BAU Journal - Creative Sustainable Development

Volume 3 | Issue 1
ISSN: 2664-9446

Article 4

November 2021

ERADICATION OF ENTEROCOCCUS FAECALIS IN CONVENTIONAL ENDODONTIC RETREATMENTS (A RANDOMIZED CLINICAL TRIAL)

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Enterococcus faecalis, Conventional endodontic retreatment, sonic agitation, laser activation, bacterial eradication, EndoActivator, Irrigation

ERADICATION OF ENTEROCOCCUS FAECALIS IN CONVENTIONAL ENDODONTIC RETREATMENTS (A RANDOMIZED CLINICAL TRIAL)

Abstract

Enterococcus faecalis is the most common bacteria isolated in conventional endodontic retreatments. Aim: To compare the impact of three irrigation modalities on the elimination of the isolated microbial strains of Enterococcus faecalis. Materials and Methods: Thirty patients requiring conventional endodontic retreatment for one of their mandibular premolars and tested positive for E. faecalis were chosen. Patients were randomly divided into three groups (n=10) according to the irrigation modality to be used; group I: syringe irrigation (NaOCl 2.625%), group II: NaOCl 2.625%+EndoActivator and group III: NaOCl 2.625%+diode laser. Before obturation, samples S2 were collected and PCR analysis was performed to identify the persistence of E. faecalis post- irrigation. Fisher exact test was used for differences in proportion between the three irrigation modalities. Analysis was achieved at the 0.05 significance level. Results: A significant statistical difference was revealed between group I and each of groups II and III whereas the statistical difference between groups II and III was not significant. Conclusion: Agitation with EndoActivator or activation with diode laser are necessary for a better eradication of E. faecalis in conventional endodontic retreatments.

Keywords

Enterococcus faecalis, Conventional endodontic retreatment, sonic agitation, laser activation, bacterial eradication, EndoActivator, Irrigation

1. INTRODUCTION

A successful endodontic treatment or retreatment is based on an adequate shaping, an optimal cleaning and a hermetic filling of the root canal system in order to preserve the tooth functional and asymptomatic in the oral cavity. Among these three phases, irrigation is a major factor when promoting the curing of pulpal and periapical pathologies. A successful healing of these pathologies assumes a complete eradication of different bacteria present in the complex endodontic system. In this context, one of the biggest irrigation challenges is that it has to reach areas that the mechanical instrumentation does not reach, like isthmuses, lateral ducts and apical deltas where microorganisms have a greater survival chance (Mohammad *et al.*, 2014).

According to Ghorbanzadeh *et al.* in 2020, eradication of microorganisms or decreasing their amount in the root canal system is one of the essential goals of the endodontic therapy. In fact, remaining germs may induce a reinfection of the root canal system causing therefore a failure of endodontic treatments. In such cases, endodontic retreatments are indicated and resistant bacteria are usually present in root canals. One of the most commonly isolated species is *Enterococcus faecalis* (Molander *et al*., 2007). It is a resistant bacterium that is able to live under various nutritional conditions (Hendi *et al.*, 2021). In their study in 2016, Arias-Moliz *et al.* concluded that the endodontic therapy is best achieved if preventive measurements and treatment procedures are based on a detailed understanding of the pathogenesis and etiology of endodontic diseases. Consequently, in conventional endodontic retreatments, in order to perform a successful therapy we have to eliminate the causative agents of disease by eradicating the *Enterococcus faecalis* strains. For most cases, fulfilling this goal seems utopic with available instrumentation and techniques.

Frequently, syringe irrigation with sodium hypochlorite as an antimicrobial agent is the most commonly used method for irrigation. But, Alghamdi and Shakir in their systematic review in 2020, showed that *Enterococcus faecalis* has specific properties permitting it to escape the conventional disinfection means. For this reason, adjunctive irrigation techniques to improve the effectiveness of irrigants are recommended to eliminate the residual bacterial strains. Many irrigating solutions and irrigation modalities are available. Each delivery system has its own advantages and disadvantages. Efficiency and safety are two essential criteria when choosing the irrigation modality. Warming, sonic agitation, ultrasonic activation and laser application on the irrigant are among those techniques.

