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Comparison between three methods of diode laser 810 nm, photodynamic therapy with laser 660 nm, and hypochlorite solution for disinfection of pulp canal of primary teeth

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ABSTRACT

Background: The presence of treatment-resistant microorganisms is known as the main cause of pulpectomy failure in the endodontic treatment of deciduous teeth. The usage of lasers can contribute to reducing these microorganisms. This study aimed to compare the effect of three disinfection methods for deciduous teeth canals using laser diode 810 nm, photodynamic therapy with laser 660 nm and methylene blue, and sodium hypochlorite.

Materials and Methods: In this experimental study, 58 single-root deciduous teeth with no root resorption were investigated in four groups, including one control group of 10 and three intervention groups of 16. Preparation of the samples was done using manual files up to three numbers after the initial file. After sterilizing the samples in an autoclave, *Enterococcus faecalis* bacteria were cultured in the canals. In the first group, irrigating with hypochlorite 2.5% was done; in the second group, photodynamic therapy was performed using a laser diode and 0.1 mg/mL methylene solution; and in the third group, high-intensity laser 810 nm direct radiation was done into the canal. Next, samples were taken from all canals. The colony formation unit (CFU) of the bacteria was counted in the blood agar culture medium. The data were analyzed using Kruskal–Wallis and negative binomial regression test (α =0.05).

Results: The mean CFU differed significantly between the four groups. The rate of incidence of *E. faecalis* colonies showed a reduction in all three intervention groups compared to the negative control. In the high-intensity laser 810 nm group, there was 68.4%; in the photodynamic therapy with diode 660 nm and methylene blue, there was 88%; and in the hypochlorite group, 98.3% reduction was observed compared to the negative control group.

Conclusion: Based on the results of this study, to compare three disinfection methods of the deciduous teeth canals without preparation of canals, sodium hypochlorite had greater efficiency. All three groups of laser, photodynamic therapy, and sodium hypochlorite showed reductions of *E. faecalis* bacterial colony compared to the control group. The reductive effects of CFU were greater in the hypochlorite sodium group, followed by photodynamic and direct laser radiation groups.

Key Words: *Enterococcus faecalis*, low-level light therapy, methylene blue, photodynamic therapy, sodium hypochlorite

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INTRODUCTION

The primary goal of pulp treatments is to preserve the health of the teeth and their supporting structures.[1] Debridement and disinfecting the root canal are the prerequisites for achieving a successful endodontic treatment.[2] Even after precise mechanical preparation of the canal, the pulp residuals, dentine debris, and bacteria may remain in the root canal.[3] In addition, any pulp remaining in the tooth root canal can be a bacterial feed source.[4] Since there is a positive relationship between the presence of bacteria and failure of endodontic treatments, and such treatments are done routinely in dentistry for deciduous teeth, the root status bacteriologically before filling in the root is an essential factor in the success of the treatment.[5] Antimicrobial disinfectants, especially sodium hypochlorite, due to their proteolytic properties, are very effective on reducing the microbial population. Although sodium hypochlorite directly affects the bacteria, factors such as anatomical complexities, deep penetration of microorganisms into the depths of dentinal tubules, and biofilm formation in the root complicate the complete removal of microorganisms and eradication of the periapical region.[6]

The root canal treatment failure probably occurs by *Enterococcus faecalis* bacterium, which is a Gram-positive facultative anaerobic microorganism which is also part of the normal flora of the mouth. This bacterium has shown resistance to various intracanal drugs and is found stably in deciduous root canal infections. The depth of penetration of sodium hypochlorite in the dentinal tubules is about 100 nm, while the penetration depth of *E. faecalis* reaches as large as 300–400 nm.[7,8] Although sodium hypochlorite and chlorhexidine have shown antibacterial effects against this bacterium, studies have described this antibacterial effect as insufficient for complete clearance of this bacterium.[9-11]

Today, the positive effect of laser and photodynamic therapy in root canal disinfection has been proven.[12] Laser diodes have attracted attention because of their good bactericidal features, being economical, and elevation of temperature[13,14] Futhermore, the laser allows deeper penetration into dentinal tubules.[12] Photodynamic therapy has found interest as an auxiliary method for the bactericidal improvement of the canal, especially against treatment-resistant bacteria such as *E. faecalis* bacterium.[15] It is practical in disinfecting the root canal system. Oxygen singlets cause degradation of the bacterial membrane and their DNA.[16,17] The oral bacteria are sensitive to photodynamic therapy, and it should be noted that photodynamic therapy is a safe antimicrobial treatment. This treatment acts selectively on microorganisms and the host tissue. This is because it only kills the cells that have been specifically aggregated using the light-sensitive material and undergo irradiation. Furthermore, compared to high-intensity lasers, it is not associated with heat generation and protects the adjacent tissues.[18,19]

Anand *et al*.[20] compared the effect of photodynamic therapy, laser, and sodium hypochlorite on intracanal *Candida albicans* and found no significant difference between the studied groups. Attiguppe *et al*.[21] compared the effect of direct irradiation by laser diode, photodynamic therapy, and sodium hypochlorite irrigating in endodontic treatment of deciduous teeth. In the groups receiving laser irradiation with an intermediate material, a better outcome was found in reducing the number of *E. faecalis* bacteria.

