Antibacterial effectiveness of different final irrigation activation techniques

Bizhyan Mahdi Mustafa [(1)] Bahar Jaafar Selivany[(1)] Abduloma Baraka Ali[(2)]

Background and objective: To evaluate and compare the antibacterial effectiveness of ultrasonic, diode laser , XP endo finisher and finisher gentle file brush against Enterococcus faecalis in root canal dentin.

Methods: Fifty-five single-rooted extracted mandibular premolars were decoronated to have a length of 12 mm, instrumented biomechanically with protaper Next up to size X3. After sterilization, five roots were selected as negative controls and the remaining teeth were inoculated with E. faecalis and incubated for 48 hr and at 37 ºC. The roots were then randomly divided into five groups (n=10) , GI: Control group ,GII: 5.25% NaOCl irrigation was activated by the ultrasonic system , GIII: By 980-nm diode laser , GIV: By Xp-endo finisher and GV : Activated by Finisher gentle file-brush. The antimicrobial efficacy was tested by collecting transfer fluid saline from the canals and counting the colony forming units (CFUs) of viable E. faecalis on agar plates. The statistical calculations were performed in JMP pro 14.3.0. using Analysis of Variance (ANOVA) followed by a Tukey test to define the differences between the tested groups.

Result: The mean bacterial CFU were 10.6 X 10^7 for Finisher gentle file brush, 35.6×10^7 for Diode laser, 39.6×10^7 for ultrasonic and 42.3×10^7 for XP Endo Finisher. Finisher gentle file brush showed a significant reduction in the colony count compared to the other groups.

Conclusion*:* None of the irrigation activation techniques removed the bacteria completely from the canal . The four tested irrigation activation techniques were shown to be effective in the disinfection of the E. faecalis-contaminated root canals. Finisher gentle file brush was the most effective against E. faecalis, while XP endo finisher was the least effective against E. faecalis.
treatment against *E. faecalis* in the root canal.

[(1)]Department of conservative dentistry, College of Dentistry, University of Duhok , Duhok, Iraqi Kurdistan Region.
[(2)]College of Dentistry, Almustafa University College, Baghdad, Iraq.

Introduction

infections are polymicrobial in nature, With intricate bacterial interactions and a predisposition for anaerobic bacteria. One of the primary goals of endodontic treatment is to eliminate any threat to treatment success by creating a bacteria-free environment in the root canal system. Endodontic infection removal differs greatly from that of most other sites in the human body. Because of the unique anatomy and physiology of root canals, host measures sufficient to eliminate the infectious organism from other sites are insufficient to eliminate endodontic infection completely.[1]

The goal of biomechanical preparation is to clean, shape, and disinfect the root canal system. This goal is rarely met due to the anatomical complexities of the root canal system, and the eradication of microorganisms is at best insufficient. During oval root canal enlargement, endodontic files cannot instrument 65% of the root canal system; these untouched zones can harbor microorganisms whose by-products can cause persistent inflammation.[2]

Numerous irrigation solutions have been introduced in endodontic practice, and the development of their formulations is guided toward improving their properties, such

as dentinal tubule penetration, antibacterial effect, smear layer removal, stability, and substantive efficacy. None of the irrigation solutions seems to provide all these desired properties. Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) solutions have gained wide acceptance, and their use is advocated as an effective irrigation protocol to eradicate the fragments of the smear layer. These solutions remain the gold standard for root canal irrigation due to their antibacterial action and necrotic and organic matter-dissolving activity within the smear layer.[3]

Traditionally, the root canal is irrigated with a syringe-needle combination. Syringe irrigation, on the other hand, has a limited effect because the solution can only reach 1 mm beyond the tip of the needle. Activating root canal irrigants may remove apical vapor lock, increasing irrigation effectiveness, and is thus the recommended method for improving canal system irrigant distribution. As a result, various activation techniques to improve irrigant efficacy have been proposed. [3]

Passive ultrasonic irrigation (PUI) has been used to improve the chemical and mechanical effects of irrigation solutions.[4] The application of an ultrasonic device to a file that has been placed within an irrigant-filled canal is known as passive ultrasonic irrigation (PUI). Even though the process is ultrasonically activated, the file remains non-cutting. For root canal cleansing, this technique has been shown to be more effective than conventional needle irrigation.[5]