The purpose of this study was to evaluate and compare the effectiveness of three irrigation techniques- syringe irrigation, sonic agitation and diode laser activation of sodium hypochlorite- on the elimination of the microbial strains of *Enterococcus faecalis* isolates in conventional endodontic retreatments.

2. MATERIALS AND METHODS

2. 1 Study Design

The patients chosen for this randomized clinical trial were selected arbitrarily from patients assigned to the Endodontic Clinics at the Faculty of Dentistry at Beirut Arab University for conventional endodontic retreatment of one of their mandibular premolars.

2. 2 Screening and Ethical Issue

All procedures used in this study confirmed to protocols approved by the Institutional Review Board of Beirut Arab University (IRB approval number: 2014-H-001-D-P-0010). The purpose and the scope of the study were explained to the chosen patients who were also informed about each step of treatment. Each patient's medical history was reviewed. A clinical examination including periapical radiographs was performed to confirm the need for a non-surgical endodontic retreatment of the mandibular premolar. Before sampling, the chosen patients agreed to participate in the study by giving a written consent. In order not to expose patients' data, each patient was randomly given a number which was used all through the study. For each patient, data such as tooth type and working length was recorded apart.

2. 3 Patient Selection Criteria

Thirty patients having one of their mandibular premolars requiring conventional endodontic retreatment were chosen according to inclusion parameters. At the time of treatment, the selected patients' age ranged between twenty and forty-five years old and having not received any antibiotic treatment in the past three months. The chosen teeth were restorable with no signs of fracture. The depth of periodontal pockets was less than 3 mm. A preoperative digital X-ray was taken by the bisecting angle technique for each tooth (Kodak 2100 intraoral X-ray system, UK). All the treated teeth had completely formed single roots (Type I). The radiographs revealed improper fillings of the root canals but without presence of broken instruments, perforations or apical transportations and without presence of periapical lesions. Medically compromised patients presenting any systemic disease and pregnant women at the time of enrollment were excluded from this study. The same operator performed all the steps for all patients in order to avoid bias in the results.

2. 4 Tooth Preparation

The tooth was isolated with a rubber dam (Hygenic, Coltene Whaledent). The field was disinfected using 30% H_2O_2 (Fluka, Germany) and 5% tincture of Iodine (Sigma, USA) then 2.625% NaOCl (Clorox, Vernon, CA, USA). Following the removal of caries and previous restorations, the disinfection sequence was repeated (Cogulu *et al*., 2007). A new number four sterile diamond round bur (Komet Dental, Germany) was then used to access the pulp chamber. Previous root canals' fillings were removed using sterile Protaper Universal instruments for retreatment (Protaper D, Dentsply Maillefer, Ballaigues, Switzerland) mounted on an X-SMART motor (Protaper D, Dentsply Maillefer, Ballaigues, Switzerland). Using the bisecting angle technique, radiographs were taken to make sure of the complete removal of the previous root canals' fillings (Kodak 2100 intraoral X-ray system, UK). During the retreatment procedure, no chemical solvents were used. The working length (WL) was determined using an apex locator (Root ZX, Morita, Tokyo, Japan), and radiographically to be within 0.5 mm of the radiographic apex for each canal.

2. 5 Collection of Initial Bacterial Sample (S1)

Following determination of the working length, a sterile K-file size 20 (Dentsply Maillefer, Ballaigues,Switzerland) was inserted one mm shorter than working length and pumped five times with minimal reaming motion in order to accumulate dentin chips and intra-canal debris. Three successive paper points (size 20) (Dentsply Maillefer, Ballaigues, Switzerland) were then placed to the full working length for two minutes. The three sterile paper points and the K- file (size 20) (Dentsply Maillefer, Ballaigues, Switzerland) were immediately transferred to a vial containing two ml of Liquid Dental Transport (Thioglycollate medium, Oxoid, England). The vial was labeled with the patient's number, time and date. This constitutes the initial bacterial sample (S1) for each patient (Cogulu *et al*., 2007). Samples were conveyed and processed at the microbiology laboratory within one hour of collection.

