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Original Article

Bacterial Count following Photoactivated Oral Disinfection versus Sodium Hypochlorite Solution on Root Canal Bacteria: An Invitro Study

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Abstract

Aim: To determine bacterial count and inhibition zone following photoactivated oral disinfection versus sodium hypochlorite solution on root canal bacteria. **Methodology:** This invitro study was conducted at Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Cairo University and Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University. Bacterial count and zone of inhibition were performed on Enterococcus faecalis and Streptococcus intermedius. A total number of 36 samples were divided into three main groups. The first group was photoactivated oral disinfection, second group was photoactivated oral disinfection with sodium hypochlorite and finally control group was sodium hypochlorite. All these groups were tested for both bacterial count and inhibition zone. **Results:** Regarding Enterococcus faecalis and Streptococcus intermedius count, the highest value was in photoactivated oral disinfection, followed by photoactivated oral disinfection with sodium hypochlorite, whereas the lowest value was in sodium hypochlorite in 24 and 48 hours. Regarding Enterococcus faecalis and Streptococcus intermedius inhibition zones, the highest value was in sodium hypochlorite, followed by photoactivated oral disinfection with sodium hypochlorite, while the lowest value was in photoactivated oral disinfection in 24 hours. **Conclusions:** Sodium hypochlorite may be the best available irrigant owing to its wide antibacterial spectrum followed by photoactivated oral disinfection with sodium hypochlorite, whereas the least effective was photoactivated oral disinfection.

Keywords: Photoactivated oral disinfection, sodium hypochlorite, root canal bacteria, bacterial count, inhibition zone

INTRODUCTION

Root canal treatment is important to save the teeth after pathological exposure due to dental caries. There are two scenarios unavoidably influenced by the caries micro-organisms, commensal bacteria, or opportunistic bacteria, first of all pulpal infection and/or infection following root canal treatment ***(Le Goff et al., 1997)***.

The severity of pulpal and periapical inflammation, infection and symptoms is determined by the number of bacterial cells in the root canal system, their virulence, and host responses. Bacterial toxins, hydrolytic enzymes, and several cell surface proteins and carbohydrates are examples of bacterial virulence factors that protect microbial attachment leading to bacterial biofilms ***(Casadevall and Pirofski, 2009)***. Thus, the primary goal of root canal treatment is to decrease the number of bacteria and bacterial biofilms and make the root canal system less conducive to bacterial growth.

Enterococcus faecalis (*E. faecalis*) is the most common micro-organism in post-treatment infections. *E. faecalis* is a facultatively anaerobic gram-positive cocci. It has the ability to invade human dentinal tubules ***(Love and Jenkinson, 2002***), adhere to dentine ***(Brändle et al., 2008)*** and high resistance to alkaline stress ***(Weckwerth et al., 2013)***.

Streptococcus intermedius (*S. intermedius)* is a type of oral bacteria that penetrates dentinal tubules both individually and in co-aggregates. Their survival in the root canal is due to their adaptive response to extreme environmental change, as well as its attachment and co-aggregation mechanism. Such organisms are shown to be resistant to root canal therapy ***(Pinheiro et al., 2003; Narayanan and Vaishnavi, 2010).***

In endodontics, sodium hypochlorite (NaOCl) is the most often used gold standard irrigation. It has several favorable traits and characteristics. NaOCl has antibacterial properties, aids in the breakdown of organic waste, and improves minor lubrication ***(Barnard et al., 1996)***. The endodontic system cannot be completely cleaned with NaOCl on its own ***(Ayhan et al., 1999)***. When used as an endodontic irrigant, NaOCl transforms into a powerful antibacterial with the ability to dissolve tissue. However, the cytotoxic action of NaOCl increases with its concentration (Shih et al., 1970; Soukos et al., 2006). The optimal concentration of NaOCl is still debatable. However, the effective concentration ranges from 2.25 % to 5.25% ***(Chaugule et al., 2015)***.

Sodium hypochlorite accident occurs when sodium hypochlorite comes into direct contact with the periapical tissues (soft tissue surrounding the root apex of teeth), eyes, or extraoral tissues. We must suspect a sodium hypochlorite accident if the patient experiences sudden severe pain and/ or swelling during the root canal procedure ***(Janani et al., 2017)***.

Photoactivated Oral Disinfection (PAD) selectively eliminates bacteria and has no harm or damage and toxicity on surrounding tissue, it does not encourage the development of resistant strains. PAD has the benefit of not causing bacterial resistance to reactive oxygen or free radicals, which is a disadvantage of the antibiotics ***(Schlafer et al., 2010).***

Such controversy about the root canal irrigants encourages us to assess the bacterial count and inhibition zone following PAD versus NaOCl solution on root canal bacteria.

