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Original Article

Antibacterial Efficacy of Sodium Hypochlorite at Different Temperatures Against Enterococcus Faecalis in Single Rooted Teeth (A Comparative In-Vitro Study)

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Abstract

Aim: This in vitro study aimed to evaluate and compare the antibacterial efficacy of intracanal heated sodium hypochlorite, cryo-treated sodium hypochlorite and sodium hypochlorite at room temperature with and without passive ultrasonic activation against *E. faecalis* in single rooted teeth.

Subjects and methods: forty-two extracted human single-rooted teeth were prepared by Protaper next rotary system. The teeth were sterilized and inoculated with *E. faecalis* bacteria for 2 weeks and divided randomly into six groups according to the final irrigation protocol. Group I1, cryotreated NaOCl at 2°C - 4°C, group I2, cryotreated NaOCl with ultrasonic activation, group I3, intracanal heated NaOCl at 180°C by fast pack, group I4, intracanal heated NaOCl with ultrasonic activation, group I5, NaOCl at room temperature with ultrasonic activation, and a control group, NaOCl at room temperature. First sample was taken from each canal before the tested protocols. Second sample was taken after implementing the irrigation protocol using paper points. The number of colonies forming units per milliliter was calculated on the agar medium using unaided eye. Significance level was set at $P \leq 0.05$.

Results: Intracanal heated NaOCl with ultrasonic activation showed the highest bacterial reduction, followed by cryotreated NaOCl with ultrasonic activation, intracanal heated NaOCl, cryotreated NaOCl, and NaOCl at room temperature with ultrasonic activation with no significant difference between them ($p > 0.05$). NaOCl at room temperature showed significantly the least bacterial reduction than all other groups.

Conclusion: Intracanal heated NaOCl at 180°C with passive ultrasonic activation showed the highest post-irrigation bacterial reduction.

Keywords: Irrigation, Intracanal heated, Cryotreated, Passive ultrasonic activation.

I. INTRODUCTION

For endodontic therapy to be successful, the root canal system must be completely cleaned and disinfected [1)]. The goal of root canal treatment is to get rid of microorganisms and their waste products and stop the spread of infection [2)]. Cleaning is challenging in canal system with an isthmus, oval canals, and curved canals, so chemomechanical approaches are used in

conjunction with one another to improve cleaning efficacy in infected root canals [3].

Enterococcus faecalis is the most prevalent bacterial species in resistant or recurrent infections because it gets into deep dentinal layers, which are hard to be cleaned and disinfected that ultimately results in the failure of endodontic therapy [4].

Sodium hypochlorite (NaOCl) is the most widely used irrigant in endodontics owing to its superior antibacterial properties and tissue dissolving action, on the other hand, it has a low penetration depth into the dentinal tubules due to its high surface tension[(5)]. It has been discovered that the concentration, duration of contact, volume, and temperature all have an impact on its antibacterial efficacy [(6)].

Several investigations observed that elevating the temperature of NaOCl through intracanal heating utilizing Elements System B heat source at 180°C enhances its ability to dissolve tissue faster, as well as its ability to remove organic material and eliminate bacteria [(7,8)].

Passive ultrasonic activation (PUA) is used in endodontics as it enhances the material exchange in the canal, hence enhancing the antimicrobial and tissue disintegrating effects of NaOCl and eliminating dentin debris and smear layer[(5)].

Cryotherapy has been utilized for its efficacy in post-operative pain management. Cryotreated NaOCl for final irrigation was observed to be efficient in lowering postoperative pain, edema, and analgesics intake [910)]. Only one in vivo study has evaluated its effect on bacteria and showed that using NaOCl at various temperatures (2ºC, 25ºC, and 45ºC) as a final irrigant had comparable antimicrobial effect, on the other hand, using cold NaOCl (2˚C) is superior to NaOCl 45˚C in case of pain levels after treatment, but no in vitro studies has been found [10)]**.** So, the aim of this study was to assess the antibacterial efficacy against *E. faecalis* between intracanal heated cryotreated NaOCl and NaOCl at room temperature with and without PUA in teeth with single root canals.

