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Comparative Evaluation of Microbial Reduction Using Different Irrigation Activation Techniques Like Needle Irrigation and Ultrasonic Irrigation Using Sodium Hypochlorite, Chlorhexidine and EDTA as Irrigants.

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KEYWORDS

Irrigation, Ultrasonic activation, Sodium Hypochlorite, Chlorhexidine, EDTA.

ABSTRACT:

Background: Endodontic infections are polymicrobial diseases characterized by apical tissue inflammation influenced by microbial, immunological and environmental factors. Microorganisms often colonize as biofilms, deeply embedded in areas inaccessible to mechanical instrumentation, which enhances their virulence and resistance. Therefore, the use of effective irrigation solutions is essential, as they aid in disrupting biofilms, eliminating microorganisms and improving overall disinfection of the root canal system.

Objectives: The aim of this study was to evaluate the microbial reduction using different irrigation activation techniques like Needle irrigation and Ultrasonic irrigation using Sodium Hypochlorite, Chlorhexidine and EDTA.

Methods: 18 patients with primary endodontic infection were selected randomly for this study and further assigned into 3 groups accordingly with needle and Ultrasonic irrigation methods. Group 1 (Sodium hypochlorite), Group 2 (Chlorhexidine), Group 3 (EDTA).

Results: Sodium hypochlorite showed the greatest bacterial reduction with ultrasonic irrigation, though the difference was not statistically significant. In contrast, chlorhexidine demonstrated a significant advantage of ultrasonic irrigation over needle irrigation ($p = 0.047$; change in values $p = 0.012$). EDTA showed no significant differences between groups.

Conclusion: Ultra sonic irrigation has the ability to disrupt the microbial colonies within the canal when compared with the needle irrigation. While, Sodium hypochlorite is the gold standard in cleaning the canals effectively by ultrasonic irrigation followed by Chlorhexidine then EDTA.

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Inroduction

Endodontic infections represent a complex disease process characterized by apical tissue inflammation influenced by microbial, immunological and environmental factors. [1,2] These infections are polymicrobial in nature, with microorganisms typically organized as biofilms that colonize inaccessible areas of the root canal system. Such biofilms increase microbial virulence, resistance to antibiotics and survival potential, making eradication particularly challenging. [3–5]

Successful endodontic therapy therefore requires significant reduction of microbial load, which may be achieved through a combination of instrumentation, irrigation regimens and occasionally intracanal medicaments. A comprehensive antimicrobial approach remains essential for effective treatment outcomes. However, the complex root canal anatomy limits the efficacy of chemomechanical preparation alone, emphasizing the need for irrigation techniques that deliver solutions effectively into inaccessible areas of the canal system.[6]

Conventional syringe and needle irrigation, which introduces irrigant solutions passively or with agitation through needles of varying gauges, has been widely used due to its simplicity, ease of manipulation and control over irrigant depth and volume. Nonetheless, the technique carries the risk of irrigant extrusion into periapical tissues due to positive pressure delivery, potentially causing severe tissue damage and postoperative discomfort.[7]

In contrast, Passive Ultrasonic Irrigation (PUI) has been shown to enhance the efficacy of Sodium Hypochlorite (NaOCl). Ultrasonic activation heats the irrigant, increasing its antibacterial potential, while simultaneously promoting removal of dentin debris and smear layer which are the common sites of microbial retention, thereby achieving more effective canal disinfection.[8]

Sodium Hypochlorite (NaOCl) is the most used root canal irrigant during the chemomechanical debridement of root canals because of its broad antimicrobial spectrum and its ability to promote organic tissue dissolution. However, NaOCl is highly irritating when in contact with periapical tissues, reduces the resistance of teeth to fracture and interferes negatively with the bond strength of adhesive restorations to dentin.

