG laser (1064 nm) is one of the laser systems that have many applications in the dental field. Irradiation with Nd: YAG laser is absorbed by protein (high affinity to melanin) and mineral structures, such as phosphates and carbonate hydroxyapatite, as well as water (low affinity). Utilizing thermal stresses created inside the tissue, Nd: YAG disorganizes the skeleton of bacterial cells. In this process, protein denaturation takes place and the bacterial cells die [8].

MATERIALS AND METHODS

This study contains 3 groups, each group containing 30 individuals. In the first group, sodium hypochlorite 5.25% was used alone as a bactericidal agent, in the second group, Nd: YAG laser was used as a bactericidal agent, and in the third group, both of them were used. All the teeth that have been used were single-rooted, primarily infected. The teeth with chronic pulpitis, secondary endodontic infection, endo-perio lesions were excluded. Bacterial swabs were taken before treatment with each bactericidal agent. These swabs were streaked on agar plates and incubated, and the colony forming units (CFU) were recorded. The test of the inhibition zone was also applied. This test depends on measuring the diameter of the non-growth area that was created by the action of the bactericidal agent. In the first group, NaOCl was used alone as a bactericidal agent. Total 1 ml of NaOCl 5.25% solution was added to 1 ml of the bacterial suspension and left for 20 min. The bacterial suspension was contained in an Eppendorf tube, 2 ml capacity. After 20 min, a swab was taken and streaked on agar plates and incubated. The number of CFU was then counted. The zone of inhibition test was also applied. A paper disk 6 mm in diameter was immersed in NaOCl 5.25% solution for 24 hours and then placed on the agar surface. After incubation, an area of non-growth appeared. This area was measured and documented.

In the second group, Nd: YAG laser was used alone as a bactericidal agent. And 1 ml of the bacterial suspension was placed in an Eppendorf tube, and a swab was taken and streaked on agar plates. The number of colony forming units was recorded. The Eppendorf tubes were irradiated by Nd: YAG laser for 2 minutes and a swab was taken after irradiation to count the number of CFU. The zone of inhibition test was also applied to this group. The agar plates were irradiated for 2 minutes. The laser device used in this study was long pulsed and the parameters were 110 joules, 2 Hz, 40 m/sec, 6 mm spot size, 3.8×10^4 J/cm^2 energy density.

In the third group, both of the NaOCl 5.25% and Nd: YAG laser were used. Total 1 ml of bacterial suspension was placed in an Eppendorf tube, and 1 ml of NaOCl 5.25% was added to it, then the tube was irradiated by Nd: YAG laser. This group was subdivided into 3 subgroups; each group contains 10 samples. All the samples were irradiated by laser for 2 minutes. The only variable was the time of NaOCl treatment. The first subgroup irradiated by laser immediately after treatment with NaOCl. The second subgroup was irradiated by laser after treatment with NaOCl for 2 minutes. The third subgroup was irradiated by laser after treatment with NaOCl for 5 minutes (Figure 1).

