Journal of Research in Medical and Dental Science
2022, Volume 10, Issue 8, Page No: 266-270

Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

Evaluation of the Antibacterial Efficacy of Electrolyzed Oxidizing Water as an Irrigant against *Enterococcus faecalis* (An *In vitro* Study)

Noor A Khait*, Muna Saleem Kalaf

Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad, Iraq

ABSTRACT

Background: Disinfection of the pulp space by extirpation of the infected pulp, microorganisms and their toxins all are essential basis for successful endodontic treatment.

Objective: This study aimed to investigate the antibacterial effect of electrolyzed oxidizing water and sodium hypochlorite in lowering bacterial infectious disease of the root canal.

Materials and methods: 45 single rooted permanent human teeth were properly cleaned, shaped, and disinfected. All of the teeth samples were contaminated with Enterococcus faecalis for two weeks at 37°C. After that, the teeth were categorized into 3 groups. 15 for each of 3% sodium hypochlorite, 15 for electrolyzed oxidizing water, and 15 for normal saline as a control group. Pre-and post-irrigation samples were collected using paper points. After 24 hours, the bacterial growth was assessed. The number of bacteria colonies was then counted. The data was evaluated with SPSS and tested utilizing the One-Way ANOVA, Shapiro-Wilk test, and Dunnett's T_3 posthoc test, with a significance level of 0.05.

Results: Antibacterial effectiveness: Both sodium hypochlorite and electrolyzed oxidizing water displayed better effectiveness when compared to normal saline (P<0.05), comparatively, 3% sodium hypochlorite demonstrated the greatest efficacy against E. faecalis biofilm. Electrolyzed oxidizing water than normal saline, percentage of antibacterial effectiveness was 97. 923%, 97.018% and 31.614 respectively with significant difference between them.

Conclusions: Electrolyzed oxidizing water had comparable effectiveness against biofilm of E. faecalis to sodium hypochlorite when it was used as an endodontic irrigants solution.

Key words: Sodium hypochlorite, *E. faecalis*, Electrolyzed oxidizing water, Electrolysis

HOW TO CITE THIS ARTICLE: Noor A Khait, Muna Saleem Kalaf, Evaluation of the Antibacterial Efficacy of Electrolyzed Oxidizing Water as an Irrigant against *Enterococcus faecalis* (An *In vitro* Study), J Res Med Dent Sci, 2022, 10 (8): 266-270.

Corresponding author: Dr. Noor A Khait
E-mail: ahassann19@gmail.com
Received: 02-Jun-2022, Manuscript No. JRMDS-22-55468;
Editor assigned: 06-Jun-2022, PreQC No. JRMDS-22-55468 (PQ);
Reviewed: 20-Jun-2022, QC No. JRMDS-22-55468;
Revised: 05-Aug-2022, Manuscript No. JRMDS-22-55468 (R);
Published: 09-Aug-2022

INTRODUCTION

The main goal of chemo mechanical endodontic treatment is to remove or significantly reduce the microorganisms present in the infected root canal. The delicate morphology of the root canal space, as well as the presence of bacteria in some areas such as, dentinal tubules, ramifications, dentinal tubules and accessory canals may prevent this goal from being fully completed [1]. *Enterococcus faecalis (E. faecalis)* is gram-positive, anaerobic cocci, facultative that is the most common cause of periradicular lesions following Root Canal Treatment (RCT). *E. faecalis* can survive starvation due to physicochemical characteristics such as antibacterial resistance, biofilm formation, and the ability to invade dentinal tubules [2]. Sodium hypochlorite (NaOCl), one of the most commonly used endodontic irrigating solutions, is well-known for it has the ability to organic tissues dissolution during chemo-mechanical root canal debridement [3]. The tissue dissolving activity of NaOCl, on the other hand, is accompanied by cytotoxicity in vital tissue [4]. Because all endodontic irrigant products on the market have limitations, It is important to make an irrigant with a broad antimicrobial activity spectrum, a quick disinfection time, and good biocompatibility [5]. Electrolyzed Oxidizing Water (EOW) is generated by passing an electrical current in to aqueous solution of Sodium Chloride (NACL) in special electrolysis units. Due to its high level of Reduction Oxidation Potential (ORP) of about 1100 mV, EO water containing Hypochlorous Acid (HOCl) has a remarkable bactericidal action, as a chlorine-based sanitizer, it is comparable to NaOCl [6]. HOCl, a powerful oxidant and deproteinizer produced by neutrophils, has high microbicidal action within these cells. It interacts with a variety of biological substances such as heme proteins, thiol, carbohydrates, amino groups, and thiolether, as well as infections and pathogens infections [7]. Whereas HOCl has no electrical charge,

since the hypochlorite ion has a negative electrical charge, it repels microorganisms with negatively charged cell walls, making it less effective at destroying germs [8]. The goal of this research was to assess the effectiveness of Electrolyzed Oxidizing (EO) water versus sodium hypochlorite as an irrigant in massively reducing bacterial infection in the root canal.