Considering the high importance of pulpectomy treatment in preserving deciduous teeth for maintaining function, space, and esthetics, it seems essential to use methods that increase the success of this treatment. Meanwhile, considering the complex anatomy of the deciduous teeth canals and the inability to complete pulp removal even using irrigators, the usage of a method that contributes to the activation of the irrigators and greater removal of the pulp tissue as well as reduction of microorganisms is of great interest. Lasers with progressive applications and economical usage can be an effective means in this regard. Due to the limited number of studies in the field of laser application in the primary teeth and since in the past studies, a high dose of the laser was used. Thus, this study aimed to compare the effect of three disinfection methods for deciduous teeth canals using laser diode 810 nm, photodynamic therapy with laser 660 nm and methylene blue, and sodium hypochlorite.

MATERIALS AND METHODS

In this experimental study, 58 single-root deciduous teeth, which had been extracted for orthodontic purposes or coronal decay, were collected and then kept inside an artificial saliva solution. The teeth with severe curves, internal or external root resorption beyond one-third of the apical one-third of the root, severe resorption that had caused perforation, and previous history of root canal therapy were excluded.

To clear the teeth from any kind of debris or plaque, they were scaled and transferred to the normal saline solution (Ariadent, Iran). The selected teeth were categorized into four groups, including three intervention groups of 16 and one control group of 10. The teeth with different canal dimensions were categorized in each group such that every group included central, lateral, and canine teeth, where the number of each of the central, lateral, and canine teeth in each intervention group was identical to that of another intervention group. To prevent intracanal contamination with the external environment, the apex of the teeth was sealed with light-cure cement (Gc Fuji 9, Japan). Instrumentation of the samples was done until three numbers after the initial file and until the functional length of 1 mm shorter than the apex. During the preparation of the samples, they were irrigated with 10 ml sodium hypochlorite 2.5% (Ariadent, Iran) and transferred to the laboratory.

Each tooth was transferred to a test tube containing 4 mL of culture medium (brain heart infusion [BHI]) and sterilized. Next, *E. faecalis* bacterium (ATCC-29212), which had been grown in culture medium (Tryptic soy broth) at 37°C in an incubator with 0.5 McFarland concentration (1.5×10^8 colony-forming unit [CFU]/mL) for culture, was transferred to inside of the canal, after which the foramen of the canals was sealed with Cavit. Next, for biofilm formation, the canal of the teeth was kept inside a culture medium (BHI) containing a test tube for 72 h. After withdrawing the teeth from the bacterial culture medium, samples were taken from the control group (which had not received any kind of intervention).

For counting the number of *E. faecalis* bacterial colonies in the control group, sterilized Ringer solution was injected into all canals, and absorbent paper points were embedded into the canals for 60 s, so that they would absorb the content of canals. The Ringer solution functions as an intermediate for carrying bacteria into the Agar culture medium. These culture media were incubated at 37°C in a CO_2 container for 48 h. The bacterial colonies are calculated with CFU/mm^3 using a colony digital counter. Once the colony count was done in the control group in each tooth, the desired intervention in each group was performed. In the first group, the canals were rinsed with 5 mL of sodium hypochlorite 2.5% for 1 min. This was followed by rinsing with 5 mL of saline solution.

In the second group, photodynamic therapy was used with a low-level laser. For this purpose, laser diode 660 nm LT-R (Behsaz Gostar, Iran) was used with the wavelength of 660 nm, power of 200 mW, frequency of 0, and energy of 90 J for 450 s (considering about 7% drop in the intraoral series, the output dose was around 30 J in the irradiated zone) as the source of light, and 0.5 mL of methylene blue 0.1 mg/mL solution (MBO, MERK, Germany) as the light-sensitive material. The methylene blue solution was injected into the canal using a syringe 5 min before the low-level laser radiation.[22] Since endotype was not available for the intracanal radiation, the radiation was done as 30 J close to the canal entry and in the tooth longitudinal axis.[18,22]

The third group received direct laser radiation into the canal; a high-intensity diode laser as continuous wave (Dr. Smile Co., Italy) was used with a wavelength of 810 nm and output power of 1 W. The irradiation was guided spirally at 2 mm/s from the apex region until the foramen.[23]

After performing the above interventions, for evaluating the samples from inside the canal similar to the control group, they were incubated inside sterilized Ringer's solution and absorbent paper point, whereby the *E. faecalis* bacterial colonies were counted and calculated [Figures 1-3].