Lasers produce a high energy density light beam through the induced emission of atoms in the laser medium. The physical interaction between laser and tissue is determined by the tissue's adsorption spectrum. If the laser wavelength corresponds to the tissue's adsorption spectrum, a linear biological effect on tissue cells is induced, characterized by hyperthermia (37-60C), coagulation (60-100C), carbonization (100-400C), and evaporation (>400C). It has been proposed to use diode laser irradiation as an adjunctive antibacterial disinfectant in the root canal. The antibacterial effect of diode laser irradiation has been attributed to its greater depth of penetration into dentinal tubules of up to 1000 □m when compared to chemical disinfectants, which were limited to 100 □m. Gutknecht et al. demonstrated that a diode laser with a wavelength of 980 nm can effectively eliminate E. faecalis up to a penetration depth of 500 □m.[6]

The XP-endo Finisher file (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is intended to be used after any root canal instrumentation to achieve improved root canal cleaning while preserving dentin. When the file tip of an XP-endo Finisher curved bulb is squeezed, it can expand its diameter by 6 mm, or 100 times the size of a corresponding sized file. The XP-endo Finisher features a small core size (ISO 25 in diameter and zero taper) and increased flexibility. A proprietary NiTi alloy is used to create the XP-endo Finisher file (Martensite-Austenite Electropolish-FleX). The XP-endo Finisher file is said to be highly flexible and performs well at different temperatures.[7]

The Gentlefile system also includes a brush (Finisher GF Brush) made of six strands of stainless steel that open outwards automatically when used in a handpiece at 6500 rpm. The original purpose of the GF Brush was to aid irrigation, but due to its design, it is expected to perform exceptionally well in the removal of substances from root canals. After root canal preparation, Neelakantan et al,[8] investigated the efficacy of irrigant agitation with the GF Brush and concluded that using the GF Brush resulted in significantly less pulp tissue remnant than syringe irrigation. A combination of flexibility and centrifugal movement would make an access and cleaning in irregular parts easier. The present study aimed to evaluate and compare the antibacterial effectiveness of ultrasonic, diode laser, XP endo finisher and finisher gentlefile brush against Enterococcus faecalis in root canal dentine.

METHODS

Sample selection and preparation

Fifty-five single-rooted completely formed, straight mandibular premolars freshly extracted for orthodontic demands were used in this study. Teeth with any signs of cracks, internal and/or external resorption, root caries,root canal calcification or obstruction, pulp stones, and previous endodontic treatment were excluded. Immediately after collection, the teeth were

cleaned by washing with distilled water, and the remnants of soft tissues were removed using a cumine scaler. Finally , the samples were stored in a plastic container containing 0.1% thymol solution at room temperature.[9]

Using a diamond disk and a digital caliper, samples were decoronated to have a length of 12 mm coronally to the apex. A barbed broach was used to extract the remaining pulp. Following the initial of the pulp extirpation patency attempt, the patency of each root was confirmed to reach the apex using a 10 K file (Dentsply, Bllaigues, Switzerland), and the working length was determined using a 10 K file. The new working length was determined by subtracting 1 mm from the full length of the root, which was initially set at 12mm, until the tip of the file was visible from the apex .[10]

The root apical foramen was sealed with acrylic resin,[11] molds for holding the teeth were constructed from a water pipe and a heavy body impression with a catalyst was used. These close-ended molds make the tooth to be fixed in place during the preparation and create a standardized position and orientation, and those samples were mounted later on by using a table jack.[12]

Root Canal Instrumentation

Protaper NEXT rotary files (Dentsply Maillefer, Ballaigues, Switzerland) was used to prepare all root canals in crown down technique according to the manufacturer's instructions, using DENTSPLY X-Smart IQ Cordless Endo Motor.The instrumentation started by adjusting the rotation speed of the endo motor according to the manufacturer in which the speed was 300 rpm, and the torque was 3.5 Ncm. In this study, three main files of Protaper NEXT fifth generation were used, which were X1, X2, and X3, each of those files has its own tip size and taperness.[13] For standardization purposes, every set of rotary files was used to prepare five canals and then discarded. Using a 27-gauge needle that was held 2 mm shorter than the working length, all root canals were irrigated with 3ml of 5.25% NaOCl , 1ml of 5.25% NaOCl used between each file and 1mL of 17% EDTA during instrumentation. [14] To counteract the effects of NaOCl, 1ml of normal saline was flushed through the canals at the end of the instrumentation. Following that, the samples were dried using X3 paper points.