Because of the adverse effects of this irrigant, researchers have developed alternative endodontic irrigants.[9] targets multiple cellular sites, reducing the likelihood of resistance. Being both hydrophilic and lipophilic, CHX interacts with cell membrane phospholipids and lipopolysaccharides, disrupting the membrane and entering the cell to induce intracellular damage, such as cytoplasmic coagulation. At low concentrations, CHX is bacteriostatic, while at higher concentrations, it is bactericidal against both gram-positive and gram-negative bacteria, with stronger activity against gram-positive species. [10]

Another irrigating solution is Ethylenediaminetetraacetic acid (EDTA) which is a synthetic amino acid, commonly used in dentistry in the form of its sodium salts (Na_2EDTA). It acts as a chelating agent, binding metallic ions such as calcium to form stable ring-shaped complexes, and is non-corrosive to instruments. While EDTA is neither bactericidal nor bacteriostatic, it inhibits bacterial growth by depriving microorganisms of essential metal ions, ultimately leading to their death.[11]

Despite extensive research on endodontic irrigation, there remains considerable variation in the reported effectiveness of different irrigants and activation techniques in achieving predictable microbial reduction. While Sodium Hypochlorite continues to be regarded as the gold standard, its performance may vary significantly depending on the method of activation used. Similarly, Chlorhexidine and EDTA, though well-established in endodontic protocols, demonstrate differing antimicrobial nature and their efficiency when combined with various irrigation methods remains insufficiently explored. Additionally, conventional needle irrigation often fails to deliver irrigants into the deeper complexities of the canal anatomy, whereas Ultrasonic Activation has shown promising results in enhancing irrigant penetration and biofilm disruption. However, limited clinical evidence exists directly comparing these irrigants under different activation systems in patients with primary endodontic infections. Therefore, a systematic comparison is needed to better understand their relative antimicrobial effectiveness, optimize clinical protocols and provide evidence-based guidance for achieving superior root canal disinfection.[4]

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Thus, the aim of this present study was to compare and evaluate the microbial reduction with Ultrasonic irrigation and Needle irrigation using Sodium Hypochlorite, Chlorhexidine and EDTA as irrigant solutions and efficacy within them.

Material & Methodology:

The study was carried out in the Department of Pediatric and Preventive Dentistry after getting necessary approval from the Institutional Ethics Committee. Total 18 patients with primary endodontic infection while carrying out pulpectomy, were selected for this study then randomly assigned into 3 groups accordingly with needle and Ultrasonic irrigation methods. Group 1 (Sodium hypochlorite), Group 2 (Chlorhexidine), Group 3 (EDTA).

Inclusion criteria:

- Primary Molars
- Co-operative children

Exclusion criteria:

- Primary Anteriors, Fractured or badly decayed teeth that could not be isolated with a rubber dam.

Medically comparomised patients

All the steps of dental intervention were performed under completely aseptic conditions. The tooth was completely isolated using a rubber dam.

The access cavity was prepared using long shank rose head bur size 2 and Endo Z bur attached to high speed contra angle hand piece. The access cavity preparation was performed without using water spray but under manual irrigation with sterile normal saline. Working length was determined using initial apical file (MANI Inc., Utsunomiya, Japan) sizes 15, 20 or 25 according to each canal size.

The first microbiological sample (S1) was obtained following the creation of the access cavity in the primary molars using absorbent paper points. The canal was filled with the paper point for derived sample under aseptic condition and biomechanical preparation was done with Kedo SH rotary file system.

The second microbiological sample (S2) was taken after irrigation activation with Sonic irrigation done in 9 patients and randomly further divided into 3 groups using Sodium hypochlorite, Chlorhexidine and EDTA as root canal irrigants. The same procedure was repeated with the needle irrigation method in 9 patients using Sodium hypochlorite, Chlorhexidine and EDTA as root canal irrigants.