2. 6 Isolation of *E. faecalis* and PCR Analysis

At the microbiology laboratory, the tubes containing samples in the transport medium were pre-incubated for 30 minutes at 37 °C. The samples were then shaken vigorously in a vortex mixer (FALC Instruments S.R.L., Italy) for one minute then divided into two parts each of one ml volume (Cogulu *et al*., 2007). PCR analysis was performed to identify the existence of *E. faecalis*. All samples were tested by the polymerase chain reaction (PCR Thermal cycler, Bioer Technology, Hangzhous, China) to detect the presence of *E. faecalis*. Two genes were employed in our PCR analysis (Universal 16 S rDNA, and *E. faecalis*). Special features of primers that were used in the study are as indicated in Table 1.

Table 1*: E. faecalis* genes and their primers
Reference: Cogulu *et al*., 2007.

Target	Sequence (5′ to 3′)
Universal 16S rDNA	AGA GTT TGA TCC TGG CTC AG ACG GCT ACC TTG TTA CGA CTT
Enterococcus faecalis	GTT TAT GCC GCA TGG CAT AAG AG CCG TCA GGG GAC GTT CAG

2.6.1 Isolation of DNA

One milliliter of the vortexed sample was transferred aseptically into an Eppendorf tube and centrifuged at 5000 rpm for five minutes then the supernatant was decanted. Forty microliters of sterile double distilled water were added then heated at 95ºC for 10 minutes. This was followed by centrifugation at 5000 rpm for five minutes and the supernatant was collected for further analysis.

2.6.2 Preparation of the reaction mixture

The reagents as indicated in Table 2 were added subsequently into a 0.2 ml PCR tube under aseptic conditions**.**

Table 2: PCR Reaction mixture
Reference: Done by the Authors.

Reagent	Volume	Final concentration
5xFIREPol®Master Mix	4µl	1x
Forward primer	1µl	0.1-1 µM
Reverse Primer	1µl	0.1-1 µM
Isolated DNA	5µl	
Water	q.s	
Total volume	20µl	

The prepared reactions were run with respect to the conditions as indicated in table 3 and according to the gene to be detected against standard strain *Enterococcus faecalis* ATCC 29212 (American Type Culture Collection).

Table 3: *E. faecalis* genes and conditions for the amplification cycles.
Reference: Cogulu *et al*., 2007.

Target	Size (bp)	Amplification cycles
Universal 16S rDNA	1505	30 cycles Denaturation: 94°C 15 sec, Annealing: 54°C 15 sec, Extension: 72°C 45 sec.
Enterococcus faecalis	310	36 cycles Denaturation: 95°C 30 sec, Annealing: 60°C 1 min, Extension: 72°C 1 min.

2. 7 Gel Electrophoresis of PCR Product

After PCR amplification, 2.5 µl of each reaction was separated by gel electrophoresis (Bio-Rad, Italy) in 1.5 % agarose gel for 45-60 minutes at 100 V in TBE buffer (Tris – Borate – EDTA buffer). Ethidium bromide (1 µg/ml) (Sigma, USA) was used to stain DNA and the bands were identified using UV transilluminator (Cleaver Scientific Ltd). Samples that contained *E. faecalis* DNA showed positive amplification.

2. 8 Cleaning and Shaping

E. faecalis was detected in the root canals of the 30 patients among 62 selected before applying any irrigation modality. Those 30 patients, were then arbitrarily allocated to one of the following groups (group I, group II and group III, of n= 10 patients each) according to the final irrigation modality to be used. For all groups, and during the whole shaping process, five ml of 2.625% NaOCl solution (Clorox, Vernon, CA, USA) were used for irrigation of root canals (Zand et al., 2016). Irrigation was performed with a three ml luer lock syringe and a 30-gauge lateral side Max-i-Probe needle (DentsplyMaillefer, Ballaigues, Switzerland), (0.05 ml/sec). The needle was inserted 2mm shorter than the working length.

2. 9 Grouping of Patients

Group I: Canals were irrigated with three ml of 2.625% NaOCl solution (Clorox, Vernon, CA, USA) using a three ml luer lock syringe and a 30-gauge lateral side Max-i-Probe needle (DentsplyMaillefer, Ballaigues, Switzerland), (0.05 ml/sec) for two minutes. The needle was applied without pressure and in a vertical movement of three mm, at two mm from the working length.