MATERIALS AND METHODS

Study setting:

This study was conducted in Pediatric Dentistry and Dental Public Health Department Faculty of Dentistry, Cairo University as the photoactivated oral disinfection device is present in the department and Medical Microbiology & Immunology Laboratory, Medical Microbiology & Immunology Department, Faculty of Medicine, Cairo University for all the microbiological procedures.

Research ethics approval:

This research was approved by the Research Ethics Committee, Faculty of Dentistry – Cairo University with approval number 1-10-20.

PICO:

P Root canal bacteria (*Enterococcus faecalis and Streptococcus intermedius*)

I_1 Photoactivated Oral Disinfection

I_2 Photoactivated Oral Disinfection with Sodium hypochlorite solution

C Sodium hypochlorite solution

O Outcomes are shown in table (1)

Table (1): showing the outcome measured in the study.

Prioritization of Outcome	Outcome	Method of Measurement	Unit of Measurement
Primary outcome	Bacterial Count ***(Balakrishna et al., 2017)***	Count	CFU
Secondary outcome	Inhibition zone ***(Wassef and Fouad, 2019)***	Ruler (Diameter)	Mm

Sample size calculation:

Sample Size Calculation was 36 samples (6 for each group) was estimated using G Power 3.1.9.4 program according to the results of Balakrishna et al., 2017 (with α set at 0.05 & power set at 0.8).

Photoactivated oral disinfection device:

The device is called aseptim TM combi system which consists of aseptim TM solution of dilute pharmaceutical grade tolonium chloride (vital stain), water and sodium phosphate buffer supplied in the form of a syringe containing 0.8 ml aseptim solution (Aseptim is distributed exclusively by SciCan Ltd and manufactured by Denfotex Light Systems Ltd, Inverkeithing, Scotland). The aseptim TM Low power laser diode red light system of a specific wavelength (635 nm) to activate the aseptim solution. It is supplied by a special handpiece with disposable tips for endodontics for light application inside the root canal.

Microorganisms:

E. faecalis and S. intermedius were isolated at the Medical Microbiology & Immunology Laboratory from clinically infected root canals.

First Intervention group:

The bacterial cultures were adjusted to the turbidity of 0.5 McFarland standard. Thirty microns of this solution was placed into a glass tube. A few drops from aseptim solution syringe were dispensed into a plastic disposable dish. Thirty microns of aseptim solution then transferred into the same glass tube by a micro pipette and then activated by the aseptim laser red light system for 120 seconds. Then thirty microns from the final solution after laser activation was placed at the center of the plate (either the blood agar or Mitis Salivarius agar plate). The glass rod was used to streak on the blood agar and Mitis Salivarius agar plates (6 agar plates for each bacterial population). Then the specimens were incubated at 37°C for 24 to 48 hrs.

Second intervention group:

The bacterial cultures were adjusted to the turbidity of 0.5 McFarland standard. Thirty microns of this solution was placed into a glass tube. A few drops from aseptim solution syringe were dispensed into a plastic disposable dish. Thirty microns of aseptim solution then transferred into another glass tube by a micro pipette and then activated by the aseptim laser red light system for 120 seconds. Thirty microns of 2.25% NaOCl were added to the aseptim solution. Thirty microns of this aseptim and NaOCl solution were added to the bacterial solution in the glass tube. Then thirty microns from the final solution was placed at the center of the plate (either the blood agar or Mitis Salivarius agar plate). The glass rod was used to streak on the blood agar and Mitis Salivarius agar plates (6 agar plates for each bacterial population). Then the specimens were incubated at 37°C for 24 to 48 hrs.

Control group:

A concentration of 2.25% of NaOCl was used as a control group by dilution of 5% with a ratio (1:1). The bacterial cultures were adjusted to the turbidity of 0.5 McFarland standard. Thirty microns of this solution was placed into a glass tube. Thirty microns of 2.25% NaOCl were added to the bacterial solution in the same glass tube. Then thirty microns from the final solution was placed at the center of the plate (either the blood agar or Mitis Salivarius agar plate). The glass rod was used to streak on the blood agar and Mitis Salivarius agar plates (6 agar plates for each bacterial population). Then the specimens were incubated at 37°C for 24 to 48 hrs.

A- Bacterial Count:

Colony-forming units (CFUs) of *E. faecalis* and *S. intermedius* were counted for each sample in each group after 24 and 48 hours. Asepsis was maintained throughout the procedures using standard precautions with two flames in a biosafety cabinet.

B- Measurement of bacterial inhibition zone:

Thirty microns of bacterial solution was placed at the center of the plates. The glass rod was used to streak the solution on the whole plate. Sterile templates were used to perform a well (5mm in diameter). Thirty microns from each solution of the three groups was placed inside the well (6 plates were prepared for each bacterium). The plates were then incubated at 37°C for 24 hrs. Inhibition zones around the wells containing the test materials were measured using a ruler and recorded after 24 hrs.