Sterilization and disinfection of the root canal samples

To avoid cross-contamination, all roots were wrapped in aluminum foil after the chemo-mechanical treatment before being placed in separate sterilization pouches. They were ready to be placed in the autoclave and sterilized for 45 minutes at 121 ºC under 1.5 psi pressure to ensure that no microorganisms remained.[11]

As a negative control, five samples were brought out after sterilization and randomly selected to be tested to determine whether they were fully sterilized. The samples were kept in sterile Brain Heart Infusion(Neogen food safety, Lansing, USA), which is used in the laboratory to cultivate a wide range of fastidious organisms. BHI is a suitable nutrition medium because it can grow bacteria, yeasts, and pathogenic fungi. To ensure sterilization, each sample was immersed in BHI in a sterile tube and incubated for 48 hours at 37 ºC. After 48 hours, the samples were removed from the incubator and cultured on a sterile Mueller-Hinton agar plate before being returned to the incubator and incubated for another 48 hours. It revealed no bacterial growth, indicating that it had been completely sterilized. [10]

Infecting the root canal with Enterococcus faecalis

After grouping and numbering of the samples, all the samples were brought and made ready to be infected with the special strain of Enterococcus faecalis EF-D1 that was prepared in the Media laboratory from root canal failure,10 µL of the prepared solution was taken by a 10 µL volume micropipette and using ready-made disposable micropipette tips and the entire procedure was done inside an isolated and sterile hood in the laboratory. The injection of the solution inside the experimental root samples was carried out gently inside the root canal samples . When the infection of each sample was finished, then each sample was placed in a special tube that was previously sterilized, to isolate each sample from another to avoid cross-contamination, and the lid of each sample tube was sealed. Then those

samples were put in a special holder and were held in an upright position to ensure the bacteria solution stays in the canal at all times. And then, the special holder with the samples was put in the incubator for 48 hr and at 37 ºC. when the incubation period was done, the samples were transferred in care for test conduction and performing the disinfection procedure.[15]

Disinfection of the root canal samples

The specimens were finally irrigated on the same day, and fifty teeth were randomly divided into five groups, as follows.

Group I Positive Control : Ten samples were kept for the positive control group, in which the root canals were rinsed with a sterile saline solution (no disinfection procedure for this group was performed),then swaps were taken from the control group and cultured on Mueller-Hinton agar.

Group II Ultrasonic activation: Each root canal was irrigated with 3 ml of 5.25% NaOCl solution and ultrasonically activated with a woodpecker ultrasonic with tip E12 (Ufile size 25) inserted into the canal 1 mm short of the WL, and the irrigant was ultrasonically activated for 20 s per cycle. To avoid contact with the canal walls, the tip was kept as central as possible. The cycle was repeated three times. The canal before each activation cycle was irrigated with 1 mL of 5.25% (NaOCl). [16]

Group III Diode Laser: Each root canal was irrigated with 3 ml of 5.25% NaOCl solution and activated with a 940 nm diode laser; the delivery was by a fiber-optic endodontic tip, E2, with a tip diameter of 200 m, specimens were irradiated with 1.08 W, continues wave (CW) mode; the fiber tip was inserted 2 mm from the apex, in contact mode, and helicoidal movement at 1mm/s. According to the manufacturer's instructions, this was completed in 18 seconds and then repeated for a total irradiation time of 36 seconds.[16]

Group IV Xp-endo Finisher: Each root canal was irrigated with 3 ml NaOCl solution (5.25%) and activated by using XP-endo finisher. At temperatures above 35ºC, the file is in its austenitic phase, which is difficult to be inserted in the canal .So, after the working length adjustment to 11 mm inside the tube, the finisher file was cooled down with ethyl chloride spray while it is inside the tube. The file was used according to the manufacturer's instructions, and the settings of the endodontic micro motor were set at 800 rpm and 1.0 Ncm for speed and torque respectively . The activation time inside the canal was 60 seconds. [3]

Group V Finisher gentle file brush (disinfectant brush): Each root canal was irrigated with 3 ml NaOCl solution (5.25%) and activated by using the Finisher gentle file brush (Gentle File, France) placed 1 mm shorter than the working length. The activation time was 60 seconds inside the canal , and the brush was moved up and down, as per the manufacturer's instruction.[3] All of the canals were rinsed with 3 ml of normal saline immediately after activation to avoid erosion of dentin and crystal formation .