The obtained data were analyzed using Kruskal–Wallis and negative binomial regression tests through SPSS 22 (SPSS Inc., Chicago, IL, USA)., where the significance level was considered 5%.

RESULTS

When comparing the *E. faecalis* colony count in the four groups, the mean CFU differed significantly across the four groups ($P < 0.001$) [Table 1].

When comparing the incidence of *E. faecalis* CFU of the groups compared to the negative control, based on the negative binomial regression model, the difference in CFU incidence in the negative control compared to other groups showed a significant reduction; the mean CFU was significantly higher in the negative control group compared to others.

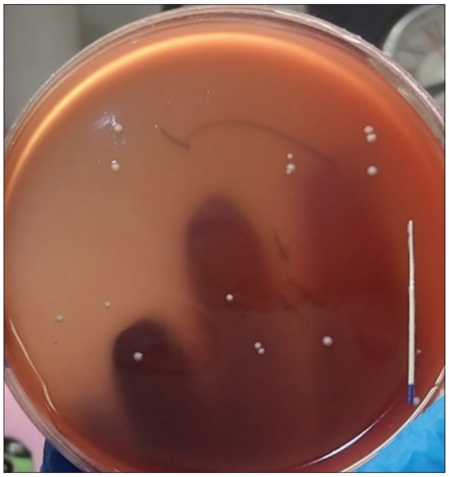

Figure 1: Bacterial colony of sodium hypochlorite experimental group.

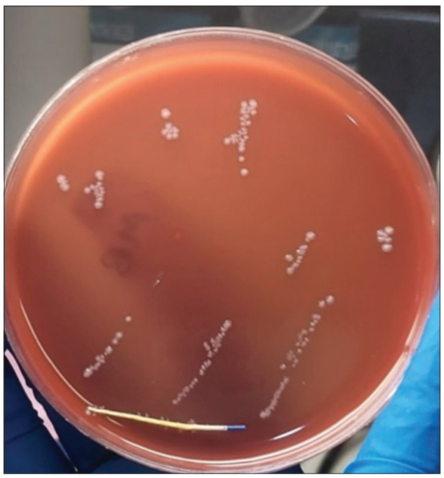

Figure 2: Bacterial colony of experimental photodynamic therapy group.

Figure 3: Bacterial colony of high-power laser experimental group.

In comparing the *E. faecalis* CFU of the groups, the colony incidence rate difference between the laser group and others was significant. The colony incidence rate of *E. faecalis* was 3.169 times greater in the negative control group compared to the laser group. Furthermore, the colony incidence rate showed a 94.7% reduction (1–0.053) in the hypochlorite group and 62% (1–0.38) in the photodynamic therapy group, compared to the laser group.

Table 1: Comparing the *Enterococcus faecalis* colony count in the four group

Group	Mean	SD	*P*
Laser	204.63	43.44	<0.001
Sodium hypochlorite	10.81	24.43	
Photodynamic	77.75	33.18	
Negative control	648.50	38.32	

SD: Standard deviation

When comparing the colony incidence rate of *E. faecalis*, the colony incidence rate difference between the hypochlorite group and others was significant; the colony incidence rate of *E. faecalis* was 18. 925, 7.191, and 59.977 times greater in the laser, photodynamic therapy, and negative control groups, respectively. Thus, the hypochlorite group has had the minimum colony incidence rate compared to other groups.

When comparing the colony incidence rate of *E. faecalis*, the colony incidence rate difference between the photodynamic therapy group and others was significant; the colony incidence rate of *E. faecalis* was 2.632 and 8.341 times greater in the laser and negative control groups compared to the photodynamic therapy group. Furthermore, the colony incidence rate showed an 86.1% (1–0.139) reduction in the hypochlorite group compared to the photodynamic therapy group.

DISCUSSION

Based on the results of this study, the usage of laser, photodynamic therapy, and sodium hypochlorite methods for root canal therapy resulted in the reduction of *E. faecalis* bacterial colonies; the extent of reduction was greatest in the sodium hypochlorite group, followed by photodynamic therapy and then direct laser irradiation, though all of them have antiseptic effects.