Statistical analysis:

Numerical data were presented as mean and standard deviation values and were explored for normality by checking the data distribution and by using Shapiro-Wilk tests. Data showed parametric distribution and were analyzed using one-way ANOVA followed by Tukey's post hoc test. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows[1].

RESULTS

Bacterial count:

1- *Enterococcus faecalis* count:

At 24 and 48 hours:

There was a statistically significant difference between different groups ($p<0.001$). The highest value of bacterial counts was found in PAD (61.60±7.43), followed by PAD with hypochlorite (32.45±0.85), while the lowest value was found in the control group (13.06±0.34). Post hoc pairwise comparisons showed difference in groups to have significantly different values from each other ($p<0.001$) as shown in table (2).

[1]R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Table (2): Mean and standard deviation (SD) values for the *E. faecalis* count

Time	*E. faecalis* count (Mean±SD)			*p*-value
	Control	PAD	PAD with Hypochlorite	
24 hours	13.06±0.34^{C}	61.60±7.43^{A}	32.45±0.85^{B}	<0.001*
48 hours	13.06±0.34^{C}	61.60±7.43^{A}	32.45±0.85^{B}	<0.001*

Means with different superscript letters within the same horizontal row are significantly different.
*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

2- *Streptococcus intermedius count*:

At 24 hours:

There was a statistically significant difference between different groups ($p<0.001$). The highest value of bacterial count was found in PAD (47.30±7.13), followed by PAD with hypochlorite (32.01±7.57), while the lowest value was found in the sodium hypochlorite (13.72±0.57). Post hoc pairwise comparisons showed difference in groups to have significantly different values from each other ($p<0.001$) as shown in table (3).

At 48 hours:

There was a statistically significant difference between different groups ($p<0.001$). The highest value of bacterial count was found in PAD (47.30±7.13), followed by PAD with hypochlorite (30.25±6.40), while the lowest value was found in the sodium hypochlorite (13.72±0.57). Post hoc pairwise comparisons showed difference in groups to have significantly different values from each other ($p<0.001$) as shown in table (3).

Table (3): Mean and standard deviation (SD) values for the *S. Intermedius count*

Time	*S. Intermedius* (Mean±SD)			*p*-value
	Control	PAD	PAD with Hypochlorite	
24 hours	13.72±0.57^{C}	47.30±7.13^{A}	32.01±7.57^{B}	<0.001*
48 hours	13.72±0.57^{C}	47.30±7.13^{A}	30.25±6.40^{B}	<0.001*

Means with different superscript letters within the same horizontal row are significantly different.
*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

Inhibition zones:

***Enterococcus faecalis* and *Streptococcus intermedius*:**

At 24 hours:

There was a statistically significant difference between different groups ($p<0.001$). Regarding *E. faecalis*, the highest value of inhibition zone was found in the control group (11.50±0.55), followed by PAD with hypochlorite (10.00±0.00), while the lowest value was found in PAD (8.00±0.00). Post hoc pairwise comparisons showed difference in groups to have significantly different values from each other ($p<0.001$) as shown in table (4). Regarding *S. intermedius*, the highest value was found in the control group (12.00±0.89), followed by PAD with hypochlorite (10.33±0.52), while the lowest value was found in PAD (8.00±0.00). Post hoc pairwise comparisons showed different groups to have significantly difference in values from each other ($p<0.001$) as shown in table (4).

Table (4): Mean and standard deviation (SD) values for the inhibition zone of *E. faecalis* and *S. Intermedius*

Time	Inhibition zone of *E. faecalis* (Mean±SD)			*p*-value
	Control	PAD	PAD with Hypochlorite	
24 hours	11.50±0.55^{A}	8.00±0.00^{C}	10.00±0.00^{B}	<0.001*
Time	**Inhibition zone for *S. Intermedius* (Mean±SD)**			***p*-value**
	Control	PAD	PAD with Hypochlorite	
24 hours	12.00±0.89^{A}	8.00±0.00^{C}	10.33±0.52^{B}	<0.001*

Means with different superscript letters within the same horizontal row are significantly different.
*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

DISCUSSION

This invitro study was performed to assess the bacterial count following photoactivated oral disinfection versus NaOCl solution on root canal bacteria. The disinfection of the root canal system is essential for important endodontic operations. The bacteriostatic/bactericidal properties of the agents are crucial for efficient root canal disinfection ***(Galler, 2016)***.