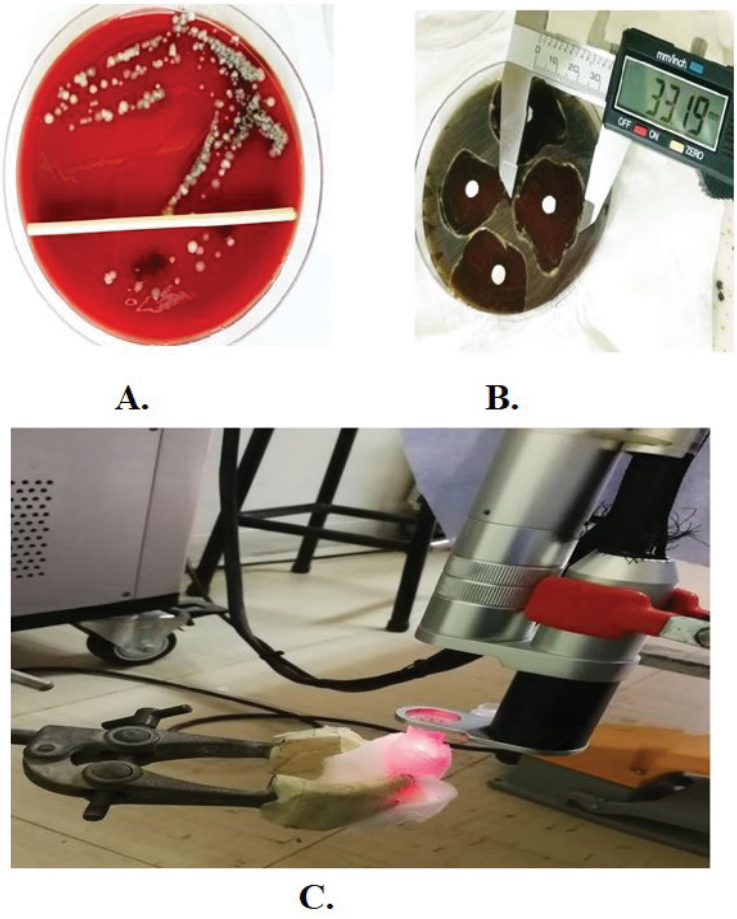

Figure 1 (A) CFU on agar plates, before and after treatment, (B) zone of inhibition, (C) laser-sample set up

RESULTS

This study showed different levels of antibacterial activity measured as CFU/ml and as inhibition zone diameter for each bactericidal agent. The NaOCl was about 89% bactericidal when used alone while Nd: YAG laser reveal weak effect (about 25% bactericidal). The combination of NaOCl and laser exhibit different levels of lethality according to the time of NaOCl exposure. The bactericidal efficacy in group 3.1 (immediate irradiation after NaOCl exposure) was about 49%, while in group 3.2 (irradiation after 2 min NaOCl exposure) was about 73%. The most effective bacterial killing regime was in group 3.3 (irradiation after 5 min of NaOCl treatment) where the percent raised to about 96%. The diameter of inhibition zone test was biased to NaOCl side, also. The biggest diameter recorded (for NaOCl) was about 40 mm. While the diameter of inhibition zone for laser beam was nearly limited to the diameter of spot size used (about 6 mm) (Figures 2 and 3).

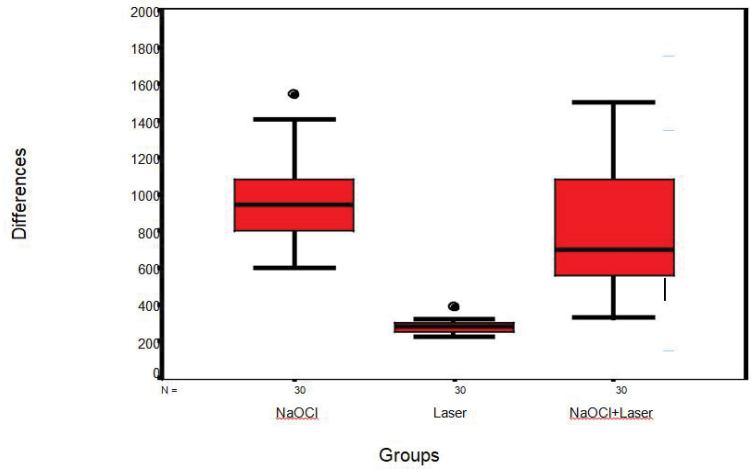

Figure 2 Stem-leaf plot for exploring the behavior of CFU/ml parameter before and after treated with different groups

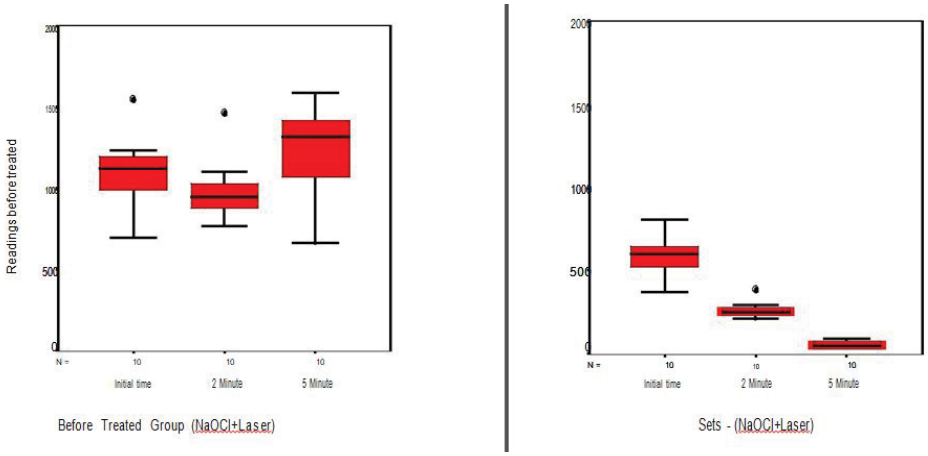

Figure 3 Stem-leaf plots for exploring the behavior of CFU/ml parameter before and after treated with different sub-groups of treated with (NaOCl+laser)