A sterile paper point X3 ProTaper NEXT (Dentsply, Maillefer, Switzerland) was used to evaluate root canal disinfection after finishing each root canal disinfection immediately. The first paper point was used to dry the canal and decrease the effect of the irrigation in the canal for 10 seconds. Swap by second sterile paper-point was taken . Then, it was transferred to a test tube containing 1 mL of sterile saline solution (0.9% NaCl). The test tube was then shaken for 30 seconds in a vortex mixer. Finally, on an agar plate, 200 µL of vortexed normal saline was cultured. Culturing was done by spreading method using a swab on Mueller-Hinton agar plate and incubated aerobically at 37˚C for 48 hrs. Following incubation, the number of colony-forming units (CFU) was determined by counting the colonies by necked eyes. [15,17]

All the culture plates were compared to the control group, and disinfection evaluation was done using statistical calculations to determine the outcome of the testing procedure of each group and compare it with the control group to determine the amount of bacterial colony-forming units reduction achieved by the disinfection regimes used in this study.[18]

Statistical analysis

The antibacterial levels were presented as a mean and standard deviation. ANOVA one-way was used to compare antibacterial results among study groups. The Tukey test was used to compare post-doc positions. A p -value of less than 0.05 was used to deter-

mine the significance of the difference. JMP pro 14.3.0 was used to perform the statistical calculations.

Results

Table 1 and Figure 1 display the mean and standard deviation of CFU in each group. The study showed that the mean values of antibacterial results were significantly different among study groups (P<0.0001) .

The Finisher gentle file brush activation of sodium hypochlorite had the lowest amount of bacteria population left in the canal (10.6 X 10^7) ,followed by Diode laser (35.6×10^7), and ultrasonic (39.6×10^7), while XP Endo Finisher left the most bacteria colonies (42.3×10^7) in the canal.

Table 2 showed that there was a significant difference when the control group was compared to ultrasonic, Diode laser, XP EndoFinisher, and Finisher gentle file brush, as well as when Finisher gentle file brush was compared to ultrasonic, Diode laser, and XP EndoFinisher, while there were no statistically significant differences

Table 1: Comparisons of antibacterial results among

Study groups	Antibacterial CFU		p-value (two-sided)
	Mean	Std Dev	
Control (n=10)	139.9×10^7	48.86×10^7	<0.0001
Ultrasonic (n=10)	39.6×10^7	20.74×10^7	
Diode laser (n=10)	35.6×10^7	23.29×10^7	
XP EndoFinisher (n=10)	42.3×10^7	9.33×10^7	
Finisher gentle file brush	10.6×10^7	14.82×10^7	
ANOVA one-way was performed for statistical analysis			

Table 2: Pairwise comparisons of antibacterial results between study groups

Study groups		Mean ±SD	Mean ±SD	p-Value
Control	Ultrasonic	139.9 (48.86)	39.6 (20.74)	<0.0001
Control	Diode laser	139.9 (48.86)	35.6 (23.29)	<0.0001
Control	XP EndoFinisher	139.9 (48.86)	42.3 (9.33)	<0.0001
Control	Finisher gentle file brush	139.9 (48.86)	10.6 (14.82)	<0.0001
Ultrasonic	Diode laser	39.6 (20.74)	35.6 (23.29)	0.7427
Ultrasonic	XP EndoFinisher	39.6 (20.74)	42.3 (9.33)	0.8246
Ultrasonic	Finisher gentle file brush	39.6 (20.74)	10.6 (14.82)	0.0209
Diode laser	XP EndoFinisher	35.6 (23.29)	42.3 (9.33)	0.5828
Diode laser	Finisher gentle file brush	35.6 (23.29)	10.6 (14.82)	0.0448
XP EndoFinisher	Finisher gentle file brush	42.3 (9.33)	10.6 (14.82)	0.0120
The post-hoc comparisons were performed using the Tukey test.				

