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Original Article

A COMPARATIVE STUDY OF THE EFFECT OF THE NEGATIVE PRESSURE IRRIGATION SYSTEM (ENDOVAC) AND ULTRASONIC-ACTIVATED IRRIGATION WITH SODIUM HYPOCHLORITE ON INTRA-ROOT CANAL ENTEROCOCCUS FAECALIS

(AN IN VITRO STUDY)

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ABSTRACT

Aim: The aim of this in vitro study was to compare the ability of irrigation with sodium hypochlorite employing the (EndoVac) irrigation system and the ultrasonic activated irrigation in comparison with conventional syringe/needle irrigation to eradicate intracanal *Enterococcus faecalis*.

Methods: Fifty-one extracted human maxillary central incisors with single straight root canals and completely formed apices were used. The teeth were allocated into three groups at random (N=17), group (1) irrigated with the conventional needle irrigation, group (2) irrigated with the passive ultrasonic irrigation, and group (3) irrigated with the EndoVac negative pressure irrigation. 5.25% NaOCL was used in this study. A suspension of *E. faecalis* ATCC 29212 grown on brain heart infusion (BHI) broth was prepared to equal the turbidity of a 0.5 McFarland standard. The root canals were inoculated with this solution. The teeth were then incubated at 37°C for 7 days. After the irrigation procedures, the amount of *E. faecalis* was calculated/sample by colony-forming units (CFU). The dentinal fragment samples were collected using H- files size 40. After incubation at 37°C for 24 h, colony-forming units (CFU) were counted and the actual bacterial count were calculated/sample.

Results: There was a statistically significant difference in the bacterial reduction between the experimental groups (PUI and EndoVac) and the control group (conventional needle irrigation) (p=0.00). There was no statistically significant difference between group (2) PUI and group (3) EndoVac in the remaining bacteria after irrigation method.

KEYWORDS: *E. faecalis*, EndoVac, Passive Ultrasonic irrigation, Side vented needle, negative pressure irrigation, passive irrigation, scanning electronic microscope

Introduction

The primary goal of endodontic treatment in infected root canals is eradicating intracanal bacteria or reducing their percentages to be well suited to periradicular tissue healing and don't stimulate or maintain a disease. The treatment outcomes may be negatively influenced by some types of bacteria that resist the chemomechanical preparation of root canals [(1)].The key component of an efficient endodontic therapy is irrigation because it performs numerous crucial chemical, mechanical, and microbiological tasks. Additionally, mechanical instrumentation cannot reach the portions of the root canal walls that require irrigation [(2)]. *Enterococcus faecalis* is a species of bacteria typically found in persistent, asymptomatic root canal infections. Its prevalence in such infections varies between 24 and 77%. This spread is a result of these bacteria's survival factors, which include their competitiveness with other microorganisms, their penetration of dentinal tubules, and their resistance to nutrient deprivation [(3)]. EndoVac is an irrigating system produces a negative pressure which is an alternative to deliver the irrigant through the needles and syringes that produce positive pressure. Through a thin needle with a unique design, the irrigating solution in the EndoVac system is pulled down the root canal and retrieved again. The EndoVac system reduces the risks correlated to irrigation at the apical third of the root canal when compared to conventional needle irrigation as well as some other systems. Endodontics has a lengthy history of utilizing ultrasonic devices for root canal cleaning and to enhance debridement. Several previous studies have compared the relative efficacy of ultrasonic and manual instrumentation techniques. Most of these studies reached to a conclusion that ultrasonics and an irrigant together produce a more thorough cleaning of the root canal space than irrigation and manual instrumentation alone [(4)].

MATERIALS AND METHODS

Preparation of teeth: Fifty-One extracted maxillary central incisor teeth were divided into three groups according to the method of irrigation: Group one: conventional side vented needle group which is the control group. Group two: ultrasonic group with U files 35. Group three: EndoVac group. Periodontal curettes were used to clean the teeth, which were then placed in 10% formaldehyde solution for two days before being washed and kept in sterile saline solution until use. In order to standardize the root length at 15 mm, the teeth were decoronated. Root canals were examined with #10 K-file for patency. ProTaper S1-S2-F1-F2-F3-F4 was used to prepare the canals to their full working length. Instrumentation included irrigation with a 2.5 percent NaOCl solution. Canals were irrigated with 1 mL of a 17 % EDTA solution for 1 minute to remove the smear layer, followed by 5 mL of a 5.25 percent NaOCl solution. Final irrigation was done with 3 mL of saline, then the canals were dried with paper points. All of the specimens underwent

autoclave sterilization at 121°C and 15 lb/in 2 pressure for 20 minutes. Six additional teeth,

were prepared to assess the effectiveness of smear layer removal by scanning electron microscopy (SEM) to confirm the bacterial penetration into the dentinal tubules.