II. SUBJECTS AND METHODS

Selection of the sample

Freshly extracted human single-rooted teeth with a single canal advised for extraction owing to orthodontic or periodontal disorders were collected with inclusion criteria: Permanent teeth with single root, straight canal and completely formed root. Sample size was calculated using the (PS software). As regarding the primary outcome (bacterial reduction), we found that 5 teeth per group will be appropriate sample size for the study with total sample size 30 teeth "the power is 80% and αerror probability =0.05", the effect size = 0.735. 30 teeth increased to 42 teeth to compensate for missing anticipated data 30%, the magnitude of the effect to be detected was estimated as mean and standard deviation of the variable of interest and obtained from the scientific literature[4)].

Sample preparation

First, the teeth external root surfaces were cleaned with a curette and soaked in 5.25% NaOCl for 30 minutes to remove soft tissue debris. Crowns of all teeth were decoronated using a high-speed hand piece at the level of cemento-enamel junction in addition to standardizing the root lengths at 15 mm. #10 hand file (MANI, Japan) was used to establish patency, the working length (WL) was determined. Root canal preparation was done using Protaper Next rotary system (Dentsply Maillefer, Switzerland) till size X4. After each instrument, 2 ml of 5.25% NaOCl solution was used to irrigate canals using side vented needle (Fanta, china), and 3 ml of 17% EDTA for 1 min as a final irrigant. The apical foramen was sealed using a Glass Ionomer and the external surfaces were covered with colorless nail varnish to prevent liquid penetration[4)].

For sterilization process, all teeth were sterilized in autoclave at 121°C for 30 minutes. *E. faecalis* (ATCC 29212) strain was cultured aerobically on Brain heart agar at 35°C for 2 days, the turbidity was adjusted to 0.5 McFarland. Twenty microns of bacteria were inoculated into each root canal and transferred to tube filled with BHI and was kept at 37°C for 14 days [4].

Randomization and Sequence generation

A computer random sequence generator application performed random allocation and sequence generation using (https//: www.random.org). Where the samples (n= 42) were randomly distributed into six groups with 7 samples in each group (n= 7): I1: cryotreated NaOCl, I2: cryotreated NaOCl with (PUA), I3: intracanal heated NaOCl, I4: intracanal heated NaOCl with PUA, I5, NaOCl at room temperature with PUA, and as control group NaOCl at room temperature.

Allocation concealment:

To avoid selection bias, the assigned sequence was safeguarded and disguised until the assignment by utilizing sequentially numbered opaque sealed containers where the teeth were placed.

Implementation:

The Co-supervisor performed sequence generation and allocation concealment.

Blinding:

The person evaluating the outcomes and the statistician performing the analysis were blinded.

Intervention for root canal disinfection

The concept is to standardize the total time and the volume the irrigant touchs the canal walls for all groups to be 6ml for 3 mins.

Intervention group 1(I 1): Cryotreated NaOCl

After removing the irrigation syringes from the refrigerator, they were put in an ice tank and utilized with a thermometer put inside to ensure the 2°C-4°C temperature range. Irrigation with 5.25% cryotreated NaOCl using side vented needle was performed. The needle was placed 1 ml less than the WL with up and down movement with a flow rate 2ml/1min. This step was repeated for 3 cycles with a fresh solution (2ml/cycle/1min).

Intervention group 2 (I 2): Cryotreated NaOCl with PUA

The same steps were done as I1 but with a flow rate 2ml/40 sec and NaOCl was activated within the time of the cycle for 20s per cycle [(11)], by using a woodpecker ultrasonic system (woodpecker, china) with tip E12 (Ufile size 25) 1 mm shorter than the WL. This step was repeated for 3 cycles with a fresh solution.