MATERIALS AND METHODS

Teeth selection and preparation

Root canals of 45 single rooted permanent human teeth were used in this study. The study did not include teeth with cracks, internal resorption, external resorption, or calcification. Soft tissues, calculus, and bone were gently removed from the root surfaces using periodontal curettes. After that, the teeth were placed in 5.25% sodium hypochlorite for 60 minutes to sterilise the root surface before being stored in normal saline [9]. The crowns were dissected at the Cemento Enamel Junction (CEJ), and the teeth's root lengths were trimmed to 13-14 mm. The working length of each tooth was determined by subtracting one millimetre from the distance passed by the K-file; one millimetre from the apical foramen [10]. Pro-Taper rotary files (CICADA) were used to prepare the canals up to F3 (master apical file). The canals were irrigated with 5.25% NaOCl using a 5 ml syringe and endodontic needle in between the use of the rotary files. Following preparation, To remove the smear layer, each canal was irrigated for 3 minutes with 1 ml 17% EDTA, 5 ml of 0.9% normal saline solution, and 1 ml NaOCl, respectively, using a 30 gauge double side vented endodontic needle. Finally, 5 ml of saline solution was sprayed into each canal. After completing biomechanical instrumentation the apical foramen was sealed with composite restorative fillings and two layers of fingernail polish were applied to the root surfaces [11]. After that, the teeth were autoclaved for thirty minutes at 12°C with 15 IB in screw cup glasses [12].

Bacterial isolation: Infected root canals were used to isolate *E. faecalis.* Several samples were collected from roots that had been contaminated for a long time and suspected of harboring *E. faecalis,* the bile esculin azide test (which test that selective for isolation of *E. faecalis*) was used to identify the bacteria after 24 hours of culture when a black deposit appears on the agar plate [13]. The Vitek 2 system was then used to detect *E. faecalis* with greater accuracy [14].

Samples inoculation: Colonies of *E. faecalis* were cultured for 4 hours at 37°C in 5 mL of Brain/Heart Infusion Broth (BHI). The level of turbidity of the *E. faecalis* suspention was adjusted to 0.5 according to the McFarland standard. The bacterial suspension was then transferred using a sterile micropipette to the mechanically enlarged lumen of the root canals. The teeth were then placed in brain heart infusion broth and cultured at 37°C for two weeks, as this time frame could allow for the formation of a well-known and significant bacterial colony [15].

Irrigants antibacterial efficacy: Following the incubation period, all teeth sample removed carefully from the tubes in aseptic condition and washed with sterile saline solution to remove non adherent bacteria and culture medium. Following that, a sterile saline was injected into each specimen; the sterile normal saline solution serves as a bacterial colony transport medium from the root canal to the blood agar plate in this experiment. A premedication sample (S_1) was achieved by introducing a sterilized #25 paper point into root canals for 60 seconds then The teeth were separated into three experimental groups at random (n=15 for each) [16].

Irrigation procedure:

Group I: 15 teeth will be treated with copious irrigation with 3% sodium hypochlorite Irrigation with normal saline was then performed.

Group II: 15 teeth will be treated with copious irrigation of electrolyzed oxidizing water solution (contain 200 ppm available free chlorine in form of hypochlorous acid) followed by irrigation with normal saline. Electrolyzed water was prepared by the electrolysis of pure water containing 2 grams of NACL in electrolyzing device.

Group III (control): 15 teeth will be treated with copious irrigation of 0.9% normal saline.

For each sample, the irrigation time will be 5 minutes. Each solution will be delivered into the canal lumen using sterile 5 ml plastic syringes and endodontic needles. All irrigation procedures will be performed by the same operator at room temperature and under aseptic conditions [5]. Then, using sterile paper points, post irrigation sampling was obtained (S_2) In Eppendorf tubes containing 1 ml of normal saline, both the before and after samples were vortexed for 30 seconds [16]. Following that, repeated dilution was carried out. Each dilution was then pipetted and grown on blood agar plates for 24 hours at 37°C. The Colony Forming Units (CFU) were counted and the CFU/ml was computed (Figure 1) [1].