Preparation of bacteria: A suspension of *E. faecalis* ATCC 29212 grown on brain heart infusion (BHI) broth was prepared to equal the turbidity of a 0.5 McFarland standard (~ 1.5×10^8 colony forming unit (CFU)/mL). The root canals were inoculated with this solution; teeth were incubated for 7 days at 37°C. To maintain the bacterial viability, the solution was renewed every two days. **The irrigating methods:**

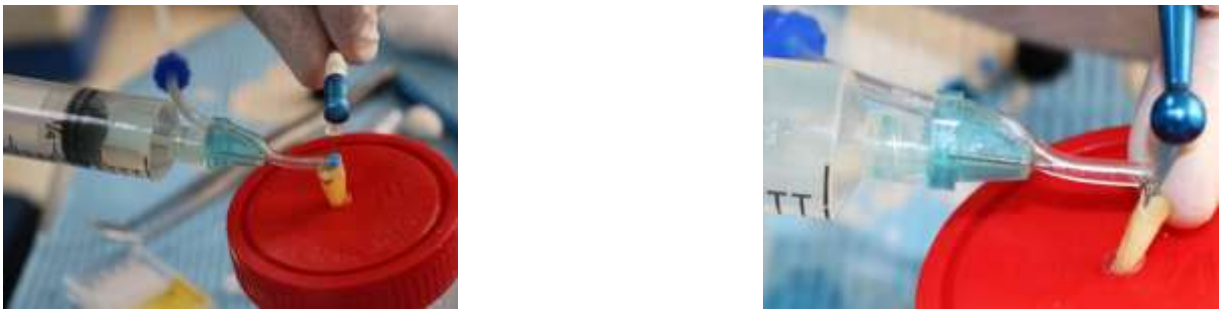
Group (1) control group, Conventional syringe/ side vented needle irrigation (N= 17): 5mL of 5.25% NaOCl for 1 min, this was done 1 mm short of the working length. The final irrigation was performed with 5 mL of sterile saline in all groups. Group (2) Passive Ultra-Sonic irrigation system (N= 17): Canals were filled with 5ml of 5.25% NaOCl and the ultrasonic irrigation protocol was established using the ultrasonic tips E11 and U file size #35; 3 cycles of 20s. The U file tip was placed 1mm short from the working length (Fig.1).

Figure (1): ultrasonic irrigation

Group (3) EndoVac irrigation system (N = 17):

A. Macro-cannula step: 5.25% NaOCl was constantly delivered for 30 seconds and the macro-cannula was placed approximately at the mid-canal level with up and down (pecking) motion.
B. Micro-cannula step: the micro-cannula was used to insert the delivered solution into the root canals. Suction was then used to remove the solution. 5.25% NaOCl was flooded into the root canals for 15 seconds before being withdrawn with a microcannula. The root canals were once more flooded with 5.25 % NaOCl for 15 seconds by inserting the micro-cannula till the end of the working length (total volume: 5 ml; irrigation time: 1 min) (Fig.2).

(a)



(b) (c)

Figure (2) (a, b, c): (a): EndoVac system (b): macro-cannula step (c): micro-cannula step.

Evaluation of remaining *E. faecalis*: After the irrigation procedures, the amount of *E. faecalis* was calculated by colony-forming units (CFU). The dentinal fragment samples were collected using H-files#40. The dentinal shavings were transferred by sterile paper points and H files into tubes containing 500 µL phosphate-buffered saline (PBS) solution (pH ~7.4) and vortexed for 1 min. A 10-fold serial dilution was prepared from each sample and 20 µ of each dilution was plated onto BHI agar. After incubation at 37°C for 24 h, colony-forming units (CFU) were counted and the actual bacterial count was calculated.

