Original Article

Comparative Molecular Study of *Enterococcus faecalis* in Endodontic Treatment Pre and Postdiode Laser Application

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Abstract

Background: Endodontic infections are polymicrobial, with the majority of bacteria being anaerobic and others being facultative. **Objective:** To detect and measure the level of *Enterococcus faecalis* in infected root canals during traditional cleaning techniques and after diode laser application by using real-time polymerase chain reaction (RT-PCR). **Materials and Methods:** Ninety samples were collected from 30 participant patients complaining of acute or chronic pulpitis with an age range of 18–50 years old; the teeth indicated endodontic treatment, including both anterior and posterior teeth. Thirty swabs were taken preinstrumentation, and then 30 swabs were taken after instrumentation and canal irrigation using sodium hypochlorite 5.25%, and then 30 swabs were taken in the same manner after the root canal sterilization with the diode laser. **Results:** This study included 30 patients with root canal infection presented with a mean age of 31.7 ± 8.6 years and a range of 18–50 years; Female patients were more than males (34.3% vs. 36.7%). The mean *E. faecalis* copy number was significantly reduced from precleaning status to postcleaning with sodium hypochlorite (5.25%) and postlaser therapy (P = 0.05). **Conclusions:** RT-PCR assay is a highly effective, dependable, and rapid technique for accurate detection and copy number counting of *E. faecalis* in the infected root canal; this molecular and clinical study supports the diode laser application of 940-nm WL as an adjuvant tool combined with sodium hypochlorite (5.25%) in canal disinfection during root canal treatment to reduce *E. faecalis* copy numbers.

Keywords: Diode laser application, *E. faecalis*, real-time PCR, root canal treatment

Introduction

Microbes that entered the pulp chamber and occupied the system of the root canal cause root canal infections. A variety of inflammatory responses are triggered when these microbes and their metabolic secretion reach the periradicular tissues via lateral foramina and apex.[1] Endodontic infections are categorized by the anatomy, which includes intraradicular or extraradicular infection, and the length of time it makes bacteria reach the root canal system (primary, secondary, or persistent infection). Primary and secondary infections are usually found in the intraradicular area, but if left untreated or improperly treated, they can develop into extraradicular infections.[2]

Microorganisms convoluted in early pulp assault and the eventual formation of necrotic tissues cause primary endodontic infections. Microorganisms entered the root canal secondarily to professional intervention and either during surgical procedures iatrogenically or afterward by coronal filtration via restorations microleaking, causing secondary root canal infections. Microbes that cause either a primary or minor infection have struggled with chemomechanical debridement techniques and have lived inside the limited situation of cured root canals, causing persistent endodontic infections. Since persistent and minor infections are difficult in the direction of identification clinically, they are likely to be grouped together below the similar disease category.[1] *Enterococcus faecalis* is a Gram-positive anaerobic coccus that usually starts in the human oral cavity, intestinal system, as well as vaginal cavity since it has developed a respectable version

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in such conditions with abundant nutrients, little oxygen levels, and compound ecology.[3] In many investigations, in unsuccessful endodontic treatment instances, *E. faecalis* was found to be more prevalent than in cases of initial infections. *Enterococcus faecalis* is the bacteria most commonly found in cases of postendodontic therapy pain and infection, with prevalence rates as high as 90%. Asymptomatic individuals were more likely to have *E. faecalis* than symptomatic instances among all instances of primary endodontic infection.[4,5]

Endodontic treatment of pulp and periapical illness requires acceptable root canal preparation.[1] The goals of canal preparation are to eliminate infection and provide obturation with a smooth, continuous, funnel-shaped taper that keeps the root canal outline.[6,7]

Attavar *et al.*,[8] mentioned that the 16SrDNA gene is used in the newer tool for bacterial lineage identification in the molecular approach to the detection of endodontic microorganisms. Polymerase chain reaction (PCR) is a very sensitive process for amplifying a little amount of DNA into a billionfold particular known sequence of microbial DNA and detecting it using electrophoresis.

The usage of laser in the dental root canal is one of these applications. The basic objective of dental root canal preparation is to remove bacteria, smear layer, and expand the canal so that canal filling material may reach the root's end. The laser is used to clean and disinfect the tooth canal as well as eliminate the smear layer. For cleaning and disinfecting dental root canals, a diode laser with a wavelength of 635–980 nm has just been introduced.[9]

Materials and Methods

The present study includes 90 samples that were collected from 30 participant patients who complain of acute or chronic pulpitis with an age variety of 18–50 years old. They attended the Teaching Clinics, Collage of Dentistry,University of Babylon, and certain private clinics in Hila city, Babylon, Iraq, for an extended period from December 2021 to February 2022.