Group II: Canals were irrigated with three ml of 2.625% NaOCl solution (Clorox, Vernon, CA, USA) using a three ml luer lock syringe and a 30-gauge lateral side Max-i-Probe needle (DentsplyMaillefer, Ballaigues, Switzerland), (0.05 ml/sec) for two minutes. The irrigating solution was agitated by the EndoActivator (Dentsply Tulsa Dental Specialties, USA) for 60 seconds. This cordless sonic system was used according to the instructions of the manufacturer by vibrating the tip and moving it up and down in short vertical strokes at 10,000 cycles per minute and 167 Hz with a 25/04 polymer tip. The procedure was repeated 3 times.

Group III: Canals were irrigated with three ml of 2.625% NaOCl solution (Clorox, Vernon, CA, USA) using a three ml luer lock syringe and a 30-gauge lateral side Max-i-Probe needle (DentsplyMaillefer, Ballaigues, Switzerland), (0.05 ml/sec) for two minutes. The diode laser (SIROLaser Xtend, Dentsply Sirona, USA) was applied to the irrigating solution according to the following technical characteristics stated by the manufacturer: The root canal decontamination was performed with an optical fiber of 200μm diameter inserted two mm shorter than the working length. Laser irradiation was performed with the irrigant in the canal for a period of 20 seconds, repeated three times at intervals of 10 seconds between each one (Neelakantan *et al*., 2015)**.** Emission and withdrawal of the tip were done with a helicoidal and circumferential motion with a speed of 2mm / second in order to permit maximum irradiation of the walls. The fiber emitted from its distal end (the beam diverged 15-22^{o} upon exciting the tip). Safety goggles (Dentsply Sirona, USA) were used for both the patient and the practitioner.

2. 10 Microbiological Collection of Post-Irrigation Sample (S2) for the Three Groups

Root canal preparation was completed in a crown down rotary instrumentation with Protapers (DentsplyMaillefer, Ballaigues, Switzerland) mounted on the X-SMART MOTOR (DentsplyMaillefer, Ballaigues, Switzerland) to master apical file size 35 (DentsplyMaillefer, Ballaigues, Switzerland). At the end of instrumentation a second microbial sample **(**S2**)** was taken as follows: Three successive size 35 sterile paper points (DentsplyMaillefer, Ballaigues, Switzerland) each inserted for two minutes inside the prepared canal were collected and directly transmitted to a vial containing two ml of Liquid Dental Transport (LDT). The samples were then processed in the microbiology laboratory within one hour of sample collection.

2. 11 Completion of Root Canal Therapy

At the end of cleaning and shaping, root canals were irrigated with three ml of distilled water (Zand *et al.*, 2016). Obturation of root canals was performed by the standard cold lateral condensation of gutta percha points (Meta-Biomed, South Korea) and AH Plus® as a resin root canal sealer (DentsplyMaillefer, Ballaigues, Switzerland). All the teeth were restored with universal dental composite (3M, USA) to grant proper coronal seal. An immediate periapical X-ray for the treated teeth was taken (Kodak 2100 intraoral X-ray system UK) using a digital intracanal sensor (Kodak RVG 5100#1UK).

2. 12 Statistical Analysis

Data were coded and entered into computer database for analysis. The statistical analyses were performed using Statistical Package for the Social Sciences (IBM-SPSS V25) software. Frequency and percentages were used to summarize categorical data. Difference in percentages of teeth with persistence *E. faecalis* status after treatment was tested using the Fisher exact test due to the small number of expect cell counts. All analyses were carried out at the 0.05 significance level.

3. RESULTS.

3. 1 Persistence of *E. faecalis* Strains after Different Irrigation Modalities

After treating the 30 teeth -10 per group- according to the irrigation modalities assigned for each group, the results have showed that: in group I, *E. faecalis* strains remained in seven out the 10 teeth (70%), in group II, one tooth only remained positive (10%), and in group III, no teeth remained positive (0%) as indicated in Table 4 and as shown as Fig.1. The differences between the three groups were tested using the Fisher exact test, which returned a (*p*-value of 0.001) showing statistical significance between group I versus group II and group I versus group III.