Statistical analysis: Values were presented as mean, standard deviations (SD) and confidence intervals. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that data were normally distributed (parametric data), therefore, ANOVA and Tukey's post hoc tests were used for intergroup comparisons. The level of significance was set at $p \leq 0.05$.

RESULTS

Evaluation of the smear layer removal by SEM: Scanning electronic microscopic (SEM) evaluation of mechanically and chemically prepared root canals by NaOCL and EDTA solutions before and after bacterial inoculation showed smear layer removal and absence of bacterial growth (Fig.3 and 4).

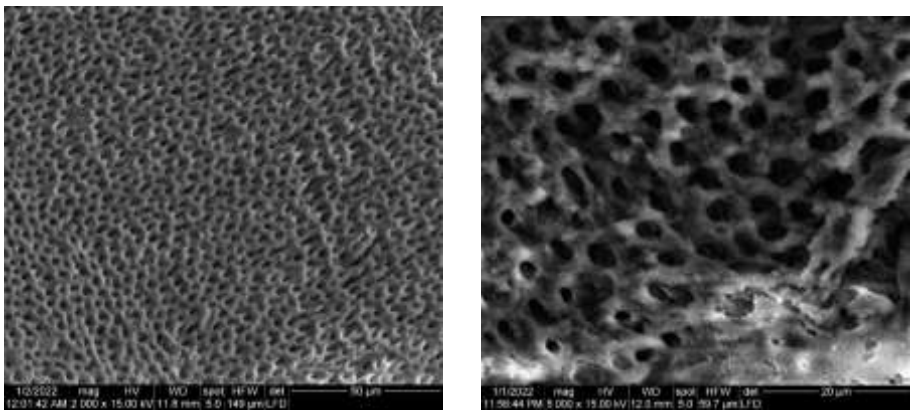


Figure (3): SEM of root canal walls showing open dentinal tubules after smear layer removal and sterilization.

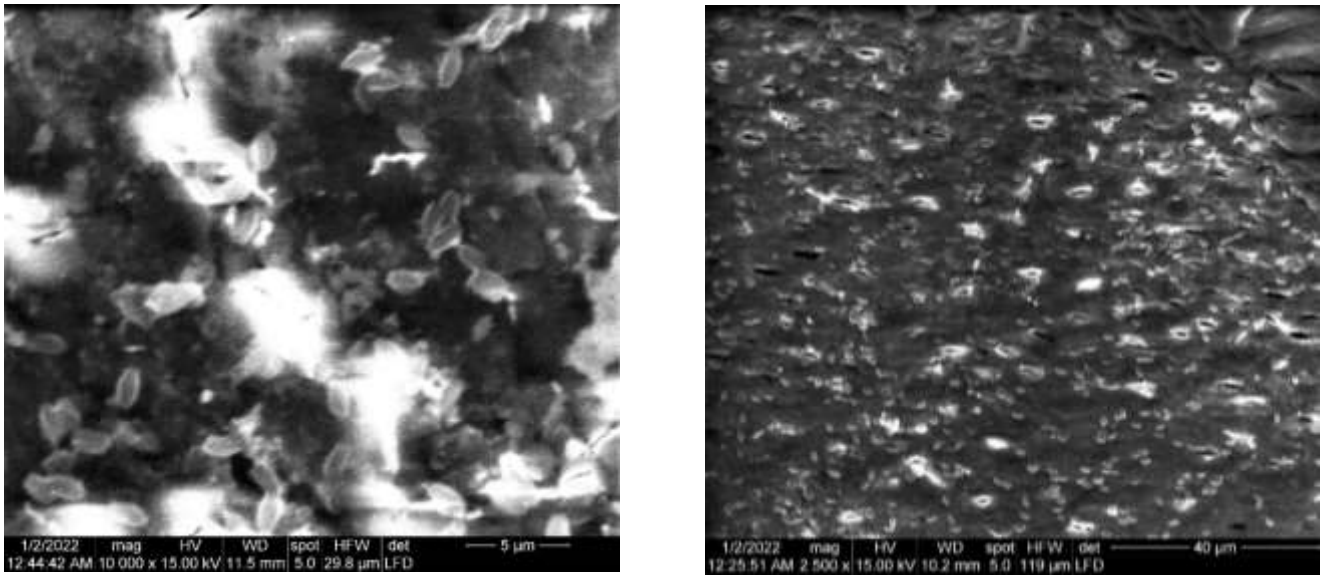


Figure (4): SEM of root canal walls exhibiting colonization of bacteria on root canal walls and in dentinal tubules after bacterial contamination**.**