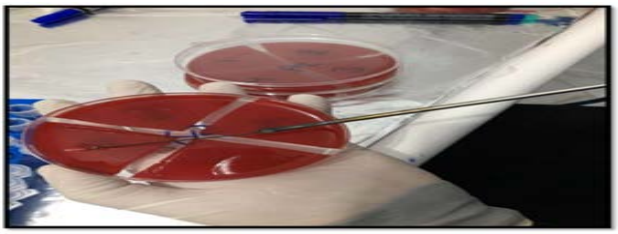

Figure 1: 10 µl of each dilution spread on a blood agar.

RESULTS

Table 1 show that all three approaches reduced the means of the post-samples of bacterial colonies, with the NAOCL group having the biggest effect size, followed by the EOW group, and the normal saline group having the smallest effect size. Meanwhile, the efficacy of each sample was determined using the Dunavant, et al. equation.

Table 1: Descriptive and statistical test of bacterial count change by groups.

Paired Samples Statistics										
Groups	Post irrigation (CFU\ml)			Pre irrigation (CFU\ml)						
	Mean	± SD	± SE	Mean	± SD	± SE	% of change	Paired t test	P value	Effect size
Saline	25740	4667.64	1205.18	37780	2850.61	736.025	31.614	9.113	0.000 Sig.	2.353
NAOCL	780	265.115	68.452	37826.7	2847.92	735.331	97.923	49.928	0.000 Sig.	12.891
HOCL	1113.33	350.238	90.431	37906.7	2834.35	731.825	97.018	47.525	0.000 Sig.	12.271

Sodium hypochlorite had the highest mean percentile of antibacterial efficiency, followed by Electrolyzed oxidizing water and normal saline. Normal saline revealed the lowest percentage (Table 2). As indicated in Table 3, there was a significant difference in antibacterial efficacy between the three groups (p=0.000).

Table 2: Bacterial count descriptive statistics among groups.

		N	Mean	± SD	± SE	Minimum	Maximum
Pre Irrigation (CFU \ml)	Saline	15	37780	2850.614	736.025	32200	41500
	NAOCL	15	37826.67	2847.923	735.331	32200	41500
	HOCL	15	37906.67	2834.347	731.825	32500	42000
Post irrigation (CFU\ml)	Saline	15	25740	4667.639	1205.179	12100	31000
	NAOCL	15	780	265.115	68.452	400	1300
	HOCL	15	1113.333	350.238	90.431	400	1900

Table 3: Statistical test of bacterial count among groups using One-Way Analysis Of Variance (ANOVA).

ANOVA						
		Sum of Squares	Df	Mean Square	F	P value
Pre Irrigation (CFU \ml)	Between Groups	123111.1	2	61555.56	0.008	0.992 NS
	Within Groups	3.4E+08	42	8090063		
	Total	3.4E+08	44			
Post irrigation (CFU \ml)	Between Groups	6.15E+09	2	3.07E+09	419.562	0.000 Sig.
	Within Groups	3.08E+08	42	7326603		
	Total	6.46E+09	44			
Levene test=14.175, p value=0.000 Sig.						

The multiple comparisons between the groups, on the other hand, by Dunnett's T_3 posthoc test revealed that there was a significant difference between each irrigant with other That largest bacterial count found in normal saline followed by HOCL while the lowest was NAOCL with significant distinction between them (Table 4).

Table 4: A comparison of the antibacterial efficacy of the irrigation groups.

Dunnett's T_3 posthoc test was used to compare the post-irrigation bacterial count (CFUml) between groups.