Sample 1(access opening) and Sample 2 (after active irrigation) were collected into 10 ml sterile vials. The first vial for preoperative sample and second vial for postoperative sample and were stored in the thermocol icebox for transportation to the microbiological lab within 2 hours. These samples were cultured on blood agar aerobically and incubated for 24-48 h at 37 degree, growing colonies were counted using digital colony counter. They were recorded as colony forming units (CFU). The values thus obtained were tabulated and statistically analysed.

Statistical analysis

Data entries were done in Microsoft Office Excel 2010 and analyses of results was done using Statistical Product and Service Solution (SPSS) version 22 software. Descriptive statistics such as mean and standard deviation was calculated for quantitative variables. The p value was fixed at 0.05. Data normality was checked using Shapiro Wilk test. Data was found to be following normality, hence parametric test were used. Unpaired t test was used for intergroup comparison between two study groups in relation to microbial efficacy. Overall intragroup comparison between three study groups will be done using One way ANOVA F test followed by tukeys post hoc test for pairwise comparison.

Results-

The mean and standard deviation of contamination levels between different treatment protocols are expressed in log10 CFU/mL in Table 1. n Group A (Ultrasonic Irrigation), the mean pre-operative colony counts ($\times 10^3$) were highest in Group 1 (Sodium Hypochlorite) [106.67 ± 90.18], followed by Group 3 (EDTA) [100.0 ± 87.17], and lowest in Group 2 (CHX) [30.0 ± 10.0]. Post-operatively, the mean colony counts were reduced to 1.36 ± 1.48 in Group 1, 1.66 ± 0.57 in Group 2, and 33.3 ± 40.4 in Group 3.

The mean reduction in colony counts (change in values) was maximum with Sodium Hypochlorite (105.3 ± 89.8), followed by EDTA (66.66 ± 47.25), and least with CHX (28.33 ± 9.5). On statistical analysis, one-way

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ANOVA revealed no significant difference among the three groups at baseline ($p = 0.414$), post-operatively ($p = 0.236$), or in the overall change in values ($p = 0.344$). Pairwise comparison between groups also did not show any statistically significant differences (all $p > 0.05$).

Thus, although all three irrigants showed a reduction in microbial counts following ultrasonic irrigation, the intergroup differences were not statistically significant.

Chlorhexidine (CHX) is a cationic bis-biguanide antiseptic with broad-spectrum antimicrobial activity. It According to the below bar Graph, comparison of antimicrobial efficacy between ultrasonic irrigation (Group A) and needle irrigation (Group B) with different irrigants showed that Sodium Hypochlorite demonstrated the maximum reduction in microbial counts.

In Group A (Ultrasonic irrigation with Sodium Hypochlorite), the mean pre-operative colony count was 106.67×10^3, which was markedly reduced to 1.36×10^3 post-operatively, yielding a mean reduction of 105.3×10^3. Similarly, in Group B (Needle irrigation with Sodium Hypochlorite), pre-operative values were much higher (253.33×10^3), but post-operatively reduced to 3.66×10^3, with a mean reduction of 249.67×10^3.When compared with CHX, Group A (Ultrasonic irrigation) showed a reduction from 30.0×10^3 to 1.66×10^3 (28.33×10^3 reduction), whereas Group B reduced from 166.6×10^3 to 26.6×10^3 (140×10^3 reduction). For EDTA, Group A reduced from 100×10^3 to 33.3×10^3 (66.6×10^3 reduction), while Group B reduced from 53.33×10^3 to 29.66×10^3 (23.66×10^3 reduction).

On overall comparison, Sodium Hypochlorite exhibited the greatest antimicrobial efficacy in both ultrasonic and needle irrigation groups, with needle irrigation showing higher absolute reduction values due to higher baseline microbial counts. Among the other irrigants, chlorhexidine showed moderate reduction, while EDTA demonstrated the least antimicrobial effect.