Table 4: Frequency and percentages of the persistence of *E. faecalis* after irrigation in the three groups.
Reference: Done by the Authors

Group / *E. faecalis*	*group I: NaOCl Syringe Irrigation*		*group II: NaOCl + Endo Activator*		*group III: NaOCl + diode laser*		*Total*	
	Frequency	*%*	*Frequency*	*%*	*Frequency*	*%*	*Frequency*	*%*
Negative	*3*	*30.0*	*9*	*90.0*	*10*	*100.0*	*22*	*73.3*
Positive	*7*	*70.0*	*1*	*10.0*	*0*	*0.0*	*8*	*26.7*
Total	*10*	*100.*	*10*	*100.0*	*10*	*100.0*	*30*	*100.0*

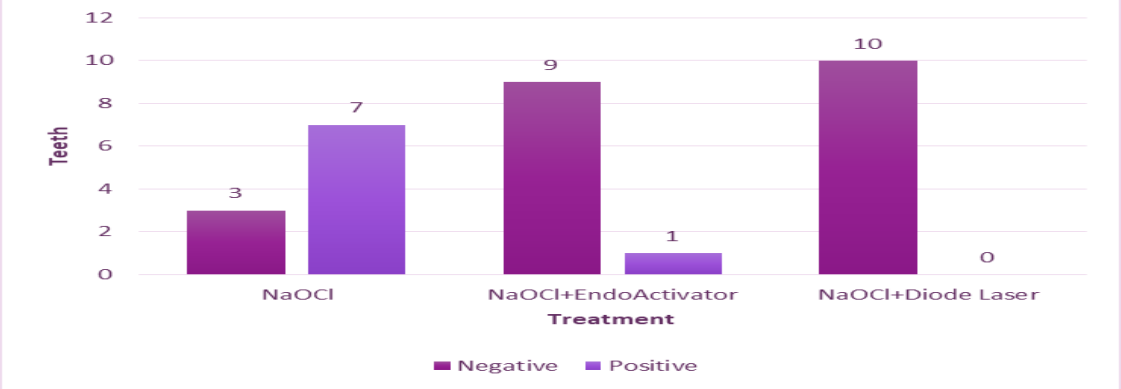

Fig.1: Bar chart showing the persistence of *E. faecalis* after irrigation in the three groups
Reference: Done by the Authors

3.2 Comparison between groups I and II

The proportion of teeth tested positive for *E. faecalis* strains after treatment was compared between group I and group II. The (*p*-value) produced by the Fisher exact test was *p*=0.020 denoting that agitation of sodium hypochlorite by the EndoActivator was able to eradicate more bacteria than syringe irrigation, and the difference was statistically significant between these two groups as shown as Fig. 2.

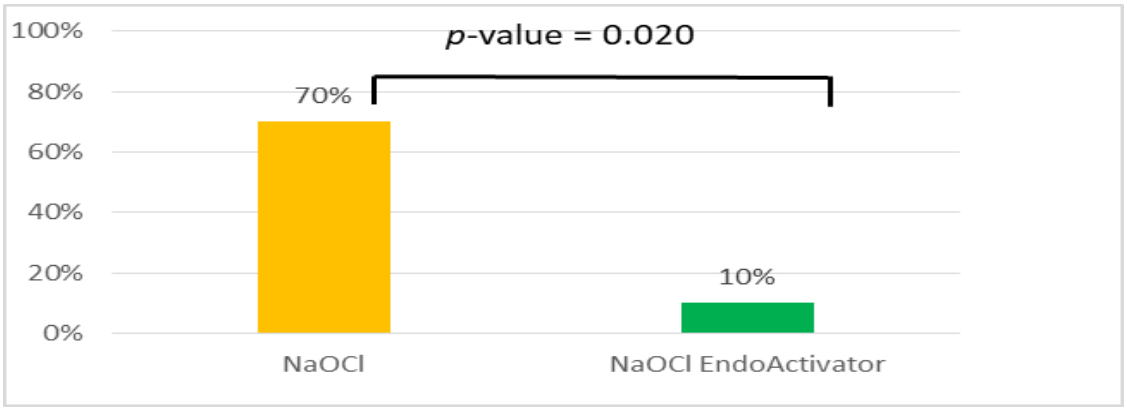

Fig.2: Bar chart comparing between group I and group II regarding persistence of *E. faecalis*.
Reference: Done by the Authors