Intervention group3 (I 3): Intracanal heated NaOCl

The canal was filled with 1ml 5.25 % NaOCl at room temperature. Using fast pack heat carrier (woodpecker, China) at 180°C, the tip was inserted 3ml less than the working length and was activated to heat the irrigant inside the canal for 10 s with small, in- and out- movements [11)], and then inactivated and the irrigant was left inside the canal for 20s [(8)] (figure 1). This step was repeated for 6 cycles (1ml/ cycle/30s) with a fresh solution.

Intervention group 4 (I 4): Intracanal heated NaOCl with PUA

The canal was filled with 1ml 5.25% NaOCl at room temperature. Using fast pack heat carrier at 180°C, the tip was activated to heat the irrigant inside the canal for 10 s with small, in- and out- movements [11)], and then inactivated and NaOCl was activated within the time of the cycle for 20s per cycle by using a woodpecker ultrasonic system. This step was repeated for 6 cycles with a fresh solution (1ml/ cycle/30s).

Intervention group 5 (I 5): NaOCl at room temperature with PUA

Irrigation with 5.25% NaOCl at room temperature using side vented needle was performed with up and down movement with a flow rate 2ml/40 sec and NaOCl was activated within the time of the cycle for 20s per cycle, by using a woodpecker ultrasonic system. This step was repeated for 3 cycles with a fresh solution (2ml/ cycle/1min).

Control group: NaOCl at room temperature:

The same steps were done as I5 but with a flow rate 2ml/1min. This step was repeated for 3 cycles with a fresh solution.

Root canal sampling procedures

It was determined using culture technique. A first sample (S1) was collected from every canal before the final irrigation protocols. Bacterial biofilm on the canal walls was loosened by H-file and collected using paper points, then put in a test tube filled with 1 ml of BHI broth. Fifty microliters of the samples after dilution were placed over BHI agar plates and cultured aseptically, then incubated at 37°C for 1 day [12] (figure 5). The number of CFUs/ml was counted on the agar medium using unaided eye [13,14]**.** The number of CFUs/ml of second sample (S2) after implementing the final irrigation was calculated as described for S1. % of bacterial reduction was calculated according to the equation (pre-)_ (post-) irrigation bacterial count/(pre-) x 100.

Figure 1: Fast pack heat carrier device

III. RESULTS

Statistical analysis:

The mean and standard deviation values were calculated for each group. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two groups in non-related samples. Paired sample t-test was used to compare between two groups in related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

There was a statistically significant difference between (S1) and (S2) groups in all groups where ($p<0.001$). The highest mean value was found in (S1) groups, while the lowest mean value was found in (S2) groups.

Relation between groups according to Postoperative bacterial count (S2):

The highest mean value was found in control group, while the least mean value was found in (I4) (figure 2) (Table 1).

One-way ANOVA revealed a statistically significant difference between groups (I1), (I2), (I3), (I4), (I5) and control group where ($p<0.001$). Tukey post hoc test revealed that I4; Intracanal heated NaOCl at 180°C with PUA showed the least post irrigation bacterial count followed by I2; cryotreated NaOCl at 2°-4°C with PUA, I3; Intracanal heated NaOCl at 180°C, I1; Cryotreated NaOCl at 2°-4°C, I5; NaOCl at room temperature with PUA with no significant difference between them ($p>0.05$). Control group: NaOCl at room temperature showed significantly higher post-irrigation bacterial count than all other groups ($p<0.001$).

Percentage of bacterial reduction:

The highest percentage of bacterial reduction was found in (I4), while the least

percentage of bacterial reduction was found in control (table 2).

One-way ANOVA revealed a statistically significant difference between groups (I1), (I2), (I3), (I4), (I5) and control where ($p<0.001$). Tukey post hoc test revealed that I4; Intracanal heated NaOCl at 180°C with PUA showed the highest bacterial percentage reduction followed by I2; cryotreated NaOCl at 2°- 4°C with PUI, I3; Intracanal heated NaOCl at 180°C, I1; Cryotreated NaOCl at 2°- 4°C, I5; NaOCl at room temperature with PUA with no significant difference between them ($p>0.05$) (figure 3). Control group: NaOCl at room temperature showed significantly lower bacterial percentage reduction than all other groups ($p<0.001$).