Thirty swabs were taken preinstrumentation (after proper tooth isolation and working length estimation). In the first group of samples, thirty swabs were taken after canal irrigation using sodium hypochlorite 5.25% as irrigant without using an endo-activator by six sterile paper points size 15–20#. For the second group of samples, thirty were then taken in the as manner like the first group but after the root canal sterilization with a diode laser.

Patient name, age, sex, occupation, and medical and dental history were taken in the case sheet.

This study includes both sexes and age ranges of 18–50 years old and both anterior and posterior teeth.

While excluded pregnant women, teeth with open apex or root resorption, immune-compromised patient, and deciduous teeth.

Access cavity and canal preparation

After definite diagnoses were rolled out and tooth isolation, the access cavity was prepared, and after initial shaping (by SX file) and canal negotiation (by 8-10-15# files), the debris and pulp tissues were flushed and removed by irrigation with sodium hypochlorite 5.25% and ethylenediaminetetraacetic acid (EDTA) by using fine side opened irrigation tip gauge 27, At this step (the second visit in 7–14 days), the root canals were well prepared by using different types of both manual and rotary files systems. After cleaning, shaping, and dryness were completed, the canal was ready for swabs collection by using a sterile absorbing paper point.[10]

Diode laser application

After canal preparation and swabs collection, the canals were disinfected by using low-power laser therapy (diode laser) with the following specifications; Probes with wavelengths 940 nm and energy density with 10 J/cm^2 on average, the principles of low-power laser therapy used in root canal disinfection was according to.[11]

In the nonstop mode, laser energy was passed through an uninitiated 200 m fiber tip (E2-14, E2-20 Endo Tip; Biolase) with a yield power of 0.1 W. The root canals were irradiated at a speed of 2 mm/s with circling motions starting from the apical to the greatest coronal region of the root canal, with the laser tip positioned at the working length. This process was frequent four times for each canal, with a 20-s break among applications. During all laser submissions, the patient and operators wore safety eyewear.[12]

Microbial sampling

Six sterilized paper points were used to collect each sample from the pulp canal by placing them individually inside the radicular pulp; each paper point was retained in the radicular pulp space for 30 s before being transferred to a sterile Eppendorf tube containing 5 ml phosphate buffer saline, kept in the refrigerator at -23°C, and sent for *E. faecalis* real-time (RT-PCR) detection and copy number counting.[13]

Genomic DNA extraction

The DNA extraction method used in this work is an improved version of the traditional phenol/chloroform method.[14] We removed the SDS/lysozyme or proteinase K lysis step and lysed cells directly with phenol. The lysate was sterilized using chloroform extraction until there was no longer a white interface; this process may need to be repeated two to three times. The aqueous phase contained pure DNA and was either utilized immediately or stored at 20°C for subsequent trials.

This study's DNA extraction method is a better variant of the standard phenol/chloroform approach.[14] We skipped the SDS/lysozyme or proteinase K lysis stage in favor of phenol lysis. Chloroform was used to sterilize the lysate until there was no longer a white interface; this process needed to be repeated two to three times. The aqueous phase contained pure DNA and was either utilized immediately or stored at 20ºC for subsequent trials.

Data analysis of qRT-PCR

The threshold cycle number (CT value) for positive amplification and the DNA copy number calculation using the DNA standard curve for each positive sample were determined using quantitative RT-PCR data analysis [Figure 1 and Table 1].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 25 program and application were used to analyze the data. Analytical results were grouped into category variables and scale variables (means and standard deviation). Categorical variables were tested using the Fishers exact test. Two independent means were compared using an independent sample *t*-test, and two consecutive means were compared using a paired *t*-test. *P*-values of 0.05 or lower were considered significant.

Ethical approval

This study was carried out in compliance with the ethical guidelines found in the Declaration of Helsinki. Prior to taking the sample, the patient's verbal and analytical consent was obtained. According to document number 115, a local ethics commission reviewed and gave its approval to the study protocol, subject information, and permission form on December 18, 2021.