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	p value	
Bacterial count (CFU\ml)	Saline	NAOCL	24960	0	Sig.
		HOCL	24626.7	0	
	NAOCL	HOCL	-333.33	0.02005	

DISCUSSION

Successful root canal treatment depend primarily on complete removal of both necrotic and vital pulp tissue, as well as microorganisms and their toxins, from the root canal space [18]. *Enterococcus faecalis* is a common oral cavity bacterium. Its presence is thought to be more common in patients undergoing initial root canal treatment and retreatment than in those who do not have a root canal problem. The presence of *E. faecalis* is lesser in primary endodontic infections (4-40%) and greater in persistent infections (24-77%) [19]. Since *E. faecalis* has unique features, it can avoid chemomechanical instrumentation during root endodontic therapy. The following are some of these characteristics: ability to colonize in peripheral, inaccessible locations away from the central canals, such as apical deltas, accessory canals, and isthmuses, and forming biofilms and the ability to be guarded by dentinal tissues, residual tissue, dead cells and human serum, which reduces the effectiveness of antibacterial agents Furthermore, to survive in harsh environments, *E. faecalis* employs a variety of mechanisms. These mechanisms include the activation of some survival genes, the use of alternative metabolic pathways, living in a nutrient-rich environment, and having bacterial synergism and aggregation capacity [20]. The most commonly used endodontic irrigant is sodium hypochlorite owing to its antimicrobial and tissue dissolving properties However, NaOCl has many drawbacks, including an objectionable odor and bad taste, extreme corrosiveness to metals and high toxicity. Furthermore, it was demonstrated that its clinical performance is lower to its *in vitro* effects [21]. There is still a need for a treatment protocol that offers an irrigation solution alternative to NaOCl that provides the same benefits as NaOCl while trying to overcome the drawbacks of storage hazard and toxic effects caused by Sodium hypochlorite extrusion outside the tooth apex. It is also necessary to use root canal irrigants that a more biologically accepted. Such as, electrolyzed oxidizing water [22]. Many of the demands for an ideal disinfectant are met by Electrolyzed oxidizing water, including activity against a broad spectrum of pathogens (bactericidal, viricidal, and fungicidal, effects). It also has a low operational cost since many of the clinic's commercially available units can generate it using only salt and electricity [23]. The principle of electrochemically activated water is to transfer liquids into a metastable state using an element or reactor, "Flow-through Electrolytic Module" and an electrochemical unipolar (cathode or anode) action. Changes water and low mineral solutions into a metastable state with changed physical-chemical properties, most notably pH and oxidation reduction potential. These variables appear to shift and change spontaneously following electrochemical activation. When it comes into contact with vital biological tissues, it is nontoxic [24]. The results of this investigation revealed that 3% NaOCl has a high antibacterial efficiency, with a mean percentage of bacterial eradication of 97.924% followed by electrolyzed oxidizing water (97.018%) with significant difference between them. Normal saline, on the other hand, had the least antibacterial action (31.614%). The activity of electrolyzed oxidizing water in the elimination of *E. faecalis* observed in the present study were close to those obtained with the use of NaOCl. The findings of this study showed that EOW have antibacterial activity against *E. faecalis*, which was consistent with previous findings of S lala and pratik in 2016 that bacteriological studies for colony forming unit show that hydroxyl ions in electrolyzed oxidizing water decrease the biofilm formed by *E. faecalis* in contaminated tooth models [5]. Another study published in 2010 by Rossi-fedele, et al. concluded that both Electrolyzed oxidizing water and sodium hypochlorite had relatively similar anti-bacterial efficiency against *Enterococcus faecalis* [8]. These findings were also consistent with the findings of Garcia, et al. in 2018, who discovered that Electrolyzed oxidizing water effectively clean the root canal surfaces while also opening the dentinal tubules and eliminates the smear layer [25]. The difference in results across the studies could be due to differences in technique, variation in the strains of the studied microorganism, and the concentrations or regimens of the irrigation solutions employed, or it could be due to variances in electrical units used to produce electrolyzed oxidizing water.

CONCLUSION

Electrolyzed oxidizing water was as active agent against *E. faecalis* as 3% NaOCl, and as a result, it can be used as an alternative to NaOCl. More *in vitro* and *in vivo* research are needed to confirm the use of electrolyzed oxidizing water as an irrigant solution against *E. faecalis*.

ACKNOWLEDGEMENTS

I would like to express my thanks to all the staff in Department of Microbiology College of Medicine University of Kufa, Special thanks to Dr. Bashar Al Mulla, who provided me with Electrolyzed oxidzing water apparatus.