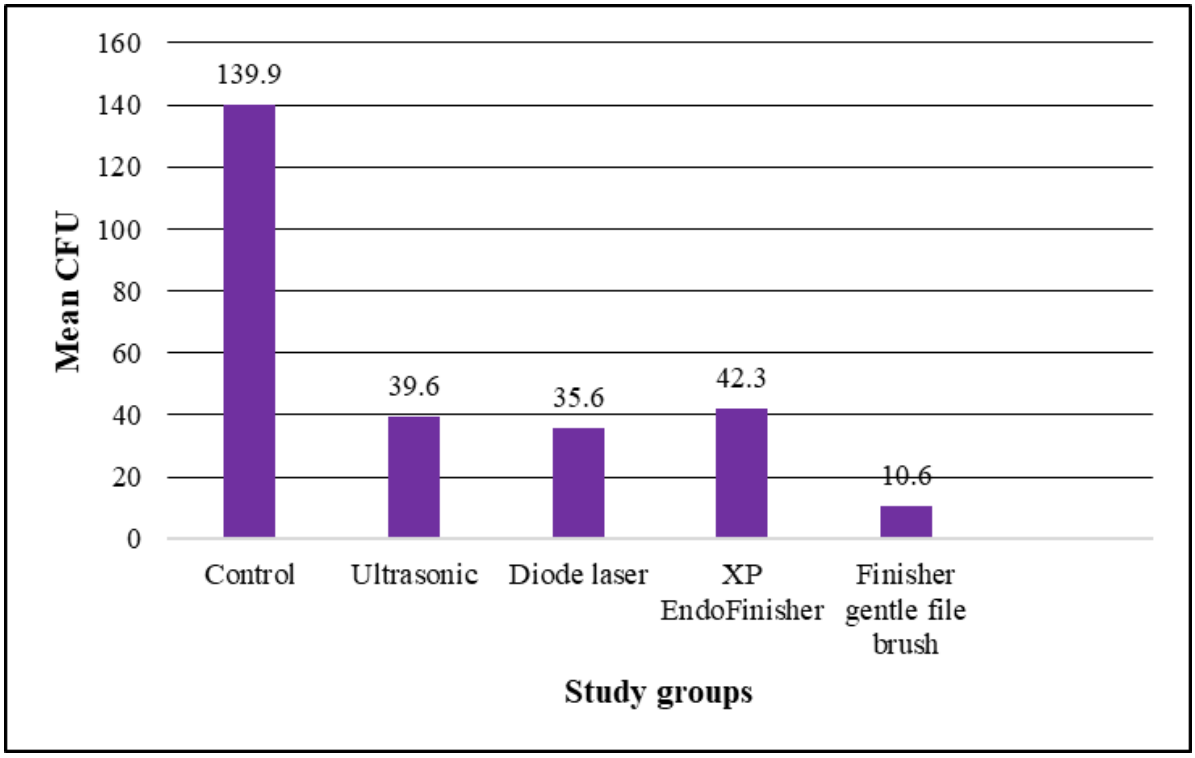

Figure 1: Comparisons of antibacterial results among study groups

Discussion

The primary goal of endodontic treatment is to clean and prepare root canal walls in order to remove necrotic and vital pulp tissue, bacteria, debris, and smear layer and prevent canal re-contamination.[19] Microorganisms in the root canals are the primary causative agents in the development of pulp and periapical lesions. The disinfection of the entire root canal system is a major goal of root canal treatment, which necessitates the removal of all root canal system contents as potential sources of infection. Because of the complex anatomy of the root canal system, various irrigant agents have been used during and immediately after mechanical instrumentation to help eliminate microorganisms that mechanical instrumentation alone cannot eliminate [29.]

In endodontic treatment, the irrigation of the root canal is considered a very important and essential part that removing tissue remnants and microorganisms during instrumentation. [21] In this study, different irrigation systems (ultrasonic, diode laser, XP- Endo finisher, and Finisher gentle file brush) were compared for their cleaning efficacy and antibacterial effectiveness.

The freshly extracted human mandibular premolar teeth were used in this study, to reduce variables through the study so the effect of different irrigation systems will be more obvious. [22] To ensure standardization of the entire experimental group, the teeth were decoronated leaving 12mm length before the instrumentation procedure.

Enterococcus faecalis, a facultative anaerobic gram-positive coccus implicated in persistent root canal infections, was used as a biological marker in this study. The pathogenicity of E. faecalis in endodontic infections is well documented. E. faecalis has demonstrated the ability to survive in a low-nutrient environment with little commonality with other bacteria. Because bacteria have been reported to enter dentinal tubules as deep as 500-1000 μm, E. faecalis has been found to be suitable for experimental penetration into dentinal tubules. This is regarded as one of the most common reasons for endodontic treatment failure. E. faecalis has been shown to survive for over 4 months in water and for extended periods of time in nutrient-limited media. The ability to withstand starvation is a

distinguishing feature that may allow E. faecalis to survive until a suitable nutrition source becomes available. [20]

In the current study, the samples were activated with final irrigation after being infected with Enterococcus faecalis, and then Bacteriologic sampling was performed by inserting sterile paper points. The use of paper points has the advantage of being a simple method that can be performed both in vitro and in vivo, but it does have a limitation in that it can only sample microorganisms found in the root canal, not those found inside dentinal tubules. [20]