Group 1 (Sodium Hypochlorite) 3 [x 10]	**Group A (Ultrasonic irrigation) Mean (SD)**	**Group B (Needle Irrigation) Mean (SD)**	**Unpaired t test**	**p value, Significance**
Pre-operative	**106.67 (90.18)**	**253.33 -224.79**	**t = -1.049**	**p= 0.353 (NS)**
Post-operative	**1.36 (1.48)**	**3.66 (1.52)**	**t = -1.870**	**p= 0.135 (NS)**
Change in values	**105.3 (89.8)**	**249.67 (224.9)**	**t = -1.032**	**p= 0.360 (NS)**
Group 2 (CHX)	**Group A (Ultrasonic**	**Group B (Needle Irrigation)**		

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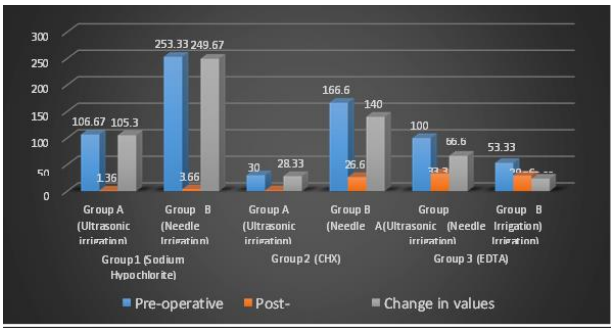
[x 103]	irrigation) Mean (SD)	Mean (SD)	Unpaired t test	p value, Significance
Pre-operative	30.0 (10.0)	166.6 (57.73)	t = -4.04	p=0.016*
Post-operative	1.66 (0.57)	26.6 (15.27)	t = -2.833	p=0.047*
Change in values	28.33 (9.5)	140.0 (43.58)	t = -4.335	p=0.012*
Group 3 (EDTA) [x 103]	Group A (Ultrasonic irrigation) Mean (SD)	Group B (Needle Irrigation) Mean (SD)	Unpaired t test	p value, Significance
Pre-operative	100.0 (87.17)	53.33 (41.63)	t = 0.837	p=0.450 (NS)
Post-operative	33.3 (40.4)	29.6 (20.5)	t = 0.140	p=0.895 (NS)
Change in values	66.6 (47.25)	23.66 (22.81)	t = 1.419	p=0.229 (NS)

Table 1: Intergroup comparison between antimicrobial efficacy in terms of number of colony count (x 10^3) respectively.

Graph 1 Comparison of antimicrobial efficacy between ultrasonic irrigation (Group A) and needle irrigation (Group B) with different irrigants



Discussion

Microorganisms and their toxic metabolites are the primary etiological factors in pulpal and periapical pathologies. The principal goals of root canal therapy are to achieve effective disinfection and to prevent subsequent microbial re-infection of the canal system. Due to the complex anatomy of root canal systems and the inherent limitations of available mechanical shaping instruments and techniques, certain areas of the canal walls often remain untouched during instrumentation. [12]

Consequently, chemomechanical debridement plays a pivotal role in root canal disinfection, and this is further enhanced by various methods of irrigant agitation. Among these, mechanical agitation has been shown to improve the efficacy of irrigation solutions, with ultrasonic irrigation proving to be more effective than traditional techniques.[13]

The total number of cultivable bacteria was assessed using a culture-based method on non-selective blood agar medium, which supports the overall growth of microorganisms. Incubation under both aerobic and anaerobic conditions allowed for separate determination of CFU counts of aerobic and anaerobic species. Accordingly, the culture method was employed in the present study.[14]

Among the various phases of endodontic therapy, root canal preparation is regarded as the most critical step for bacterial elimination. Rotary multi-instrument systems, comprising sequential nickel-titanium (Ni-Ti) files operated in continuous rotary motion, have demonstrated a highly significant reduction of bacterial load in clinical investigations. Hence, the Kedo SH rotary system was selected for canal preparation in this study.[15]