The influence of the irrigation methods on bacterial count: In all groups there was a very heavy number of *E. faecalis* = more than 2000 CFU/ sample. **Conventional needle irrigation (group1)** which is the control group exhibited the highest number of remaining bacteria after irrigation with a mean value of **(502.94±66.07)**. It significantly **showed a higher bacterial count than the other groups (p=0.00)**. **The passive Ultrasonic irrigation (group2)** exhibited the **least number of remaining bacteria** after irrigation among the other two groups. It significantly showed a lower bacterial count than the conventional needle irrigation group with a mean value of **(28.82±14.95)**. The **EndoVac (group3)** also, exhibited few numbers of remaining bacteria, yet in comparison to the ultrasonic group (group2), the number of remaining bacteria was slightly higher. Also, the EndoVac group was significantly lower than the conventional needle irrigation group with a mean value of **(55.00±16.86)**. However, **there is no statistically significant difference between the Ultrasonic group and the EndoVac group according to the Post hoc test** (Table 1, Fig.5).

Table (1): descriptive statistics and comparison between groups regarding bacterial count (CFU) after irrigation (ANOVA test).

	Mean	**SD**	**95% Confidence Interval for Mean**		**Min**	**Max**		
			Lower Bound	**Upper Bound**			**F**	**P**
Conventional needle group	**502.94[a]**	**66.07**	**468.97**	**536.91**	**400.00**	**600.00**	**744.136**	**0.000***
Ultrasonic group	**28.82[b]**	**14.95**	**21.14**	**36.51**	**10.00**	**50.00**		
EndoVac group	**55.00[b]**	**16.68**	**46.43**	**63.57**	**25.00**	**80.00**		

Significance level p≤0.05, *significant. Tukey's post hoc test: means sharing the same superscripts are not significantly different.

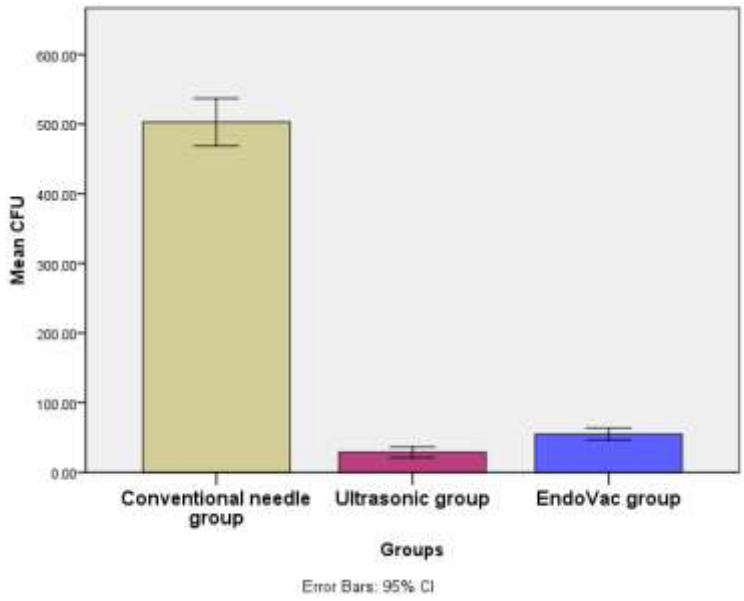


Figure (5): a bar chart illustrating the mean bacterial count (CFU) after irrigation in different groups.

Percentage of reduction of bacterial count after irrigation: All of the three groups showed **a huge** reduction in the bacterial count after the use of irrigation methods by NaOCL. A comparison of the percentage of bacterial reduction among the three groups showed: **Conventional needle irrigation (group1)** which is the control group exhibited the least percentage of bacterial reduction among the other groups, The least mean value of the percentage of reduction was recorded in the conventional needle group **(-74.85±3.3)**. **It significantly showed the lowest bacterial reduction among the other 2 groups (p=0.00**). **Passive ultrasonic irrigation (group2)** exhibited the highest percentage of bacterial reduction among the other groups. It has the greatest mean value of the percentage of bacterial reduction **(-98.56±0.75)**. **EndoVac group (group3)** exhibited a higher percentage of bacterial reduction than the conventional needle group and slightly lower than the ultrasonic group. The mean value of the percentage of reduction was recorded in this group **(-97.25±0.83)**. **There is no statistically significant difference between the Ultrasonic group (group2) and EndoVac group (group3) according to the Post hoc test** (Table 2, Fig.6).

