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EFFECT OF XP ENDO FINISHER AND PASSIVE ULTRASONIC IRRIGATION ON BACTERIAL REDUCTION: IN VITRO STUDY

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ABSTRACT

Introduction: Chemo-mechanical preparation of the root canal system to reach an almost sterile condition, is the key for better endodontic results. Thus, root canal disinfection should be aiming to use effective irrigant activation techniques that will improve root canal disinfection in addition to an efficient irrigant. Methodology: Thirty three Single-rooted extracted permanent human teeth with mature apices were instrumented to tooth length to a K- file #25 in reaming action, using water as irrigant. Teeth were divided to 3 groups, each group in a silicone mold and sterilized. Teeth were then contaminated with E.feacalis. After incubation root canal instrumentation and irrigant activation was done according to groups. Group A: Xp endo finisher, Group B: Passive ultrasonic irrigation, Group C: conventional needle irrigation. Results: group A and B showed significantly greater bacterial reduction compared to group C. Conclusion: irrigant activation with XPF and PUI caused greater bacterial reduction than conventional needle irrigation.

KEYWORDS: Activation, irrigation, ultrasonic, Xp endo finisher

INTRODUCTION

Chemo-mechanical preparation of the root canal system to reach a near-sterile condition is mandatory to obtain optimal endodontic outcome [(1)]. Studies have found that almost 60% of cases result in negative cultures after preparation and irrigation of the canals. Thus, root canal disinfection should be aiming to use efficient irrigation techniques aided with activation of irrigant that may improve root canal cleaning and disinfection. Several mechanical activation devices have been developed to improve the penetration and effectiveness of irrigation including mechanical activation through sonic or ultrasonic energy , irrigation under negative pressure as well as laser assisted irrigant activation [(2)].

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Ultrasonic irrigation has proved to be powerful by means of acoustic streaming and cavitation phenomena [3]**.** XP-endo Finisher is made of a highly flexible memory alloy, NiTi MaxWire Martensite Austenite electropolish FleX alloy. When introduced into the canal, rising to body temperature, it expands its working width 6mm in diameter or 100-fold of an equally sized file forming a very particular cleaning instrument that acts by scraping but not shaping a large surface area of the canal [2]

METHODOLOGY:

(1) Sample size calculation:

Total of 30 subjects shows 95% power to detect differences among the means versus the alternative of equal means using an F test with a 0.0500 significance level. An extra tooth was added to each group to be used for confirmation of bacterial colonization.

(2) Preparation of samples:

Single-rooted extracted permanent human teeth (n= 33) with mature apices were used for this study. Before use, each tooth was cleaned from the remnants of periodontal tissues and disinfected with NaOCl. They were then kept in jars filled with distilled water until use. The length of the teeth were reduced to 18mm for standardization using a high speed diamond stone. The W.L. was established by subtracting 1 mm from where the tooth length which was determined by the file apparent at the foramen. To standardize the size of the apical constriction, root canals were instrumented to full tooth length to 25-K file in reaming motion. Water was used as irrigant. Apical foramen was sealed with resin material to avoid bacterial contamination or bacterial leakage. For easier tooth identifying and easier tooth handling, the teeth were inserted vertically to the cervical root portion in three blocks of a putty silicone impression material (11 teeth in each block).The blocks were then put in pouches and sterilized until use to ensure the sterility of the specimen before bacterial inoculation [4]

(3) Contamination of root canals with *E. Faecalis:*

Cultures from pure strain of E.Faecalis (ATCC 29212) were left to grow at 37°C in brain heart infusion broth (BHI broth). At 24 hrs, changes in turbidity was checked to ensure bacterial growth. The root samples were removed from their sockets then immersed in the BHI broth previously contaminated with E.faecalis in six-well plates [5]**.** The silicone moulds were re-sterilized for later use.

The plates were then incubated in a shaking incubator at 37°C at 100%humidity for 21 days through which the media was replaced by 90% with fresh BHI day after day [5]**.** At the end of incubation period**,** three samples (1 from each silicone block) were ivestigated under environmental scanning electron microscope to ensure that bacterial colonies were successfully established.

(4) Instrumentation of root canals:

Laboratory bench was thoroughly disinfected with alcohol and a Bunsen burner was used to maintain a sterile field area created by the updraft of the flame to avoid any risk of contamination. The teeth were replaced in their sterile silicone sockets and mechanical preparation was done with the moulds immersed in the water bath at 37°C taking care that water from the water bath will not contaminate the teeth. Crown-down technique was used for canal instrumentation utilizing rotary Revo-S nickel-titanium instruments following the manufacturer's instructions up to size # AS40 instrument. Each canal was irrigated with 2ml of 2.5% sodium hypochlorite after each file size by means of 30-gauge side vented needle.