According to Nakamura et al. in 2018, Ultrasonic activation was more effective than needle irrigation in reducing bacterial levels.[16] **Spoleti et al. in 2003** also reported higher bacterial survival when ultrasonic activation was not applied. Likewise, **Carver et al. in 2007** found that using ultrasonic irrigation after hand or rotary instrumentation significantly decreased CFU counts in infected necrotic molars which is in accordance with present study. [17]

The findings of the present study are consistent with

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those of **Townsend and Maki in 2009** who reported that ultrasonic irrigation was significantly more effective in eliminating intracanal bacteria compared to both needle irrigation and EndoVac. Similarly, **Hockett et al. in 2008** concluded that Apical Negative Pressure (ANP) delivery of irrigants using the EndoVac system provided superior microbial control compared to positive pressure needle irrigation.[18,19]

In contrast, the present findings disagree with those of **Beus et al. 2012**, who compared non-activated needle irrigation with Passive Ultrasonic Irrigation (PUI) in achieving bacteria-free canals. Their study reported no statistically significant difference between the two groups, which may be attributed to the lack of standardization before microbiological sampling. Specifically, in the PUI group, samples were collected immediately after activation, whereas in the needle group, a hand file matching the master apical file size was used to ream the canal walls, potentially dislodging debris and bacteria before sampling.[20]

A study conducted by **Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ in 2007,** evaluating the antimicrobial efficacy of chlorhexidine gluconate gel using the agar diffusion method demonstrated that 2% chlorhexidine gel produced significantly larger zones of bacterial inhibition compared to all tested concentrations of Sodium Hypochlorite, including 5.25%. Furthermore, no significant difference was observed between chlorhexidine gel and chlorhexidine solution of corresponding concentrations, suggesting that chlorhexidine, irrespective of its form, exhibits strong antibacterial potential. These findings, however, stand in contrast to the results of the present study, where Sodium Hypochlorite—particularly when activated ultrasonically—proved more effective in reducing intracanal microbial load compared to Chlorhexidine. The discrepancy may be attributed to fundamental methodological differences, as the cited study was conducted under in-vitro agar diffusion conditions, while the present study evaluated clinical microbial reduction within the complex anatomy of infected root canals. Therefore, although chlorhexidine gel shows promising antimicrobial activity in controlled laboratory settings, the present study demonstrates that Sodium Hypochlorite remains superior in practical endodontic disinfection, especially when enhanced with ultrasonic activation.[21]

While, **Karale R et al in 2016** investigated the effect of dentin on the antimicrobial action of 3% Sodium Hypochlorite, 2% Chlorhexidine, 17% EDTA and 18% etidronic acid against C. albicans found that both NaOCl and CHX were highly effective without dentin, but their efficacy decreased significantly in its presence. EDTA showed better activity with dentin and etidronic acid demonstrated similar results in both conditions. These findings are partially in accordance with the present study in confirming the antimicrobial potential of NaOCl and CHX; however, they differ in showing dentin's inhibitory effect on these irrigants. In contrast,

the current study—conducted in vivo with ultrasonic activation—found NaOCl to remain the most effective irrigant, suggesting that clinical activation techniques may overcome the limiting influence of dentin observed in vitro.[22]

A recent meta-analysis by **Ruksakiet K. et al. (2020)** reported that Chlorhexidine and Sodium Hypochlorite exhibit comparable antimicrobial efficacy, with no significant difference in bacterial reduction between the two irrigants. These results differ partially from the findings of the present study, in which Sodium Hypochlorite—particularly when ultrasonically activated—achieved superior microbial reduction compared with Chlorhexidine. This discrepancy may be attributed to the use of activation techniques, as most studies included in the meta-analysis did not employ ultrasonic agitation, which likely enhanced the effectiveness of NaOCl in our investigation.[23]