DISCUSSION

One of the most important goals of root canal treatment is removing of these inflamed tissues from the root canal cavity and eradication of bacteria living inside. In this study, the work was achieved on a biomass, which means all the living micro-organisms were included (all species were targeted). Metal files were used to clean and shape the canal walls and chemical solutions with bactericidal effects such as NaOCl, CHX, EDTA…, etc. were used to kill the bacterial cells and also to facilitate the movement of the files inside the canal and washing of debris to the outside [9,10]. The most important chemical solution with the bactericidal effect that was used widely in the dental field is NaOCl. It has a dissolving action on the organic components present inside the canal (such as the bacteria and the pulp tissues). It has the ability to dissolve the lipids and convert them to fatty acids [11]. The removal of this debris from the root canals will facilitate the shaping of the canal and providing more access for the solution to enter dentinal tubules and sterilize them. Siqueira, et al., tested many concentrations of NaOCl (1%, 2.5%, 5.25%) to figure out which concentration has a more powerful bactericidal effect (*in vitro*) [12]. NaOCl was used on root canals intentionally infected by *E. faecalis*, and bacteria were counted (as CFU) before and after application of the solution. It was concluded that "all the concentrations were effective in reducing bacterial count, and there were no significant differences between them."

Harsh, et al., found that the 4% NaOCl was very effective in reducing the bacterial count (*in vitro*) [13]. He worked on roots contaminated with *E. faecalis*. Different concentrations of NaOCl and different irrigation techniques were used

to activate the solution and the result he found to coincide with our research. Al-Sudani, et al., prove that NaOCl is very good for flushing out the debris, removing the smear layer and reducing bacterial count [14]. It has an effective dissolving action on the organic component inside the root canal. All the previously mentioned studies agree with the result of this research that the NaOCl is very effective in reducing bacterial count. Soares, et al., figure out that the intra-canal microbiota is highly susceptible to biomechanical preparation in the presence of 1.25% and 5.25% NaOCl as an irrigant inside the root canal during treatment [15]. He took a sample from infected teeth before and after biomechanical preparation and noticed the decrease in the number of the microorganisms.

In last years, the laser has been exercised widely in different branches of dentistry, either in soft tissue treatment or in the cutting of bone and dental hard tissues. Lasers in recent time are frequently used to deal with oral lesions and infections or in the eradication of bacteria (especially in root canal treatment). Many types of lasers have been used in endodontic treatment. The most popular one is the diode laser (because of its availability and low device cost). The other types of laser devices also have been practiced in endodontic treatment such as (Er: YAG, ErCr: YSGG, Nd: YAG) in many institutions that provide dental care services. The main uses of lasers in root canal therapy are killing of bacteria, shaping of the canal, and activation of chemical irrigation solutions. The action of the laser in such treatments is explained by the thermal effects that are generated by the laser beam. The Nd: YAG laser is classified as solid-state laser because it contains a solid lasing medium (Neodymium-doped yttrium aluminum garnet). The wavelength of this type is 1064 nm and it is also available as a second harmonic generation with 532 nm. The laser beam in this wavelength could be delivered optically by using optical fibers. The Nd: YAG laser is considered as a soft tissue laser since the laser beam has a peak absorption in pigmented tissues that contains dark pigments such as melanin and hemoglobin. There are many types of bacteria that produce pigments (e.g., black pigmented bacteria), and these types can absorb the Nd: YAG beam easily [16]. The Nd: YAG laser has a good penetration depth through the dentinal tubules. Berkiten, et al., has tested the antibacterial efficiency of Nd: YAG laser and measure the penetration depth of the laser beam under SEM [17]. He used 2 powers: 1.8 watts and 2.4 watts. The 1.8 W laser beam shows a penetration depth ranged from 400 µm to 800 µm. While for the 2.4 W laser the depth was 600 µm to about 750 µm. In this study, The Nd: YAG laser shows a weak bactericidal effect when it is used alone. This indicates weak absorption of bacterial cells to Nd: YAG laser. This result agrees with the study conducted by Meire, et al., where they grew *E. faecalis* on dentin disks and expose it to ND: YAG laser [18]. The number of the surviving bacteria was counted by using plate count. He found that the Nd: YAG laser has the least effective bactericidal effect if compared to Er: YAG and NaOCl. The weak bactericidal effect of the laser (when used alone) coincides with Pirnat, et al.m who used a sapphire substrate, loaded with *E. faecalis* and irradiate it with Nd: YAG laser [19]. He found that the Nd: YAG has a weak direct bactericidal effect on the pigmented and non pigmented bacterial cells (as a biomass). Asnaashari, et al., compares between different laser wavelengths including diode (810 nm), Er: YAG (2940 nm), Er,Cr: YSGG (2780 nm) and Nd: YAG (1064 nm) in killing bacterial cells and conclude that "all lasers were efficacious in reducing bacterial population without damaging thermal effects, particularly the Erbium family" [20]. The Erbium family has more bactericidal action because the 2940 nm, 2780 nm wavelengths have very strong absorption in water (which forms the most component of any living cell). The erbium laser has an ablative action which damages the walls of the root canals during the treatment. This is considered the main disadvantage of such type of laser [21]. Asnaashari, et al., measures the penetration depth of diode laser through the dentin under SEM and he found it about 500 µm, this result indicates that the Nd: YAG laser has a preference of greater penetration depth through canal walls (as explored by Berkiten, et al., [17]) which means better sterilization ability [20].