After instrumentation, samples (n=30) were classified into 3 equal groups according to irrigation activation device used:

Group A: Root canals of this group (n=10) were finally irrigated with xp-endo finisher as final irrigation protocol at 800 rpm for 1 minute with upwards and downwards motion short 1 mm from W.L.

Group B: Root canals of this group (n=10) were finally irrigated with passive ultrasonic irrigation as final irrigation protocol 1 mm shorter than W.L.

Group C: This group (n=10) acted as a control group where final irrigation was done by 3 ml 2.5% NaOCl via 30G sidevented irrigation needle passively inserted into canal

(5) Quantification of bacterial load:

a) Confirmation of bacterial colonization: Three samples were tested under scanning electron microscope to ensure bacterial colony formation in the root canals. With care not to penetrate the canal, grooves were prepared in a longitudinal direction on the buccal and lingual surfaces of each root by a diamond disc at a low speed. Then with a chisel the root was split into 2 halves. Half of each sample was then mounted on metallic stubs and examined at random areas under 10,000X magnification.

b) Sample 1 (S1): Bacteria sampling S1 was done after bacterial incubation and before mechanical instrumentation of the canals. Canals were filled with sterile saline solution, and the 1st sample (S1) was taken by a paper point size #20 placed to the full WL for one minute. After that another paper point was inserted for one minute to obtain all the fluid in the canal. Paper points were put into tubes containing 1 mL of BHI broth. After serial dilutions up to 10-6 in saline, 50 μl of bacteria broth were plated onto brain heart infusion agar plates using glass rods. The plates were stored in the incubator for 24 hours/ 37°C. After that, grown CFUs were counted and bacterial counts were calculated based on the dilution factors previously recorded (4).

c) Sample 2 (S2): Bacteria sampling S2 was done at the end of the procedure in all groups. 1ml 10% sodium thiosulfate was used to rinse the canals before sampling in order to achieve NaOCl neutralization. To act as a transport fluid, 1 ml sterile saline was used to rinse the canal. An H-file #30 was used to file the canal walls to ensure that the medium is loaded with the bacteria which was adhered to the canal walls. Two sterile paperpoints size 35 were placed at the WL to absorb the fluids in the canalsx. Paper points were carried in test-tubes containing 1ml BHI broth. Procedure was completed as in S1 [4].

Statistical analysis:

Data is presented as mean and standard deviation. Data is explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. The data showed non-normal distribution, Kruskal Wallis test is used to compare between tested groups. Then Mann Whitney-U test for pairwise comparison with Dunn Bonferroni correction. The significance level has been set to $P \leq 0.05$. Statistical analysis has been performed using IBM SPSS Statistics in Windows, Version 26.0. Armonk, NY: IBM Corp

RESULTS

Verification of bacterial biofilm: The SEM pictures showed biofilm of well-established microbial colonies on the canal walls. There wasn't a significant difference in Log CFU/ml in samples collected before root canal preparation among tested groups (**p=0.607).**

For all groups, log CFU/ml was significantly reduced after root canal preparation with or without irrigant activation. After activation protocol, group A and B showed significantly lower log CFU/ml compared to group C **(p=0.004), (*Table 1)***

TABLE (1) Mean and SD for Log(CFU/ml)for different tested groups

		Before		***After***		***p-value***
		Mean	***SD***	***Mean***	***SD***	
Log CFU/ml	***Group A***	***2.13^{Aa}***	***0.13***	***1.42^{Ab}***	***0.13***	***<0.001****
	Group B	***2.19^{Aa}***	***0.27***	***1.47^{Ab}***	***0.25***	***<0.001****
	Group C	***2.20^{Aa}***	***0.20***	***1.80^{Bb}***	***0.25***	***0.003****
p-value		***0.607 NS***		***0.004****		

Different capital letters in each column indicate significant difference

Different small letters in each row indicate significant difference

***NS**= Non-significant, *= significant*

After instrumentation and irrigation protocol, group A and B showed significantly higher % of change in CFU/ml compared to group C (**p<0.001**). ***(Table 2)***

TABLE (2) Mean and SD for % of change in CFU/ml for different tested groups

	Group A		Group B		Group C		p-value
	Mean	SD	Mean	SD	Mean	SD	
% of change in CFU	80.23^{a}	2.27	80.59^{a}	3.31	59.44^{b}	10.42	<0.001*