Microbial reduction was achieved through mechanical preparation, which removed infected dentin from the root canal walls, in combination with the use of irrigating solutions. Sodium hypochlorite (NaOCl), in particular, was effective in dissolving organic tissue remnants, eliminating debris from prepared canals and exerted strong antimicrobial action. Moreover, the second microbial sample (S2), taken after irrigation–activation procedures, demonstrated a significant role in further reducing the microbial load.[24,25]

Results of the present study revealed that chemomechanical preparation had great role in microbial reduction, despite the detection of positive cultures which might be due to the complexity of root

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canal systems. So it was better to use adjunctive method of irrigation agitation to increase the microbial reduction.[26]

Limitations:

The limitation of this study was that neither group was able to promote complete decontamination of the root canal system. Bacterial starvation and short exposure to irrigants could help to explain the survival of microbes like E. faecalis in the root canals submitted to treatment protocols in the present study.

Conclusion

Within the limitations of this study, the following conclusions were drawn:

1. Root canal instrumentation combined with irrigation–activation plays a crucial role in reducing microbial load. The findings of this study highlight that all evaluated irrigants exhibited measurable antimicrobial effects; however, Sodium Hypochlorite demonstrated superior bacterial reduction, especially when enhanced by ultrasonic activation.
2. None of the irrigation–activation techniques tested were able to completely eradicate all microorganisms.
3. Both Passive Ultrasonic Irrigation (PUI) and needle irrigation effectively disrupted microbial communities within the canal.

   Future in vivo studies with larger sample size should focus on evaluating clinical signs and symptoms alongside microbial reduction, considering different irrigation protocols, activation techniques and the use of intracanal medicaments before and after chemomechanical preparation.

References:

1. Hahn CL, Liewehr FR. Relationships between caries bacteria, host responses, and clinical signs and symptoms of pulpitis. Journal of Endodontics. 2007 Mar 1;33(3):213-9.
2. de Mendonça A, Soares N, Santos I, de Azevedo X, Pessoa C, Marcos S, et al Assessment of microbiota in root canals with pulp necrosis by means of Gram test A.f. J Microbiol Res. 2018;12:508–511.
3. Shahsiah S, Azizi A, Moghimipour E, Abbott PV, Karamifar K, Jafarzadeh M, et al Evaluation of tissue dissolution ability of modified chlorhexidine as a root canal irrigant Biosci Biotech Res Com. 2017;10:40–48.
4. Hoedke D, Enseleit C, Gruner D, Dommisch H, Schlafer S, Dige I, et al Effect of photodynamic therapy in combination with various irrigation protocols on an endodontic multispecies biofilm *ex vivo* Int Endod J. 2018;51:e23–e34.
5. Kapralos V, Koutroulis A, Ørstavik D, Sunde PT, Rukke HV. Antibacterial activity of endodontic sealers against planktonic bacteria and bacteria in biofilms. J Endod. 2018;44:149–154.
6. Soares JA, Carvalho MARd, Soares SMCS, Pereira RD, Brito-Júnior M, Magalhães PP, et al Pattern of disinfection of root canal dentin by alternated acid-base irrigating regimen Sci W J. 2019;2019:230–238.
7. Desai P, Himel V. Comparative safety of various intracanal irrigation systems. J Endod. 2009;35:545–549.
8. Mohammadi Z, Shalavi S, Giardino L, Palazzi F, Asgary S. Impact of ultrasonic activation on the effectiveness of sodium hypochlorite: A review Ira Endod J. 2015;10:216.
9. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. J Endod. 2003;29:654–657. doi: 10.1097/00004770-200310000-00010.
10. Iqbal A. Antimicrobial irrigants in the endodontic therapy. International Journal of Health Sciences. 2012 Jun;6(2):186.
11. Shabahang S, Torabinejad M. Effect of MTAD on Enterococcus faecalis contaminated root canals of extracted human teeth. J Endod. 2003;29:576– 579. doi: 10.1097/00004770-200309000-00008.
12. Zeng C, Meghil MM, Miller M, Gou Y, Cutler CW, Bergeron BE, et al Antimicrobial efficacy of an apical negative pressure root canal irrigation system against intracanal microorganisms. J Dent. 2018;72:71–75.