3.3 Comparison between groups I and III

The proportion of teeth testing positive for *E. faecalis* strains after treatment is compared between group I and group III. The *(p*-value) produced by the Fisher exact test was (*p*=0.003) denoting that the difference is statistically significant between these two groups with a better performance of group II regarding eradication of *E. faecalis* from the root canal systems as shown as Fig.3.

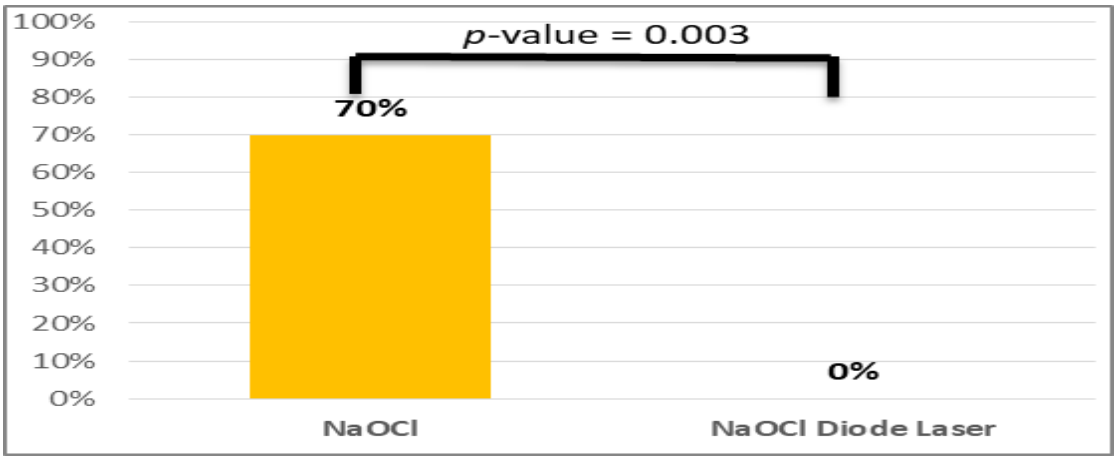

Fig.3: Bar chart comparing between group I and group III regarding persistence of *E. faecalis*.
Reference: Done by the Authors

3.4 Comparison between groups II and III

The proportion of teeth testing positive for *E. faecalis* strains after treatment is compared between group II and group III. The (*p*-value) produced by the Fisher exact test was (*p*=0.999) denoting that the difference is not statistically significant between group II and III although clinically *E. faecalis* was detected in 10% of root canals of patients in group II compared to 0% in group III as shown as Fig.4.

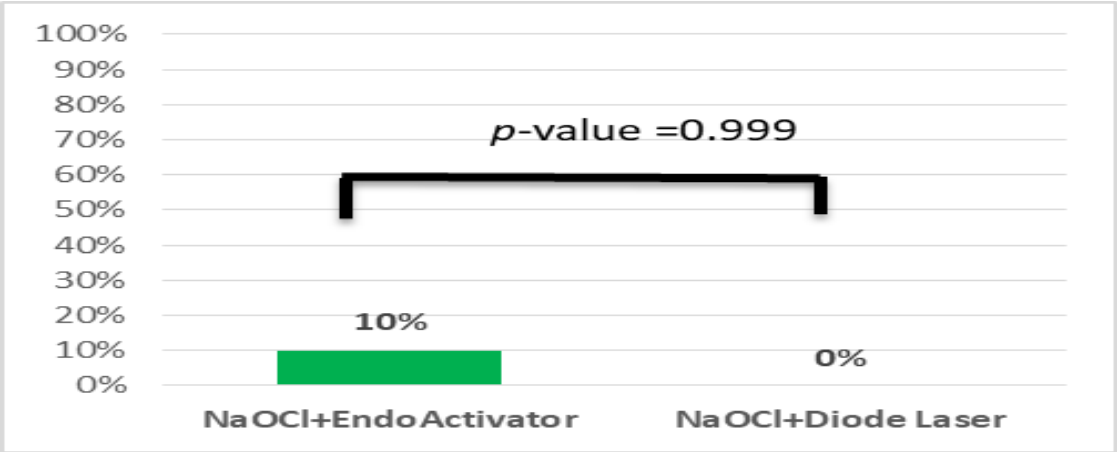

Fig.4: Bar chart comparing between group II and group III regarding persistence of *E. faecalis.*
Reference: Done by the Authors