Results

This study included 30 patients with root canal infections who were between the ages of 18 and 50 years old, with a mean age of 31.78% and an age range of (18–50 years). Forty percent of the patients were under the age of 30, and 36.7% were between the ages of 30–39 years, and 23.3% were teeth older than 40 years. Female patients outnumbered male patients (34.3% vs. 36.7%). Patients with root canal infection had anterior teeth in 16.7% of cases and posterior teeth in 83.3% of cases. The mean *E. faecalis* copy number was significantly reduced from precleaning status to postcleaning and postlaser therapy ($P = 0.05$) [Table 2 and Figure 2].

The mean *E. faecalis* copy number was significantly reduced from precleaning status to post-cleaning status ($P = 0.02$) [Table 3].

The mean *E. faecalis* copy number was significantly reduced from precleaning status to postlaser status ($P = 0.02$) [Table 4 and Figure 3].

According to study periods, male and female patients, no significant differences were observed in the mean *E. faecalis* copy number (separately, $P = 0.6$ and $P = 0.32$) [Table 5].

No significant differences were observed in mean *E. faecalis* copy number according to study periods among patients with anterior and posterior teeth ($P = 0.1$ and $P = 0.57$, separately) [Table 6].

Discussion

Thirty patients incorporated in this study complained of root canal infection at a mean age of 31.7 ± 8.6 years; the main age group of patients was 40% in the age group <30 years. Female patients were more than males (36.7%) versus (34.3%); the teeth of patients with root canal infection were anterior less than posterior teeth.

There was a significant difference in the means of *E. faecalis* copy numbers in all treatment stages which included chemomechanical cleaning and diode laser disinfection.

In Juric *et al.*,[15] study, 21 patients with "chronic apical periodontitis" and a history of root canal treatment were

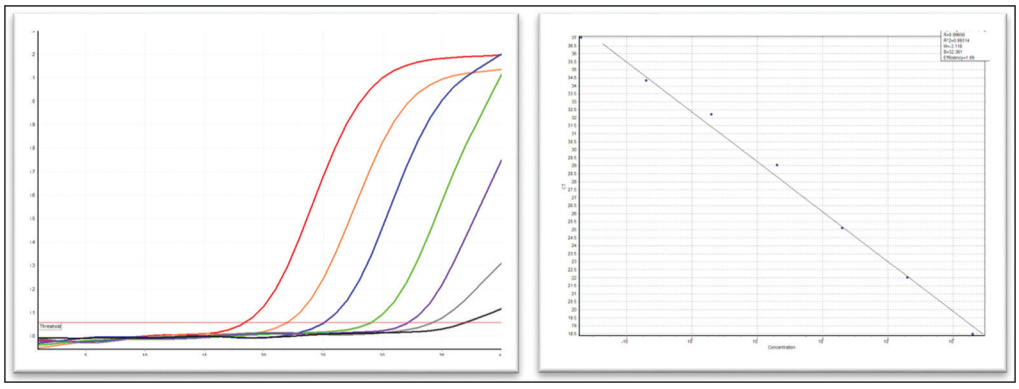

Figure 1: Amplification of *Enterococcus faecalis* genomic DNA and standard curve to calculate the copy number, the vertical axis (CT value), and the horizontal axis (DNA concentration pg/mL)

chosen at random. Initially, fourteen bacterium species were isolated from the infected root canals, averaging 4.57 species per channel. In these species, the MO ratio was as follows: G+, 43% G-, 57% facultative anaerobic, and 43% obligate anaerobic bacteria were found. After each treatment stage, a statistically significant decrease in the number of bacterial species was observed for each canal. After the usual chemomechanical preparation, the bacterial loading was reduced to 2.57 species per canal, with no bacterial growth in one of them. However, after the chemomechanical preparation combined with photo dynamic therapy (PDT), the bacterial loading was reduced to 2.57 species per canal, with no bacterial growth in one of them, no colony-forming units displayed in 11 of the 21 root canals (52.4%), and this agreed with the current study which displayed a significant reduction in *E. faecalis* copy number after chemomechanical preparation and diode laser disinfection.

Tennert *et al.*[16] studied the effect of PDT on 160 monoradicularly removed teeth inoculated with *E. faecalis*. The study discovered a reduction in bacterial viability in both original endodontic infection and re-treated root canals infected with *E. faecalis. E. faecalis* was significantly reduced after root canal therapy with NaOCl or PDT or a combination of NaOCl and PDT. In cases of initial infection, the combination of NaOCl irrigation and the PDT procedure resulted in the highest number of intracanal negative cultures (90%) compared to irrigation with NaOCl alone (80%), which coincided with the findings of a recent study that showed that the combined effect of NaOCL and diode laser therapy is enhanced.