REFERENCE

1. Tennert C, Fuhrmann M, Wittmer A, et al. New bacterial composition in primary and persistent/ secondary endodontic infections with respect to clinical and radiographic findings. J Endod 2014; 40:670–677.
2. Mahmoudpour A, Rahimi S, Sina M, et al. Isolation and identification of *Enterococcus faecalis* from necrotic root canals using multiplex PCR. J Oral Sci 2007; 49:221–227.
3. Prabjot K. Role of Irrigants in Endodontics. J Dent Probl Solut 2020; 7:100–104.
4. Vouzara T, Koulaouzidou E, Ziouti F, et al. Combined and independent cytotoxicity of sodium hypochlorite, ethylene di amine tetra acetic acid and chlorhexidine. Int Endod J 2016; 49:764–773.
5. Lata S, Mohanty SK, Pradhan PK, et al. Anti-bacterial effectiveness of Electro-Chemically Activated (ECA) water as a root canal irrigant-an *in-vitro*

comparative study. J Clin Diagnostic Res 2016; 10:138–142.

6. Rahman SME, Khan I, Oh D. Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. Compr Rev Food Sci Food Saf 2016; 15:471–490.
7. Eryilmaz M, Palabiyik IM. Hypochlorous acid-Analytical methods and antimicrobial activity. Trop J Pharm Res 2013; 12:123–126.
8. Garcia F, Murray PE, Garcia-godoy F, et al. Effect of aquatine endo cleanser on smear layer removal in root canals of *ex vivo* human teeth. J Appl Oral 2018; 18:403–408.
9. Nourzadeh M, Amini A, Fakoor F, et al. Comparative antimicrobial efficacy of Eucalyptus Galbie and *Myrtus communis* L. Extracts, chlorhexidine and sodium hypochlorite against *Enterococcus Faecalis.* Iran Endod J 2017; 12:205–210.
10. Sahebi S, Khosravifar N, Sedigh Shamsi M, et al. Comparison of the antibacterial effect of Sodium Hypochlorite and Aloe Vera Solutions as Root Canal Irrigants in Human Extracted Teeth Contaminated with *Enterococcus Faecalis.* Dent J 2014; 15:39–43.
11. Andriukaitiene L, Song X, Yang N, et al. The effect of smear layer removal on *E. faecalis* leakage and bond strength of four resin-based root canal sealers. BMC Oral Health 2018; 18:1–9.
12. Mir MA, Ashraf MW, Tripathi V, et al. Isolation, characterization and prevention of various microbial strains in NIC unit and PIC unit. Sci Rep 2021; 11.
13. Garcia-Garrote, Fernando, Emilia Cercenado, et al. Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of enterococci. J Clin Microbiol 2000; 38:2108-2111.
14. Sohrabi K, Sooratgar A, Zolfagharnasab K, et al. Antibacterial activity of diode laser and sodium hypochlorite in *enterococcus faecalis*-contaminated root canals. Iran Endod J. 2016; 11:8–12.
16. Alabdulmohsen Z, Saad A. Antibacterial effect of silver nanoparticles against *Enterococcus faecalis*. Saudi Endod J 2017; 7:29–35.
17. Buraihi MM, Alkurtas SA. The Photothermal Effect of 940 nm Diode Laser on *Enterococcus Faecalis* Biofilm in Infected Root Canal.
18. Gomes BPF de A, Herrera DR. Etiologic role of root canal infection in apical periodontitis and its relationship with clinical symptomatology. Braz Oral Res 2018; 32:82–110.
19. Kim, Sang-Wan, and Dong-Hoon Shin. Antibacterial effect of urushiol on *E. faecalis* as a root canal irrigant. Restor Dent Endod 2017; 42:54-59.
20. Siqueira JF, Rocac IN. Critical review in oral biology and Medicine: Diversity of endodontic microbiota revisited. J Dent Res 2009; 88:969–981.
21. Abuhaimed TS, Neel EAA. Sodium Hypochlorite Irrigation and Its Effect on Bond Strength to Dentin. Biomed Res Int 2017.
22. Dube, Kavita, Jain P. Electrolyzed saline… An alternative to sodium hypochlorite for root canal irrigation. Clujul Med 2018; 91:322–327.
23. Farah RF, Al-Haj Ali SN. Electrolyzed water generated on-site as a promising disinfectant in the dental office during the COVID-19 pandemic. Front Public Health 2021; 9:629142.
24. Solovyeva AM, Dummer PMH. Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: A pilot study. Int Endod J 2000; 33:494–504.
25. Rossi-Fedele G, de Figueiredo JAP, Steier L, et al. Evaluation of the antimicrobial effect of super-oxidized water (sterilo) and sodium hypochlorite against enterococcus faecalis in a bovine root canal model. J Appl Oral Sci 2010; 18:498–502.