Table (2): descriptive statistics and comparison between groups regarding the percentage of reduction of bacterial count (%) after irrigation (ANOVA test).

	Mean	SD	95% Confidence Interval for Mean		Min	Max		
			Lower Bound	Upper Bound			F	P
Conventional needle group	**-74.85[b]**	**3.30**	**-76.55**	**-73.15**	**-80.00**	**-70.00**	**744.136**	**.000***
Ultrasonic group	**-98.56[a]**	**0.75**	**-98.94**	**-98.17**	**-99.50**	**-97.50**		

EndoVac group	**-97.25[a]**	**0.83**	**-97.68**	**-96.82**	**-98.75**	**-96.00**

Significance level p≤0.05, *significant.
Tukey's post hoc test: means sharing the same superscripts are not significantly different.

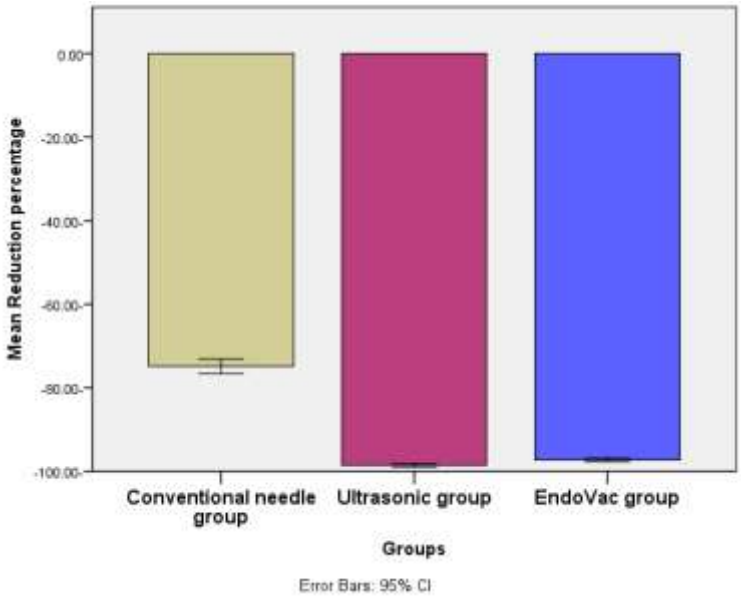


Figure (6): a bar chart illustrating the mean percentage of reduction in bacterial count (%) after irrigation in different groups.

DISCUSSION

The current study, compared the antibacterial efficacy of three irrigation techniques using an extracted tooth model with single straight roots to mimic the intracanal environment [5]. A 7-day-old biofilm was chosen in the current study according to previous studies, which have confirmed the 7-day growth phase as optimal for production of standardized *E. faecalis* biofilm for testing the efficacy of different irrigation methods [6, 7]. *Enterococcus faecalis* was selected as the bacteria to be tested in the current study being the most reported organism from persistent apical periodontitis cases, with a prevalence ranging from 24% to 77% of cases detected by culturing methods. Also due to its ability to form a stable biofilm [8, 3, 9]. Numerous studies confirmed the substantial role of the chemical irrigating solutions in enhancing the root canal debridement, and it was concluded that a chemo-mechanical preparation would facilitate the task of proper cleaning and most convenient disinfection of the root canals [10]. Sodium hypochlorite (NaOCL) was selected to be the irrigating solution in the current study being the most commonly used and accepted solution in endodontic treatment since it was first reported for use by Walker in 1936 [11]. NaOCL can dissolve organic substance as pulp remnants, microbes and biofilms. It is used in many concentrations between 0.5-6% [4]. There