4. DISCUSSION

The purpose of the current study was to compare the effectiveness of sonic and laser activation of sodium hypochlorite, against traditional syringe irrigation, on the elimination of the isolated microbial strains. The study concerned patients having single rooted mandibular premolars requiring non-surgical endodontic retreatments.

In the present study, the selection *of E. faecalis* as the bacterial strain to be investigated, was because this bacteria is frequently associated with persistent endodontic infections namely in conventional endodontic retreatments. To highlight on the association between *E. faecalis* and secondary infections during endodontic retreatments, a research by Xu *et al.* in 2019 studied the adherence of *E. faecalis* in root canals. The authors concluded that the amount of *E. faecalis* adherence was significantly higher on instrumented dentin specimens when compared with that on uninstrumented dentin. They also found that the adhesion force detected on the surface of gutta-percha and sealer was higher than that detected on the dentin surface. This may explain the high incidence of *E. faecalis* in conventional endodontic retreatments.

Recent addition to the methods of identification of bacteria is the use of molecular biology. The most common types of molecular biological techniques are PCR and DNA-DNA hybridization. In our study, the polymerase chain reaction (PCR) was used for the identification of *E. faecalis*. This molecular genetic method have several advantages over other methods with regard to microbial identification. It can provide higher specificity and accurate identification of microbial strains with ambiguous phenotypic behavior and can detect microbial species directly in clinical samples without the need for cultivation. It has higher sensitivity and can detect dead cells. It is a fast technique, less time-consuming and offering a rapid diagnosis. It does not require carefully controlled anaerobic conditions during sampling and transportation. Furthermore, when a large number of samples are to be surveyed in epidemiological studies, samples can be stored and analyzed all at once (Siqueira and Rocas, 2009).

In our study, when the syringe irrigation with 2.625% NaOCl was the only irrigation modality, *E. faecalis* strains remained in 70% of the cases. Conventional irrigation with syringes has been promoted as an efficient method of irrigant delivery by Boutsioukis *et al*. in 2010. Nevertheless, the mechanical flushing created by this irrigation technique is relatively weak. Therefore, the ability

to disinfect the root canal systems from *E. faecalis* strains is limited. Our results are in agreement with those of Parmar *et al*. in 2001 who concluded that syringe irrigation with sodium hypochlorite is not efficient to eradicate *E. faecalis* from root canals. They found that NaOCl could only penetrate up to 130 μm into the dentin while *E. faecalis* can enter up to 653 μm the depth of the dentin. This bacterium has the ability to adhere to dentinal tubules and form intra-and extra-radicular biofilms that are not easily disturbed. They are composed of a complex three-dimensional shape consisting of an extracellular polymeric matrix in which microorganisms are embedded.

In our study, when using the syringe irrigation with sodium hypochlorite without any activation, NaOCl was delivered into the canal via a 30-gauge syringe needle inserted apically and streamed at relatively high velocity. This type of delivery was, in itself, a limitation for the elimination of *E. faecalis* because the remainder of the root canal was irrigated at a lower velocity. Bago *et al*. in 2013 confirmed that fact, by showing that the highest velocity was produced only at and around the needle tip. Our results are in agreement with other studies confirming the inability to eradicate *E. faecalis* by using only syringe irrigation with sodium hypochlorite due to the fact that this irrigation modality creates the possibility of air bubbles becoming trapped in the apical region of the root canal, making it nearly impossible to remove biofilm in that area (Mohmmed *et al*., 2017).