The best result obtained in this study was when both laser and NaOCl were used together. This result coincides with Asnaashari, et al., who found that "maximum effect is obtained when laser light is used in canals in combination with sodium hypochlorite irrigating substance in an appropriate concentration" [20,22]. Combination of NaOCl and Er: YAG was used to eradicate bacterial cells from experimentally contaminated root canals. He concluded that the laser-assisted irrigation has more ability than the conventional method to remove the smear layer and opening of dentinal tubules, which in turn will permit more penetration depth for NaOCl and sealer material for the dentinal tubules. This explains the excellent bactericidal effect of this regime. Retamozo, et al., figured out that the best time for NaOCl application is 40 min to achieve very good bacterial eradication [23]. In this study, the perfect bacterial elimination has been achieved by activation of NaOCl by utilizing Nd: YAG laser and the time required was reduced to 5 min only. This research coincides with Rahimi, et al., where they worked on roots of extracted teeth and used Nd: YAG laser, NaOCl, and the combination of both to sterilize the canals [24]. They proved that the effect of Nd: YAG laser

beam on the bacterial cells is weaker than the effect of NaOCl 5.25%, and the combination of both the agents gave the best results.

Gutknecht, et al., found that the Nd: YAG laser was able to eliminate about 97% of the *Enterococcus faecalis* (*in vitro*) by utilizing optical fibers to deliver the laser beam to the root canal walls, and the efficiency of the Nd: YAG laser in killing bacterial cells was better than NaOCl solution which is in contrast to this study [25]. The different method of work that Gutknecht, et al., has followed in their study led to such a result. Optical fibers of 2 different diameters (200 μm and 400 μm) have been used. Also, a different type of laser device (short pulse) was used. In this study, the Nd: YAG laser has been tested against multi-species bacterial cells, while Gutknecht, et al., has tested the Nd: YAG against only one type. In fact, the bacterial spores should also be considered as they appear higher resistance to heat.

The zone of inhibition is a test used to estimate how much the antibacterial agent is effective in limiting bacterial growth. This test is dependent basically on measuring the diameter of the no-growth area that appears on the bacterial growth media [26]. The zone of inhibition of NaOCl 5.25% for different endodontic pathogens was measured and compared it with a mixture of tetracycline and citric acid detergent (MTAD). Results showed a broad antibacterial spectrum of NaOCl with more powerful effect on *Candida* species than the other microorganisms [27]. While comparing pure NaOCl with other types of NaOCl that contain some additives it was figured out that 5.25% NaOCl has very aggressive antibacterial behavior against *Enterococcus faecalis, Staphylococcus aureus* and *Streptococcus mutans* strains more than the other types of solutions (with additives) [28]. The antimicrobial efficiency of Chlorhexidine (0.12%, 0.5%, 1%) concentration was tested and compared with NaOCl (1%, 5%) by using the direct contact method and inhibition zone measurement. The study was done on some endodontic pathogens (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Porphyromonas gingivalis,* and *Fusobacterium nucleatum*). They found that "1% and 5% NaOCl was antibacterial against all tested bacterial strains" which agreed with the study.

