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Original Article

Comparative evaluation of the efficacy of 1% and 3% Sodium hypochlorite in reducing the microbial counts in primary teeth root canals using Bioluminometer – A randomized clinical trial

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ABSTRACT

Purpose: Sodium hypochlorite (NaOCl) is commonly used to irrigate primary teeth during pulpectomy. Although high concentrations of NaOCl are effective, they pose a risk of toxic damage to periapical tissues if NaOCl penetrates through the apical foramen. Therefore, low concentrations of NaOCl are preferred to mitigate this risk. However, concerns persist regarding the antibacterial efficacy of low concentrations of NaOCl compared to high concentrations. The objective of this study was to assess and compare the efficacy of 1% and 3% NaOCl irrigation in reducing bacterial load within primary teeth root canals.

Materials and methods: This clinical study involved forty participants divided into two groups. Group 1 (n = 20) received canal irrigation with 1 % NaOCl solution, while Group 2 (n = 20) received canal irrigation with 3 % NaOCl solution. Microbial samples were collected from the root canal using a paper point before and after irrigation. The samples were aseptically transferred to ultra-snap tubes and then analyzed using a Bioluminometer. The results were recorded.

Results: Both groups exhibited a decrease in bacterial count after irrigation. The mean colony count post irrigation for 3 % NaOCl was 258.05 ± 28.61, and for 1 % NaOCl it was 267.60 ± 30.56. However, no statistically significant difference was observed upon intergroup comparison.

Conclusion: This study shows that 1% NaOCl is equally effective as 3% NaOCl in reducing bacterial count in root canals. Thus, using 1% NaOCl as an irrigant is appropriate in clinical practice.

1. Introduction

Endodontic treatment of primary teeth is particularly challenging due to their unique internal anatomy. This complexity is marked by the presence of accessory canals and horizontal anastomoses, making thorough cleaning and disinfection difficult (Mathew and Soni, 2019; Mahesh and Nivedhitha, 2020). The primary objective of root canal treatment in primary teeth is to maintain the integrity of the dental arch and promote periodontal health. Achieving successful endodontic treatment depends on several critical factors, including accurate diagnosis, comprehensive canal cleaning, and an effective irrigation protocol (Da Silva et al., 2006). Bacterial infection is the primary cause of pulp and periapical diseases. The microbial landscape in endodontic infections is highly diverse, comprising numerous bacterial species. Research has shown that the types of microorganisms in root canals of asymptomatic teeth differ significantly from those in teeth exhibiting clinical symptoms. Specifically, the microbial population in root canals can include aerobic and anaerobic bacteria, along with facultative microorganisms (Da Silva et al., 2006; Liu et al., 2021).

Effective endodontic treatment aims to eliminate these bacterial

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agents from the root canal system. This is essential because the presence of bacteria and their metabolic byproducts can lead to persistent infections and treatment failures. Techniques for thorough debridement and disinfection, including mechanical instrumentation and chemical irrigation, are pivotal in reducing bacterial load and ensuring the success of the treatment. Thus, endodontic therapy in primary teeth is recognized as a highly effective method for removing bacteria from the root canal, thereby preventing reinfection and promoting the long-term health of the tooth and surrounding periodontal tissues.

The main objective of endodontic therapy is to thoroughly clean, shape, and seal the root canals in an ideal manner, ensuring a mechanical seal. Failure to eliminate bacteria residing within dentinal tubules may lead to potential root canal reinfection. Recent imaging techniques have revealed that mechanical preparation alone may not adequately treat certain regions of the pulp spaces. Therefore, irrigation and instrumentation are combined to ensure thorough debris removal and efficient root canal disinfection (Liu et al., 2021;Nandakumar and Nasim., 2020). While several irrigation solutions are utilized in primary teeth, sodium hypochlorite (NaOCl) is the gold standard. It is employed in various concentrations, typically 0.5 % to 5.25 %. Despite NaOCl's demonstrated effectiveness as an irrigation agent for primary teeth, high concentrations may pose a risk of toxic damage to the periapical region if NaOCl penetrates through the apical foramina of the tooth (Ahangari et al., 2008; Zehnder et al., 2002; Mathevanan et al., 2023).

It has been proposed that the effects and toxicity of NaOCl depend on the administered dosage. An ideal irrigation solution should be non-toxic, possess broad antibacterial activity, efficiently degrade necrotic pulp tissue, neutralize endotoxins, and prevent the formation of smear layers (Chang et al., 2001; Teja et al., 2021) . Consequently, low concentrations of NaOCl may reduce toxicity compared to high concentrations. However, the American Academy of Pediatric Dentistry (AAPD) recommends using 1 % NaOCl for irrigation in pediatric dental procedures, underscoring its clinical relevance and importance (American Academy of Pediatric Dentistry, 2019). Despite this recommendation, limited studies directly compare the antibacterial efficacy of 1 % NaOCl with high concentrations. Therefore, the primary objective of this study was to assess the effectiveness of 1 % and 3 % NaOCl irrigation in reducing bacterial load in primary teeth root canals.