Table (1): The mean, standard deviation (SD) values of bacterial counts.

	S1		S2		P value
	Mean	SD	Mean	SD	
I1	**1000**	**0**	**3.43**	**0.69**	**< 0.001***
I2	**1000**	**0**	**1.86**	**0.51**	**< 0.001***
I3	**1000**	**0**	**2.43**	**0.48**	**< 0.001***
I4	**1000**	**0**	**1.57**	**0.43**	**< 0.001***
I5	**1000**	**0**	**5.71**	**1.34**	**< 0.001***
Control	**1000**	**0**	**12.86**	**2.50**	**< 0.001***
p-value	_			**<0.001***	

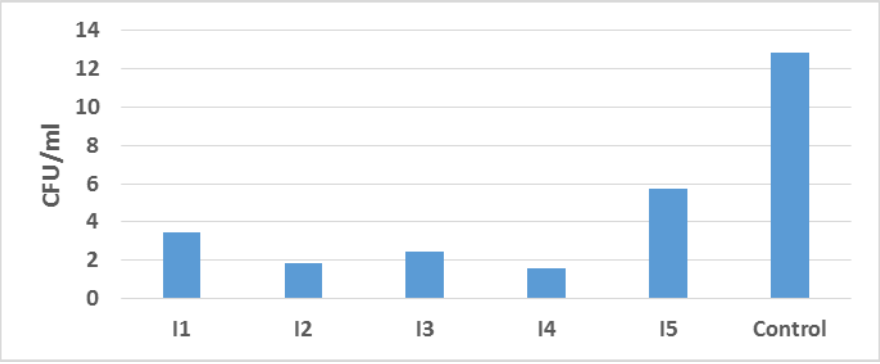

Figure 2: Bar chart representing the mean post-irrigation bacterial count.

Table (2): The mean, standard deviation (SD) values of percentage of bacterial reduction

	Mean	SD	Min	Max
I1	**99.66**	**0.07**	**99.40**	**99.90**
I2	**99.81**	**0.05**	**99.60**	**100.00**
I3	**99.76**	**0.05**	**99.60**	**100.00**
I4	**99.84**	**0.04**	**99.70**	**100.00**
I5	**99.43**	**0.13**	**98.90**	**99.80**
Control	**98.71**	**0.25**	**98.00**	**99.40**

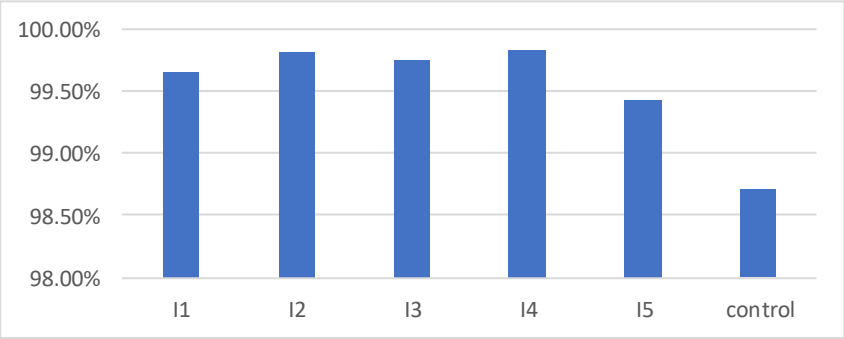

Figure 3: Bar chart representing percentage of bacterial reduction groups

IV. DISCUSSION

NaOCl is the commonly used irrigant in endodontic treatment. It has the ability to dissolve organic tissue, has lubricating and antimicrobial properties [15,16].