E. faecalis is the most resistant strain in the root canal of permanent teeth and deciduous teeth.[24] Thus, promising results that were obtained through applying disinfection techniques through laser for this species of bacteria can probably offer better outcomes with other root canal microorganisms as well. Lasers are an alternative to conventional or advanced methods for disinfecting root canals. The use of laser diodes plus sodium hypochlorite or photodynamic therapy is practical for disinfecting the regions that are impossible to access through conventional techniques. High-intensity laser diodes, due to dose-dependent heat generation, are antibacterial. Their antimicrobial effects against different microorganisms have been shown in a study by Gutknecht *et al*.[25]

Bahrololoomi *et al*.[23] positively appraised the effect of laser diode 1.5 W on reducing bacterial colony count, but this effect was less strong than conventional canal preparation (rinsing with sodium hypochlorite). Attiguppe *et al*.[21] compared the effect of three states of direct laser diode irradiation into the canal, photodynamic therapy, and laser-activated sodium hypochlorite 2.5% for endodontic treatment of deciduous teeth. They indicated the superiority of the photodynamic therapy and laser-activated hypochlorite groups compared to the direct laser irradiation into the canal. However, no significant difference was found between photodynamic therapy and laser-activated hypochlorite. In the present study, again, the superiority of photodynamic therapy and sodium hypochlorite was observed over the direct laser irradiation group. However, the hypochlorite group showed better outcomes than the photodynamic therapy group. The reason for the difference between these two study results can be the different types of photosensitizers (green indocyanine) as well as the method of disinfecting the canals with hypochlorite (filling in the pulp chamber using hypochlorite and then its activation with the laser) as well as the laser power difference, which was 200 mW in our study.

In the study by Walia *et al*.,[2] when comparing the effect of four groups, including saline, sodium hypochlorite 1%, chlorhexidine 2%, and laser irradiation on reducing the *E. faecalis* bacterial colony count of deciduous teeth, they did not observe considerable difference between chlorhexidine, sodium hypochlorite, and laser diode. However, all of them offered better results compared to saline. Nevertheless, the difference between that study and the present research was in the sodium hypochlorite concentration (1% vs. 2.5% in the present study).

Thomas *et al*.[26] when comparing the effect of three groups, including sodium hypochlorite, Triphala, and laser diode irradiation into the canal for deciduous teeth canals, it concluded that laser was more effective than hypochlorite and Triphala in reducing the *E. faecalis* bacterial colony count. In the study by Thomas *et al*.,[26] the used laser with 2W power, which is larger than the power employed in the present research. Furthermore, each tooth was irradiated five times, each time for 5s and with 15-s intervals between each time of radiation, which can justify the different results with the present research.

In the study by Anand *et al*.[20] when comparing four groups of photodynamic therapy, laser and normal saline group, an antifungal agent (clotrimazole), and sodium hypochlorite on reducing *C. albicans* fungi, they showed no significant difference between these four groups. Nevertheless, complete prevention from fungal growth was observed only in the antifungal agent group. This is not in line with the present research, which can be due to different statistical populations, different resistant microorganisms (*C. albicans* vs. *E. faecalis*), as well as the type of high-intensity laser used (wavelength 940 nm and power 0.5 W).

Kuvvetli *et al*.[27] when comparing three groups of laser diode, Er:YAG laser, and sodium hypochlorite with the control found that the laser diode offered better results over Er:YAG. Although both lasers failed to eradicate the bacteria, both of them were more effective than the control group. The most effective group in this study was sodium hypochlorite, with a concentration of 5.25%, which concurred with the present study's findings.

Tenore *et al*.[28] when comparing photodynamic therapy with a 365 nm laser and toluidine blue with the conventional endodontic chemical–mechanical process, in the photodynamic therapy groups, alongside mechanical–chemical preparation, observed the best result in reducing the bacterial colony count. This reduction was significant in these two groups and the positive control group (chemical–mechanical preparation alone) compared to the negative control group. Due to the different laser settings (power 100 mW against 200 mW and wavelength 635 nm vs. 660 nm) as well as the combination of photodynamic therapy with chemical–mechanical preparation, their results are different from the present study results.

Some of the limitations of this study were the difficulty of collecting teeth without any resorption, the low-power laser device LT-R lacking an endotype for focused radiation inside the root canal and the absence of fiber 200 in high-power laser for radiation inside the canal. To solve these limitations, it is suggested to increase the sample population and prepare suitable endotypes and fiber.

CONCLUSION

All three groups of laser, photodynamic therapy, and sodium hypochlorite resulted in the reduction of *E. faecalis* bacterial colonies compared to the group that received no kind of intervention. Nevertheless, the extent of reduction was greatest in the sodium hypochlorite group, followed by photodynamic therapy and 810 high-intensity laser direct irradiation.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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