Bacterial counting was accomplished by calculating the number of bacterial colonies forming units (CFU), which is a simple and straightforward method. [23] When compared to the positive control group, all of the activation techniques used in this study significantly reduced the CFU of E. faecalis. Finisher gentle file brush had the highest bacterial reduction, followed by Diode laser and Ultrasonic, while XP EndoFinisher had the lowest. The finisher gentle file brush works by rotating the six Stainless Steel strands and activating the sodium hypochlorite at 6500 rpm, which may scrape the canal walls to remove tissue and the rest of the debris, primarily that left in the apical third, and destroys the remnants of the biofilm sticks on the canal wall. It can also reach and approach unreachable areas, such as a small niche in the canal.[8]

As an effective tool for canal disinfection, the diode laser was introduced into root canal treatment. Because of its inherent properties of local intensity enhancement, light scattering, and attenuation, laser irradiation has superior antimicrobial efficacy and light penetration deeper into the dentinal tubules. The diode laser has a thermal photo disruptive action in dentin, which increases the root canal system's bactericidal effect.[24]

Passive ultrasonic activation of the root canal irrigant is a predominant step in the endodontic treatment procedure. One of the main reasons that the activation of the irrigant is an essential step in the endodontic procedure is to create an acoustic streaming effect that enhances the wall shear stress and also it has a great role in distrusting the biofilm within the canal .Also, during irrigation ultrasonic creates a faster flow volume of the irrigant into the root canal, resulting in better debris and smear layer removal, less apical packing, and improved chemical product reaching to accessory canals and isthmuses. This, in turn, kills bacteria more effectively than syringe irrigation. [25] However, the ultrasonic instruments are made as non-cutting heads, but they are made from a metal alloy that is harder than dentine, which might increase the risk of changing the root canal morphology. In this study woodpecker ultrasonic which is a cordless and portable device was used to activate the irrigation solution and was able to generate (45,000Hz), which is required to create sufficient acoustical streaming .

XP-endo finisher (FKG, La Chaux-de-Fonds, Suisse) universal NiTi-based instrument with many properties that allowed to reach the walls that are untouched by the round files used during canal instrumentation, and is used to scrape those walls. Moreover, those files cause turbulence of the irrigant solution, causing an enhancement in its antimicrobial properties. [26]

These results were in agreement with Tilakchand et al [27] who found that combination therapy which included irrigation with NaOCl and diode laser irradiation, was an effective treatment option for reducing E. faecalis and other bacterial flora in the root canal system. Neelakantan et al. [28] discovered that using NaOCl after or in conjunction with a chelator resulted in the greatest reduction of E. faecalis. In dentinal tubule disinfection, diode laser and Er:YAG laser activation outperformed ultrasonics. According to Rahimi et al, [29] the laser is less effective in root canal disinfection than the combined use of laser and NaOCl. Aveiro et al, [30] reported that ultrasonic Activation of 6% NaOCl was associated with a greater reduction in microbial load within root canals.Abu Hasna et al, [31] reported that Passive ultrasonic irrigation (PUI) improves NaOCl action over E. faecalis and E. coli and their endotoxins. Kesim et al, [32]

reported that the highest Reduction in colony count (RCC) was determined when NaOCl was activated with Passive ultrasonic. According to de Oliveira et al, [33] bacterial reduction was greater in the Passive ultrasonic irrigation (PUI) than in the XP-endo finisher (XPF). The agitation of the irrigant resulted in a significant bacterial reduction. These results were in disagreement with Herce-Ros et al, [34] who found that NaOCl activated with XPF was as effective as PUI in removing biofilm from the apical third of the canal as PUI. According to the findings, XPF only achieved the efficacy of PUI when NaOCl was heated. Carvalho et al, [35] discovered that single-file instrumentation using the XP-Endo Shaper and Reciproc Blue files is effective at reducing bacterial levels in oval-shaped root canals. The inclusion of XP-Endo Finisher to the irrigation/ instrumentation technique improved the cleaning efficiency of both file systems tested.

Conclusions

None of the irrigation activation techniques removed the bacteria completely from the canal .Finisher gentle file brush activation of sodium hypochlorite had the greatest effect on eliminating bacteria.Xp endo finisher activation of sodium hypochlorite had the least effect on eliminating bacteria from the canal.

Conflicts of interest

The authors reported no conflicts of interests.

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