13. Siqueira Jr JF, Rôças IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. J Endod. 2000;26:331–334.

14. Cohenca N, Silva LAB, Silva RAB, Nelson-Filho P, Heilborn C, Watanabe E, et al Microbiological evaluation of different irrigation protocols on root canal disinfection in teeth with apical periodontitis: An *in vivo* study. Braz Dent J. 2013;24:467–473.

15. Neves MA, Provenzano JC, Rôças IN, Siqueira Jr JF. Clinical antibacterial effectiveness of root canal preparation with reciprocating single-instrument or continuously rotating multi-instrument systems. J Endod. 2016;42:25–29.

16. Nakamura VC, Pinheiro ET, Prado LC, Silveira A, Carvalho A, Mayer MPA, et al Effect of ultrasonic activation on the reduction of bacteria and endotoxins in root canals: A randomized clinical trial. Int Endod J. 2018;51:e12–e22.

17. Carver K, Nusstein J, Reader A, Beck M. *In vivo* antibacterial efficacy of ultrasound after hand and rotary instrumentation in human mandibular molars. J Endod. 2007;33:1038–1043

18. Townsend C, Maki J. An *in vitro* comparison of new irrigation and agitation techniques to ultrasonic agitation in removing bacteria from a simulated root canal. J Endod. 2009;35:1040–1043.

19. Hockett JL, Dommisch JK, Johnson JD, Cohenca N. Antimicrobial efficacy of two irrigation techniques in tapered and nontapered canal preparations: an *in vitro* study. J Endod. 2008;34:1374–1377.

20. Beus C, Safavi K, Stratton J, Kaufman B. Comparison of the effect of two endodontic irrigation protocols on the elimination of bacteria from root canal system: A prospective, randomized clinical trial. J Endod. 2012;38:1479–1483.

21. Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. Comparative study of the antimicrobial efficacy of chlorhexidine gel, chlorhexidine solution and sodium hypochlorite as endodontic irrigants. Brazilian Dental Journal. 2007;18:294-8.

22. Karale R, Odedra KM, Srirekha A, Champa C, Shetty A, Pushpalatha S, Sharma R. Effect of dentin on the antimicrobial efficacy of 3% sodium hypochlorite, 2% chlorhexidine, 17% ethylenediaminetetraacetic acid, and 18% etidronic acid on Candida albicans: An: in vitro: study. Journal of Conservative Dentistry and Endodontics. 2016 Sep 1;19(5):455-60.

23. Ruksakiet K, Hanák L, Farkas N, Hegyi P, Sadaeng W, Czumbel LM, Sang-Ngoen T, Garami A, Mikó A, Varga G, Lohinai Z. Antimicrobial efficacy of chlorhexidine and sodium hypochlorite in root canal disinfection: a systematic review and meta-analysis of randomized controlled trials. Journal of Endodontics. 2020 Aug 1;46(8):1032-41.

24. Aksel H, Serper A. Concentration and time-dependent effect of initial sodium hypochlorite on the ability of QMix and ethylenediaminetetraacetic acid to remove smear layer. J Conserv Dent.. 2017;20:185.

25. Zandi H, Rodrigues RC, Kristoffersen AK, Enersen M, Mdala I, Ørstavik D, et al Antibacterial effectiveness of 2 root canal irrigants in root-filled teeth with infection: a randomized clinical trial. J Endod. 2016;42:1307–1313.

26. Machado MEdL, Nabeshima CK, Caballero-Flores H, Elmadjian-Filho M, Duarte MAH, Odinola-Zapata R, et al Instrument design may influence bacterial reduction during root canal preparation. Braz Dent J. 2017;28:587–54