The inhibition zone of Nd: YAG laser was limited to the diameter of the laser spot (+2, -2 mm difference). The ND: YAG laser beam showed no inhibitory action wider than 6.2 mm which means that it has a weaker effect than the NaOCl 5.25%. There were no references about the diameter of inhibition zone of Nd: YAG laser. The studies about this topic were limited to the inhibitory action of the Nd: YAG laser (i.e., how much the Nd: YAG laser is effective in preventing the growth of the bacterial cells). Meire, et al., compared between Nd: YAG and Er: YAG lasers in terms of irradiation dose required for microbial inactivation, to quantify these irradiation doses and to investigate the influence of certain laser parameters on the antimicrobial efficacy [29]. Agar plates containing a uniform layer of *Enterococcus faecalis, Candida Albicans* or *Propionibacterium acnes* were mounted perpendicularly under the laser handpieces (5 mm spot) and irradiated. After incubation, the growth on these agars was documented and the total inhibitory threshold was determined. Er: YAG was better than Nd: YAG in inhibiting the growth of bacterial cells.

CONCLUSION

Nd: YAG laser 1064 nm is less effective (if used alone) as a bactericidal agent in comparison to NaOCl 5.25% solution. It is not absorbed by all species of bacteria. It can be used as a co-adjunctive agent to agitate the NaOCl solution and increasing its activity. The Nd: YAG Laser was effective in reducing the time required for NaOCl application to 5 min.

Limitations and Difficulties

- Risk of getting an infection, since the study deals with extracted teeth, blood, and harmful microorganisms
- The agar plates used in growing bacteria were very susceptible to be contaminated, which gives wrong measurements
- The determination of the predominant bacterial species was very difficult and required an advanced technology (Vitek) and efficient bacteriology lab
- Lack of financial support

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

[1] Rajendran, R. *Shafer's textbook of oral pathology*. Elsevier India, 2009.

[2] Cohen, Stephen, et al. "Pathways of the Pulp (1)." *Learning,* Vol. 30, 1998, p. 10.

[3] Bergenholtz, Gunnar, Preben Hörsted-Bindslev, and Claes Reit, eds. *Textbook of Endodontology*. John Wiley and Sons, 2013.

[4] Marsh, Philip D., et al. *Oral Microbiology E-Book*. Elsevier health sciences, 2009.

[5] Narayanan, L. Lakshmi, and C. Vaishnavi. "Endodontic microbiology." *Journal of Conservative Dentistry: JCD,* Vol. 13, No. 4, 2010, p. 233.

[6] Al-Aamirry, L. M. *Effect of He-Ne laser (632.8 nm) and Diode Laser (890nm) on Streptococcus mutans isolated from patients of carious teeth*. Diss. M. Sc. Thesis, Institute of Laser, Univ. Baghdad, 2003.

[7] de Paula Eduardo, Carlos, and Sheila Gouw-Soares. "The Use of Lasers for Endodontic Applications in Dentistry." *Medical Laser Application,* Vol. 16, No. 3, 2001, pp. 231-43.

[8] Stabholz, Adam, Sharonit Sahar-Helft, and Joshua Moshonov. "Lasers in endodontics." *Dental Clinics,* Vol. 48, No. 4, 2004, pp. 809-32.

[9] Basrani, Bettina, ed. *Endodontic Irrigation: Chemical disinfection of the root canal system*. Springer, 2015.

[10] Garg, Nisha, and Amit Garg. *Textbook of endodontics*. Boydell and Brewer Ltd, 2010.

[11] Haapasalo, Markus, Trude Udnæs, and Unni Endal. "Persistent, recurrent, and acquired infection of the root canal system post□treatment." *Endodontic Topics,* Vol. 6, No. 1, 2003, pp. 29-56.

[12] Siqueira Jr, José F., et al. "Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite." *Journal of Endodontics,* Vol. 26, No. 6, 2000, pp. 331-34.

[13] Priyank, H., et al. "Evaluation of 4% Sodium Hypochlorite in eliminating Enterococcus faecalis from the root canal when used with three irrigation methods: An in vitro study." *The Journal of Contemporary Dental Practice,* Vol. 18, No. 3, 2017, pp. 214-17.