Different letters within each row indicates significant difference

NS= Non-significant, *= significant

DISCUSSION

The complete bacterial elimination from root canal system is challenging but yet crucial to achieve success of treatment and survival of endodontically treated teeth. Studies report 35- 53% of the surface of walls of root canals were remaining untouched during canal preparation which result in biofilms remaining in situ. Thorough irrigation of root canal system is very important to eradicate the bacterial biofilm and infected debris [6]. Although root canal infection is caused by bacteria of multiple species, this study used a single species model to create biofilm to minimize effects that might be resulting from multiple species bacteria [5]. Enterococcus faecalis was used in this study because it is found in different root canal treatment failures [7].It is Gram-positive, facultative anaerobic cocci [7]. Its virulence is due to formation of resistant biofilm, deeply invade dentinal tubules, and show resistance to harsh environments. Studies have found it to be common in failed endodontic cases [5]. Teeth were immersed in the broth incubated with bacteria and put in a shaker incubator. This method allows bacteria to infiltrate into the root canals and create a better biofilm, rather than injecting into the canal. Laboratory setting was done in laminar air flow cabinet in the presence of Bunsen burner to eliminate any chances of bacterial contamination because of the heat halo in the surrounding atmosphere.

Colony forming units (CFUs) method was used for quantification of bacterial load. Many studies have used CFUs method to compare amount of bacterial reduction amongst groups before and after cleaning procedures [5], [8], [9],[10].

Bacterial reduction was significant in all tested groups after root canal preparation. These results are in agreement with studies that investigated the effect of chemomechanical procedures with 2.5% NaOCl conventional irrigation in minimizing the bacterial count from root canal systems[11], [12], [13]. This is attributed to the effectiveness of mechanical debridement of the root canal with instrumentation along with NaOCl irrigation that possesses antimicrobial activity and ability to dissolve organic tissues[12]. Both activation groups showed significantly better bacterial reduction than conventional needle irrigation groups. These results are in agreement with several previous studies that found that XPF resulted in greater bacterial

reduction than conventional irrigation [2],[12],[14],[15],[16]. Other studies stated that passive ultrasonic irrigation resulted in greater bacterial reduction than conventional needle irrigation [17], [18], [19], [20].

In disagreement with the current study are the results of ***Tüfenkçi*** and ***Yılmaz*** (***2020***)[10]. They stated that XPF as an adjunctive step didn't significantly increase bacterial reduction than chemomechanical preparation alone. This might be because they used distilled water as the irrigation solution, which shows that the presence of an antimicrobial irrigant is necessary for showing the effect of irrigant agitation. Also in disagreement with the current study is ***Bhuva et al.*** (***2010***) [21] and ***Paiva et al.*** (***2013***) [22] who stated that PUI didn't increase bacterial reduction after chemomechanical preparation with conventional syringe irrigation. While comparing both activation protocols, our study's results showed no significant difference between (PUI) and XPF groups regarding bacterial reduction. This was in agreement with ***Ballal et al.*** (***2020***) [6], ***Carvalho et al.*** (***2020***) [23], ***Alves et al.*** (***2016***) [24], ***Bao et al.*** (***2017***) [25]. They found that XPF resulted in reduction in the bacterial numbers after preparation than PUI with significant difference. They concluded that the flow of the irrigant in the canal, facilitated by the 800-rpm XP file with an asymmetric helical shape, created stream of irrigation powerful enough to result in detachment of a lot of bacteria from canal walls. ***Sasanakul et al.*** (***2019***) [5] also found that XPF resulted in significantly better results than PUI but this study was aimed to evaluate disinfection prior to regenerative endodontic procedures on teeth with open apices of 0.8mm wide apical foramina. They stated that shearing forces produced by PUI is inversely proportionate to the thickness of the bounding area or width of root canal. Thus, the size of the canal, which is large in their study, might have caused diminished effect in bacterial removal by PUI. On the other hand, ***Villalta-Briones et al. (2021)***[26] found that PUI significantly reduced bacterial biofilm than XPF. Files under ultrasonic activation, produce streaming motions in the irrigant in the area next to the file, causing increased irrigant flow within the canal. Thus producing shearing forces, which might be able to damage microbial cells [27]. The inconsistency of results in literature might be due to difference in study methodology and microbial-identification techniques used, e.g. quantitative polymerase chain reaction (qPCR) [16,18,22,23], culturing [5,6,12], identification of biofilm under scanning electron microscope [15], [25]. In the current study, microbiological testing was done by counting colonies to calculate colony forming units (CFU). This technique is known to be of limitations because bacteria in the main canal can be taken for sample through paper points but not that in lateral canals and ramifications [10]. Its advantages are that no root sectioning is required so results are not altered by diamond disc and smear layer produced. DNA based techniques are known to be more reliable in detection of culture-difficult or as-yet-uncultivable bacteria; however, they are limited in that discrimination between live and dead bacteria is not possible leading to unablility to detect the effects of antimicrobial treatments [28].

CONCLUSION

Mechanical preparation caused bacterial reduction with or without irrigant activation. XP endo finisher and Ultrasonic activation cause greater bacterial reduction in comparison with conventional needle irrigation.

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