Contrary to our results, Zand *et al.* in 2016, assessed the bactericidal effect of 2.5% and 5.25% NaOCl solution against *E. faecalis*. They found that both totally eliminated *E. faecalis* biofilm with no statistical significant difference between the two concentrations ($p>0.05$). In the same context, Frough Reyhani *et al*. in 2017, studying the effect of syringe irrigation with sodium hypochlorite on *E. faecalis,* concluded that NaOCl concentrations of 2.5% and 5% showed complete elimination of *E. faecalis* (cfu count = 0).

In our study, sonic agitation of 2.625% sodium hypochlorite solution with the EndoActivator (Dentsply Tulsa Dental Specialties, USA) has significantly improved the eradication of intraradicular *E. faecalis* when compared to the syringe irrigation technique. Our results showed that 90% of the sampled teeth became free of *E. faecalis* strains when agitating the final rinse of 2.625% NaOCl with the EndoActivator (Dentsply Tulsa Dental Specialties, USA) for 60 seconds and repeating that for three times. This is in agreement with the results found by Kanter *et al.* in 2011. Our findings are also in accordance with those of Chatterjee *et al.* in 2015 who concluded that the bacterial reduction was due to the fact that the EndoActivator (Dentsply Tulsa Dental Specialties, USA) produces powerful hydrodynamic intracanal waves.

In the present study, the diode laser activation of 2.625% NaOCl showed a complete elimination of *E. faecalis* and no teeth remained positive. Our findings are in accordance with those of Vatkar *et al.* in 2016 who concluded that the diode laser activation of sodium hypochlorite achieved a total elimination of *E. faecalis*. The authors suggested a possible explanation for this ability. They found that the diode laser had the capability to reach dentinal tubule depths beyond 1000μm. They explained that once the laser light is emitted, it loses the characteristics of a concentrated beam. Instead, a "light fog'' is created inside the dentin. The dentinal tubules along with the enamel prisms will propagate this light to the peripheral root dentin, and this may eliminate the *E. faecalis* present there. As it is admitted, root canal systems possess a complex anatomy that includes challenging areas such as isthmuses, furcal canals and lateral canals. Conventional irrigants without activation are unable to reach these areas allowing the *E. faecalis* biofilm to remain undisturbed. Our results are also in agreement with those of Afkhami *et al.* in 2017 who concluded that diode laser activation of sodium hypochlorite showed high antibacterial efficacy which may be due to thermal modifications that damage bacterial cell walls.

Recent research still focus on the eradication of *E. faecalis* from the root canal system. In their *in-vitro* study in 2021, Panariello *et al.* found that the use of electromagnetic stimulation on an *Enterococcus faecalis* biofilm is effective for the disinfection of the root canal system. However, they added that the treatment they proposed should be translated to future clinical studies. In another *in-vitro* study also, Kranz *et al.* in 2021 showed that chloramine-T presented strong antiseptic activity and is also efficient in suppressing *E. faecalis* inside dentinal tubules. The authors added that further investigations are necessary in order to proof the efficacy and safety of chloramine-T clinically *in-vivo.*

In the literature, the majority of the studies concerning the efficiency of irrigation modalities on the elimination of *E. faecalis* from root canal systems, were *in- vitro* ones. Our *in- vivo* study compared the impact of three different irrigation modalities on the root canals of thirty patients. Further randomized clinical trials with larger sample sizes should be conducted. From the activation systems that we used in our study, diode laser activation and sonic agitation contributed positively to both chemical and mechanical aspects of the irrigation process. Their contribution improved the outcome of endodontic treatments. This subject merits additional research. Future studies involving the efficacy of selected irrigation modalities on multiple bacteria eradication should be directed. Development of such an approach will improve the design of future generations of irrigant agitation and activation systems.

5. CONCLUSIONS

Within the limitations of this study, we can conclude that in conventional endodontic retreatments, sonic agitation and diode laser activation of 2.625% sodium hypochlorite performed better on the eradication of intra radicular *E. faecalis* strains when compared to syringe irrigation with 2.625% sodium hypochlorite. Furthermore, when comparing between EndoActivator agitation and diode laser activation, no statistical significant difference was detected between these two irrigation regimens.

6. ACKNOWLEDGEMENT

The Authors declare that there are no conflicts of interest in this study.

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