Table 1: Specific primer with amplification conditions: for detection of *Enterococcus faecalis*

Primer		Sequence (5′-3′)	References
16S rRNA *E. faecalis*	F	CAA ACT GTT GGC ATT CCA CAA	[14]
	R	TGG ATT TCC TTT CCA GTC ACT TC	
Bacteria		Conditions	
E. faecalis		Step 1: 94°C, 1 min Step 2: 94°C, 5 s Step 3: 55°C, 30 s Step 4: 72°C, 30s Step 5: 72°C, 5 min	

Table 2: *Enterococcus faecalis* copy number distribution according to study periods

Variable	Study periods			*P*
	Pre	Postcleaning	Postlaser	
	Mean ± SD	Mean ± SD	Mean ± SD	
E. faecalis copy no. (copy/mL)	14,1075.76 ± 285,358.76	76,539.24 ± 242,107.35	68,459.98 ± 186,503	**0.05***S

*Repeated measures analysis, S=Significant

Table 3: *Enterococcus faecalis* copy number distribution and postcleaning

Variable	Study periods		*P*
	Precleaning	Postcleaning	
	Mean ± SD	Mean ± SD	
E. faecalis copy no. (copy/mL)	141,075.76 ± 285,358.76	76,539.24 ± 242,107.35	**0.02***S

*Repeated measures analysis, S = Significant

Table 4: *Enterococcus faecalis* copy number distribution to precleaning and postlaser

Variable	Study periods		*P*
	Prelaser	Postlaser	
	Mean ± SD	Mean ± SD	
E. faecalis copy no. (copy/mL)	141,075.76 ± 285,358.76	68,459.98 ± 186,503	**0.02***S

*Repeated measures analysis, S = Significant

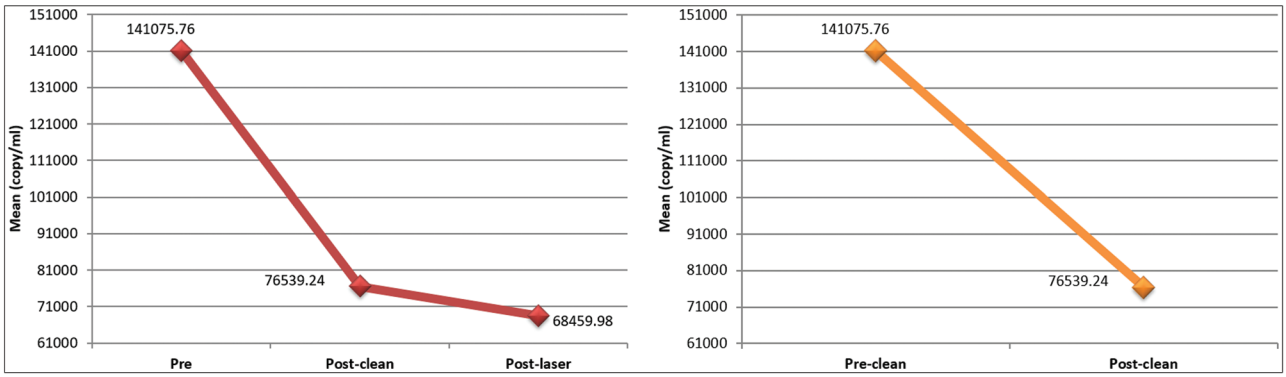

Figure 2: Mean of *Enterococcus faecalis* copy number according to study periods

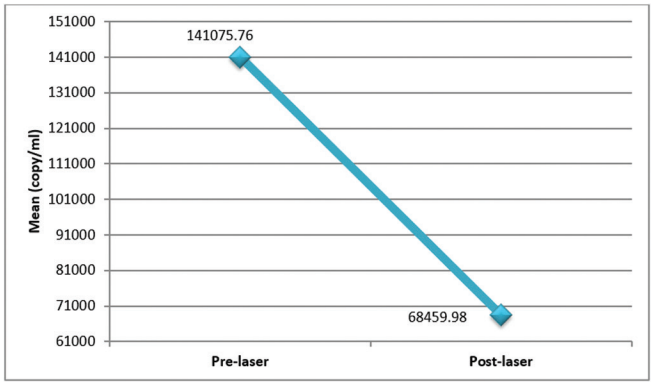

Figure 3: Mean of *Enterococcus faecalis* copy number to precleaning and postlaser therapy

Table 5: Spreading of *Enterococcus faecalis* copy number according to study periods concerning the patient's gender