The main factor for endodontic failure is the presence of bacterial species, such *E. faecalis*, inside the root canal system. These bacteria are more resistant to disinfectants, which results in a persistent intra-radicular or extra-radicular infection ***(Alghamdi***

and Shakir, 2020). *Streptococcus Intermedius* has been observed to enter the dentinal tubules both singly and in groups. They can survive in the root canal because of their ability to adapt to significant environmental changes and their attachment and co-aggregation mechanism, which is the foundation for their survival in micro-communities **(Siqueira, 1998; Siqueira et al., 2000).**

Sodium hypochlorite is a strong antibacterial agent that rapidly kills the majority of germs upon direct contact ***(Haapasalo et al., 2014)***, owing to its draw backs such as toxicity in the form of pain or swelling during extrusion of the irrigant through the periapical area. Thus, a novel disinfecting approach that uses PAD device in addition to traditional root canal therapy using NaOCl as a root canal irrigant has been explored.

A non-toxic photosensitizer is triggered using laser energy in a process known as photoactivated oral disinfection. The singlet oxygen generated from these photosensitive dyes destroys microorganisms' membranes and DNA ***(Bago et al., 2013)***. The photosensitizers are highly selective in destroying microbes without compromising the survival of the host cells ***(Lee et al., 2004)***.

Furthermore, photosensitizers kill microorganisms with high selectivity while preserving the viability of the host cell. PAD has been shown to be effective in the elimination of multidrug resistant bacteria ***(Garcez et al., 2010)***. According to ***Fonseca et al. 2008***, this method is very effective at eliminating *E. faecalis* from the root canals.

We used NaOCl 2.25% as a control group according to ***Siqueira, 1998***. Sodium hypochlorite (NaOCl), which possesses tissue-dissolving and antibacterial characteristics, is the most common irrigant. On the other hand, it weakens dentine's flexural strength and resilience and, in large quantities, is toxic, and may harm it ***(Huth et al., 2009)***.

The present study showed that NaOCl has the most effective inhibition of bacteria in comparison to PAD and even to the mixture (PAD with NaOCl). The mixture of PAD with NaOCl has a more significant effect in reducing the count than that of PAD alone. We suggest that the presence of NaOCl was the cause of such a reduction in number.

Vaziri et al., 2012 agreed with our results as they discovered that PAD was less effective than 2.5 % NaOCl at decreasing *E. faecalis* levels, and that the combination of PAD and 2.5 % NaOCl was more effective compared to PAD. Moreover, ***Meire et al., 2012*** discovered that treating *E. faecalis* with 2.5 % NaOCl was quite effective. ***Rios et al., 2011*** suggested the potential application of PAD as an alternative antibacterial method to be utilized in conjunction with traditional endodontic therapy but did not provide a detailed evaluation of PAD and NaOCl efficacy.

Gueorgieva and Gergova, 2021, also discovered that NaOCl disinfection has the greatest antibacterial effect. The PAD is ranked second. Because of its well-established antibacterial action, we believe PAD is an appropriate adjunct to conventional endodontic treatment.

On the reverse, few studies such as ***Arneiro et al., 2014*** discovered that adding PAD to NaOCl as an adjuvant during endodontic treatment had improved antibacterial properties. On the contrary, ***Bonsor et al., 2006*** showed that PAD with diode laser was found to be more efficient at lowering or removing the bacterial load from the canals. Another study showed that the PAD group experienced a larger decline in *E. faecalis* CFUs than did the standard 2.5 % NaOCl syringe irrigation according to **Bago et al., 2013.**

The findings of ***Poggio et al., 2011*** were different from our study, which compared the antimicrobial effects of photoactivated oral disinfection, traditional 5.25 % NaOCl irrigant, and a combination of both on teeth infected with *E. faecalis*, *S. mutans*, and *S. sanguis*. They discovered that PAD used for longer periods of time or PAD combined with 5% NaOCl had significantly greater antibacterial effects. However, they demonstrated that prolonged exposure could produce better results, or that using a high concentration of hypochlorite alongside could improve disinfection effectiveness.

Our study showed that NaOCl is highly effective in 24 and 48 hours in bacterial count, and the same was noticed in the inhibition zone at 24 hours. Thus, there are comparable results between the reduction of bacterial count and inhibition zone.

CONCLUSIONS

From the results of our study, we conclude that sodium hypochlorite and photoactivated oral disinfection can be used as disinfectant agent in root canal treatment. Photoactivated oral disinfection is a supplement for root canal disinfection along with NaOCl irrigation.

CLINICAL SIGNIFICANCE

The use of both the sodium hypochlorite and photoactivated oral disinfection as disinfectant agent for pulpectomy of primary teeth can be promising for eradication of resistant bacteria.

Conflict of interest: No conflict of interest.

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Ethics: This research was approved by Research Ethics Committee on 1/10/2020, Faculty of Dentistry – Cairo University with approval number 1-10-20.

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