In this study we used intracanal heated NaOCl rather than preheated NaOCl outside the canal because when NaOCl was preheated to 50°C - 70°C, it stabilised quickly to reach body temperature and the apical third, was unable to reach 40°c at any time. Higher temperature can be maintained for 57 seconds with the intracanal approach using a heat carrier [7,8]. We used intracanal heated NaOCl at 180°C in accordance with previous studies which used system B (Sybron endo) for heating[8,174]. we used Fast Pack (woodpecker, china) heat carrier, which serves a similar purpose as system B according to the manufacturer. The health of the PDL is deemed to be in danger when the outside root surface temperature exceeds 47°C for more than one minute, however the root surface temperature did not rise over 47°C, the temperature which might affect the periodontal tissues, when using the System B that was adjusted to 150°C [1811,18] , so in this study the irrigant was kept inside the canal not more than 30 seconds then refreshed.

In the present study we used NaOCl at 2°C–4°C as it was previously hypothesized that temperature drop would have an anti-

inflammatory action in the periradicular tissues and decrease postoperative pain [19]. Two studies found that cryotreated NaOCl reduced postoperative pain, and edema with significantly fewer analgesics intake [9,10]. Cryotreated NaOCl showed a good result in reduction of the amount of *E. faecalis* [10]. That's why we used in this study NaOCl at 2°C–4°C to assess its antimicrobial effect.

Only one study used cold NaOCl in comparison to extra canal heated and room temperature NaOCl for testing their antibacterial property and postoperative pain in teeth with asymptomatic apical periodontitis, they found that cold NaOCl and preheated NaOCl decreased the bacterial count but they had no significance difference in antibacterial efficacy while preheated NaOCl increased postoperative pain values [10].

In this study NaOCl was activated as PUA enhance the efficacy of disinfection of chemical irrigants and help in decreasing the bacterial count [20]**.** By using a woodpecker ultrasonic device with tip E12 (U file size 25) to be passive inside the canal, the irrigant was activated for 20s per cycle [11].

Our results showed that intracanal heated NaOCl at 180°C with PUA showed the highest anti-bacterial effect followed by NaOCl at 2°C - 4°C with PUA group with no significant difference between them. According to **Landolo et al.**[21], ultrasonic activation of intracanal heated NaOCl greatly increased NaOCl penetration into the dentinal tubules and root canal cleaning. After them came intracanal heated NaOCl at 180°c, NaOCl at 2°C - 4°C, and NaOCl at room temperature with PUA groups with no significant difference between them. This is similar to the findings of **Yared & Al Asmar** [11] as they found that the percentage of bacterial count eliminated did not vary significantly among group of heating and heating with passive activation, that means that bacterial reductions are to be attributed to rise of temperature of the irrigant. These results are also similar to **KarataŞ et al**, [10] who found that the bacterial count decreased after irrigation with heated & cold NaOCl with no significant difference.

There was a significant difference between NaOCl at room temperature without PUA and all other groups as it showed the lowest anti-bacterial effect. These results are also similar to some studies that concluded that 180°C intracanal heated NaOCl was more effective at creating clean canal walls and significantly reduced bacteria as *E. faecalis* in comparison to room temperature irrigation[4,817,22]**.**

The present study observed that activation of 5.25% NaOCl intracanal heated and cryotrated NaOCl has a superior efficiency in elimination of infection, so intracanal heated and cryotreated NaOCl could be considered as an alternative to NaOCl at room temperature. According to **KarataŞ et al**,[10] and present study results as a clinically relevant conclusion that the use of cryotreated NaOCl can have positive clinical outcomes regarding bacterial reduction, postoperative pain, analgesic intake compared to heated NaOCl. From the limitation of this study, the protocol was done in single, straight, and oval root canals only and on extracted teeth.

V. CONCLUSION

1) Intracanal heated NaOCl at 180°C with PUA showed the highest bacterial reduction.

2) NaOCl at room temperature showed significantly the least post-irrigation bacterial reduction than all other groups.

Conflict of Interest:

The authors declare no conflict of interest.

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Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry-Cairo university on: 29/3/2022 ……, approval number:19/3/2022

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