Variable	Study periods			*P*
	Pre	Postcleaning	Postlaser	
	Mean ± SD	Mean ± SD	Mean ± SD	
Male				0.6*NS
E. faecalis copy no. (copy/mL)	251,526.1 ± 3.79	132,843.9 ± 3.15	136,557.5 ± 2.5	
Female				
E. faecalis copy no. (copy/mL)	56,613.7 ± 1.48	3,343.1±7171.8	362.5 ± 723.4	0.32*NS

*Repeated measures analysis, NS = Not significant

Table 6: Distribution of *Enterococcus faecalis* copy number according to study periods about tooth number

Variable	Study periods			*P*
	Pre	Postcleaning	Postlaser	
	Mean ± SD	Mean ± SD	Mean ± SD	
Anterior				0.1*NS
E. faecalis copy no. (copy/mL)	15,365 ± 16,295.2	244.5 ± 288.3	0.00 ± 0.00	
Posterior				
E. faecalis copy no. (copy/mL)	16,6217.9 ± 3	92,601.3 ± 2.6	84,258.4 ± 2	0.57*NS

*Repeated measures analysis, NS = not significant

However, in terms of secondary infections, the single NaOCl irrigation technique had the highest number of negative bacterial cultures, while all specimens were positive after all PDT.[17]

Partial *E. faecalis* eradication was found to match the present findings.[18] On 71 single-rooted mandibular premolars inoculated with *E. faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, Oliveira, compared the efficiency of PDT with traditional irrigation. Although the combination of 5.25% NaOCl and PDT resulted in the greatest number of samples devoid of microbial growth, the pathogen was not entirely eliminated. Partially eradicating microorganisms has been connected to PDT-related characteristics such as chromophore type and concentration, irradiation time, light source wavelength, chromophore absorption peak, and exposure period.

The present study was incompatible with Sohrabi *et al.*,[19] who reported the effectiveness of a 980-nm diode laser against NaOCl in eliminating *E. faecalis* from the root canal system was tested. The occurrence of germs within the intricate root canal system and dentinal tubules is thought to be the greatest communal cause of root canal treatment failure. As a result, successful endodontic therapy requires the total eradication of germs and their toxins. The superficial layers of dentin and a portion of infected pulp tissue can be removed during chemomechanical root canal preparation; also, chemical irritants' action is confined to the root dentin's most superficial layers. *E. faecalis* has been used in numerous training to assess the decontamination potential of antibacterial drugs and various types of lasers.

So according to Sohrabi *et al.*,[19] NaOCl caused 99.87% of bacterial elimination and had a significantly greater antibacterial effect than the 980-nm diode laser, which caused 96.56% bacterial drop, suggesting that the diode laser might be used as a balancing decontamination technique in root canal treatment

In addition, the current work intersected with,[20] which used a diode laser with a wavelength of 635 nm to irradiate 46 monoradicular teeth for the removal of *E. faecalis* biofilms. A single application of the PDT technique was shown to eliminate around 45% of the initial *E. faecalis* colonies. However, the two PDT cycles strategy was substantially more efficient in eliminating bacterial biofilm by approximately 95%. Despite the fact that PDT might be regarded as an active therapy in comparison to standard root canal treatment, NaOCl remains the most effective antibacterial irrigant.

According to training by Sarda *et al.*,[21] the use of laser in endodontic treatment has significant advantages over traditional therapeutic procedures. Furthermore, we discovered that using the laser at a safe thermal threshold can effectively reduce bacterial contamination as well as the virulence factors responsible for the production of inflammatory mediators in chronic apical periodontitis, indicating that the diode laser can be used during root canal therapy.

In recent years, studies have focused on the antimicrobial effects of several wavelengths, including the PDT. Despite this, many of these investigations found that the "standard chemomechanical preparation" using NaOCl had a

considerably better disinfection impact. The authors noted that the methodological concentration variances and the action time of NaOCl in the root canal ranged from "1% to 5.25%" and "15 s to 20 min," respectively, after examining a selected publication. As a result, depending on these circumstances, the antimicrobial activity's efficiency may vary. As a result, it is critical to recognize that these circumstances may have influenced the findings of the investigations.