was a study suggesting that the effectiveness of any chemical irrigating solution cannot be achieved without a direct connection between the solution and organisms in the anatomy of root canal [12]. If used in sufficient quantities and changed frequently, sodium hypochlorite, 4% to full strength, can eliminate *E. faecalis* in the root canal. [3]. Some of the clinical problems associated with NaOCL are reported, such as the vapor lock phenomena that resulted from the reaction between NaOCL and the organic tissues in dentinal walls, causing hydrolysis, which liberates carbon dioxide and ammonia. This forms micro gas bubbles entrapment in the apical portion of the root canal that united into a large apical vapor bubble and quickly forms a column of gas in this area which does not permit further irrigating solutions to penetrate and hence isolates this area from further debridement [13]. There were some studies that explained how we can penetrate this bubble by placing the irrigation needle at or close to the apical constriction [14]. In this case it may increase the chances of extrusion of NaOCL beyond the apex causing severe pain, injury and periapical tissue destruction. These findings raised a clinical problem in achieving a safe and effective debridement of the entire root canal which included an apical constriction and fine lateral canals. So, the need for upgrading the delivery methods of irrigation to be more effective through achieving an adequate flushing action along the working length by a safe manner has become highly needed [15]. Passive ultrasonic irrigation system was chosen in the present study to test the antibacterial efficacy in comparison with EndoVac and conventional needle irrigation. The term passive indicating non-cutting action of the ultrasonically activated file. The mode of action of PUI depends on transmitting the acoustic energy through the activated file to the irrigating solution to create an acoustic microstreaming and cavitation (microbubbles) which increase

the efficacy of the irrigating solution by contacted greater surface area which is called agitation of irrigation [16]. In addition to contribution to elevation of the temperature of the irrigating solution and increasing its movement which leads to an increase in the efficacy of irrigation and enhance killing more bacteria and improving penetration ability in difficult- to-reach areas [17]. The EndoVac was chosen to be one of the methods in the present study being mainly dependent on negative apical pressure and consists of macro and microcannula. A delivery/evacuation tip is attached to a syringe of the irrigant and the high-volume suction part of the dental unit. As the macro and microcannulas are placed in the canal, negative pressure withdraws irrigant solution from the chamber, down the canal to the tip of the cannula, into the cannula, and out through the suction hose. The microcannula of this system have an ISO size of 35 and can be used at working length in a canal that is enlarged to 35 or larger [18]. Our results showed that the EndoVac system significantly reduced the higher number of bacteria in comparison with conventional needle irrigation. This finding was in agreement with previous studies [19-24]. A study by (Buldur and Kapdan 2017) [25], confirmed that the EndoVac did not produce significantly better elimination of bacteria than the conventional needle irrigation method, this finding was in disagreement with our results. This difference between results may be due to the variation in methodology between the studies, such as the concentration of NaOCL which is 5.25% in our study that was higher than their concentration which was 2.5%. Also, their study was performed on primary teeth and the current study was done on permanent teeth. Furthermore, they used a closed system model which increases the probability of vapor lock formation and hence it prevents reaching of further irrigating solution into the apex. In the current study it was noticed that the PUI system significantly reduced the number of bacteria in comparison with conventional needle irrigation and this finding was in agreement with previous studies by [17,26-29]. The results of the current study were in agreement with (Townsend and Maki 2009) [30]. They found that the ultrasonic agitation of irrigation produced significantly more reduction of bacterial load than needle irrigation and EndoVac. On the other hand, a study by (Bhuva 2010) [31], showed that there were no significant differences regarding the antibacterial activity between the PUI system and the conventional needle system and this finding was in disagreement with our results. This may be attributed to the differences in the concentrations of NaOCL. They used 1% concentration of NaOCL while a concentration of 5.25% was used in the present study. The results of the present study were also in disagreement with (Paiva et al., 2012) [32], who found that the PUI didn't achieve a significant reduction of bacteria better than that achieved by chemomechanical preparation. This is

because they used a PCR method which detected the viable and non-viable bacteria; yet it did not have a control group. In addition to using different concentrations of NaOcl. According to the results of the current study, it was found that there were no statistically significant differences in reduction of number of bacteria between PUI system and EndoVac system. This finding was in agreement with (Mikulik et al., 2019) [23], who found that there were no significant differences between PUI and EndoVac systems.

CONCLUSION

Within the limitations of this study, it could be concluded that, Both PUI and EndoVac irrigation methods have an antibacterial efficacy better than the conventional side vented needle irrigation.

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Ethics: This study protocol was approved by the ethical committee of the faculty of dentistry Cairo university

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