[14] Al-Sudani, Dina A., and Husein A. Al Omar. "Evaluation of Sodium Hypochlorite (NaOCl) Gel as an Endodontic Irrigant." *Journal of Biomaterials and Tissue Engineering,* Vol. 1, No. 2, 2011, pp. 215-18.

[15] Soares, Janir Alves, and Donaldo Rosa Pires Júnior. "Influence of sodium hypochlorite-based irrigants on the susceptibility of intracanal microbiota to biomechanical preparation." *Brazilian Dental Journal,* Vol. 17, No. 4, 2006, pp. 310-16.

[16] Schoop, Ulrich, et al. "Bactericidal effect of different laser systems in the deep layers of dentin." *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery,* Vol. 35, No. 2, 2004, pp. 111-16.

[17] Berkiten, Mustafa, Rahmiye Berkiten, and İmer Okar. "Comparative evaluation of antibacterial effects of Nd: YAG laser irradiation in root canals and dentinal tubules." *Journal of Endodontics,* Vol. 26, No. 5, 2000, pp. 268-70.

[18] Meire, M. A., et al. "Evaluation of Nd: YAG and Er: YAG irradiation, antibacterial photodynamic therapy and sodium hypochlorite treatment on *Enterococcus faecalis* biofilms." *International Endodontic Journal,* Vol. 45, No. 5, 2012, pp. 482-91.

[19] Pirnat, Samo, Matjaz Lukac, and Alojz Ihan. "Study of the direct bactericidal effect of Nd: YAG and diode laser parameters used in endodontics on pigmented and nonpigmented bacteria." *Lasers in Medical Science,* Vol. 26, No. 6, 2011, pp. 755-61.

[20] Asnaashari, Mohammad, and Nassimeh Safavi. "Disinfection of contaminated canals by different laser wavelengths, while performing root canal therapy." *Journal of Lasers in Medical Sciences,* Vol. 4, No. 1, 2013, p. 8.

[21] Hossain, Mozammal, et al. "Ablation depths and morphological changes in human enamel and dentin after Er: YAG laser irradiation with or without water mist." *Journal of Clinical Laser Medicine and Surgery,* Vol. 17, No. 3, 1999, pp. 105-09.

[22] Zhu, Xiaofei, et al. "Comparison of the antibacterial effect and smear layer removal using photon-initiated photoacoustic streaming aided irrigation versus a conventional irrigation in single-rooted canals: an in vitro study." *Photomedicine and Laser Surgery,* Vol. 31, No. 8, 2013, pp. 371-77.

[23] Retamozo, Bonnie, et al. "Minimum contact time and concentration of sodium hypochlorite required to eliminate Enterococcus faecalis." *Journal of Endodontics,* Vol. 36, No. 3, 2010, pp. 520-23.

[24] Rahimi, Saeed, et al. "Bactericidal effects of Nd: YAG laser irradiation and sodium hypochlorite solution on Enterococcus faecalis biofilm." *Photomedicine and Laser Surgery,* Vol. 30, No. 11, 2012, pp. 637-41.

[25] Carvalho, Erica dos Santos, et al. "Effect of chemical substances in removing methylene blue after photodynamic therapy in root canal treatment." *Photomedicine and Laser Surgery,* Vol. 29, No. 8, 2011, pp. 559-63.

[26] Ashari, Mohammad Asna, et al. "Evaluation of the antimicrobial effects of MTAD, NaOCl against selected endodontic pathogens." *Iranian Endodontic Journal,* Vol. 4, No. 2, 2009, p. 63.

[27] Poggio, Claudio, et al. "Antimicrobial activity of sodium hypochlorite-based irrigating solutions." *The International Journal of Artificial Organs,* Vol. 33, No. 9, 2010, pp. 654-59.

[28] Sassone, Luciana M., et al. "Antimicrobial activity of sodium hypochlorite and chlorhexidine by two different tests." *Australian Endodontic Journal,* Vol. 34, No. 1, 2008, pp. 19-24.

[29] Meire, Maarten A., et al. "In vitro inactivation of endodontic pathogens with Nd: YAG and Er: YAG lasers." *Lasers in Medical Science,* Vol. 27, No. 4, 2012, pp. 695-701.