Conclusion

The combined effect of NaOCL and a 940-nm root canal with diode laser sterilization is measured as the most effective antimicrobial action when compared with using NaOCL alone during root canal treatment. RT-PCR was a highly efficient, reliable, and rapid technique for *E. faecalis* copy number measuring.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Wong J, Manoil D, Näsman P, Belibasakis GN, Neelakantan P. Microbiological aspects of root canal infections and disinfection strategies: An update review on the current knowledge and challenges. Front Oral Health 2021;2:672887.
2. Siqueira J, José F, Rôças IN. Microbiology and treatment of acute apical abscesses. Clin Microbiol Rev 2013;26:255-73
3. Khassaf MB, Qasim BJ. Histopathological assessment of colonoscopic biopsies in patients with bleeding per rectum. Med J Babylon 2022;19:203.
4. Alghamdi F, Shakir M. The influence of *Enterococcus faecalis* as a dental root canal pathogen on endodontic treatment: A systematic review. Cureus 2020;12:3.
5. Sakko M, Tjäderhane L, Rautemaa-Richardson R. Microbiology of root canal infections. Prim Dent J 2016;5:84-9.
6. Gergi R, Abou Rjeily J, Sader J, Naaman A. Comparison of canal transportation and centering ability of twisted files, Pathfile-ProTaper system, and stainless steel hand K-files by using computed tomography. J Endod 2010;36:904-7.
7. Chandler N, Chellappa, D. Lubrication during root canal treatment. Aust Endod J 2019;45:106-10.
8. Attavar SH, Hegde MN, Shetty V. Molecular method of detection of primary endodontic infection—An ex vivo study. J Evol Med Dent Sci 2020;9:1148-52.
9. Godiny M, Khavid A, Jalilvand M. Evaluation of the effect of various powers of the diode laser in microcrack formation in the wall of the dental root canal by scanning electron microscope (SEM). Open Dent J 2020;14:1.
10. Mamoun JS. The maxillary molar endodontic access opening: A microscope-based approach. Eur J Dent 2016;10:439-46.
11. Genc Sen O, Kaya M. Effect of root canal disinfection with a diode laser on postoperative pain after endodontic retreatment. Photobiomodul Photomed Laser Surg 2019;37:85-90.
12. Mohammed HA, Sahi NM, Ahmed RT, Fauzi Al-Rubaye A. Antimicrobial activity of some nanoparticles synthesized by laser ablation technique against some bacteria isolated from oral cavity. Med J Babylon 2022;19:601-8.
13. Endo MS, Signoretti FG, Kitayama VS, Marinho AC, Martinho FC, Gomes BP. Culture and molecular detection of *Enterococcus faecalis* from patients with failure endodontic treatment and antimicrobial susceptibility of clinical isolates. Braz Dent Sci 2014;17:83-91.
14. Cheng Ha R, Jiang N. Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnol Lett 2006;28:55-9.
15. Jurič IB, Plečko V, Pandurić DG, Anić I. The antimicrobial effectiveness of photo-dynamic therapy used as an addition to the conventional endodontic re-treatment: A clinical study. Photodiagn Photodyn Ther 2014;11:549-55.
16. Tennert C, Feldmann K, Haamann E, Al-Ahmad A, Follo M, Wrbas KT, *et al*. Effect of photo-dynamic therapy (PDT) on *Enterococcus faecalis* biofilm in experimental primary and secondary endodontic infections. BMC Oral Health 2014;14:1-8.
17. Zhang D, Shen Y, de la Fuente-Núñez C, Haapasalo M. In vitro evaluation by quantitative real-time PCR and culturing of the effectiveness of disinfection of multispecies biofilms in root canals by two irrigation systems. Clin Oral Investig 2019;23:913-20.
18. de Oliveira BP, Aguiar CM, Câmara AC, de Albuquerque MM, de Barros Correia AC, Soares MF. The efficacy of photo-dynamic therapy and sodium hypochlorite in root canal disinfection by a single-file instrumentation technique. Photodiagn Photodyn Ther 2015;12:436-43.
19. Sohrabi K, Sooratgar A, Zolfagharnasab K, Kharazifard MJ, Afkhami F. Antibacterial activity of diode laser and sodium hypochlorite in *Enterococcus faecalis*-contaminated root canals. Iran Endod J 2016;11:8.
20. Prażmo EJ, Godlewska RA, Mielczarek AB. Effectiveness of repeated photo-dynamic therapy in the elimination of intracanal *Enterococcus faecalis* biofilm: An in vitro study. Lasers Med Sci 2017;32:655-61.
21. Sarda RA, Shetty RM, Tamrakar A, Shetty SY. Antimicrobial efficacy of photodynamic therapy, diode laser, and sodium hypochlorite and their combinations on endodontic pathogens. Photodiagn Photodyn Ther 2019;28:265-272.