2. Materials and methods

2.1. Study design

This study employed a double-blinded, randomized clinical trial. Forty participants were divided into two groups of twenty each using a computer-generated series of random numbers. The timeline of the randomized controlled trial is shown in Fig. 1.

2.1.1. Study setting

The research was conducted at a private dental institution in Chennai, tamil Nadu, India at the department of Pediatric and preventive dentistry.

2.1.2. Ethical clearance

The institutional ethics committee (IHEC/SDC/PEDO-2101/23/130) approved the study before its commencement. The study was registered with the Clinical Trials Registry India (CTRI) under registration number CTRI/2023/11/059520. Written informed consent was obtained from parents, and participant anonymity was maintained throughout the study.

2.1.3. Study population

Participants aged 6–9, visiting the outpatient department of Pediatric and preventive Dentistry in March– May 2023, were included in this study

2.1.4. Inclusion criteria

Children requiring pulpectomy treatment for primary mandibular posterior teeth, those classified under Frankl ratings 3 and 4, and those belonging to the American Society of Anesthesiologists 1 category were included in the study.

2.1.5. Exclusion criteria

Children with teeth exhibiting calcified canals, crowns beyond restoration, or presenting significant internal or external root resorption were excluded from the study. Additionally, children with systemic disorders or medically compromising conditions and those who had

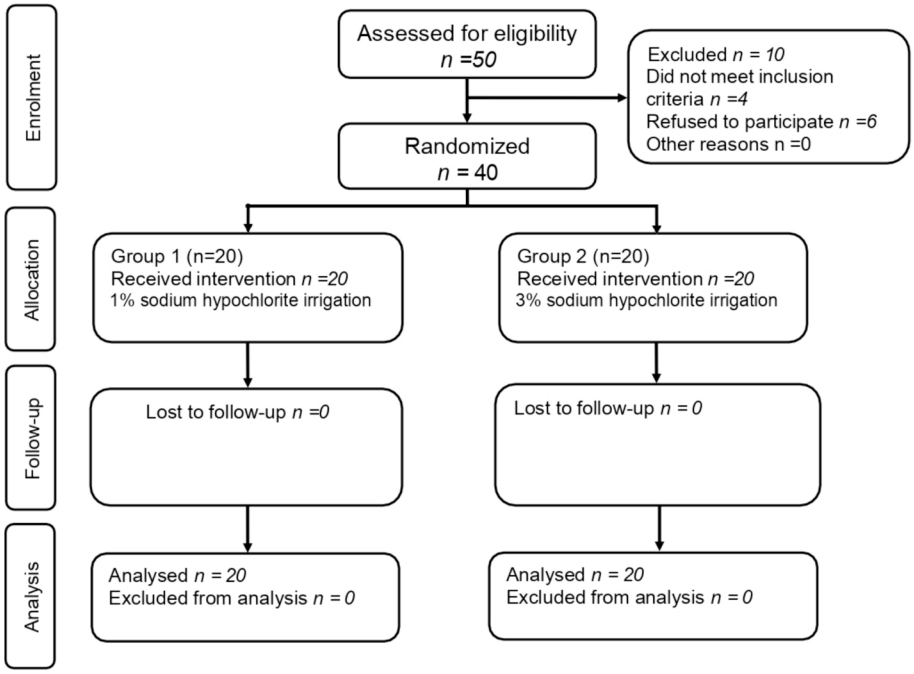

Fig. 1. Consort flow diagram showing the number of participants through each stage of the randomized clinical trial.

received antibiotics within two weeks before the procedure were excluded.

2.1.6. Sample size calculation

The sample size was determined using G Power software, according to the study conducted by Walia et al (2019). Calculations were based on a significance level p-value of 0.05, a statistical power of 90 %, and an effect size of 0.538. The calculation yielded a sample size of 20 participants in each group.

2.2. Procedure

A single visit pulpectomy procedure was performed for all patients by a trained pediatric dentist. Following local anesthesia administration, the teeth were isolated using a rubber dam. A diamond round bur (#330 bur) was used to create a precise access opening. Once the access cavity preparation was completed, the canals were successfully located.

2.2.1. Pre-operative sample collection

Once the canals were located, they were irrigated with a saline solution. Subsequently, a sterile #20 K file was carefully inserted into the canal, stopping approximately 1 mm short of the root apex as determined by pre-operative radiographs, and gentle filing was performed. Pre-operative microbiological samples were collected from within the canal using sterile Dentsply paper points (Fig. 2), which were then aseptically transferred to ultra-snap tubes (Fig. 3). These tubes contained D-luciferin liquid and an adenosine triphosphate (ATP) bioenzyme. To ensure the paper point made contact with the liquid bioenzyme mixture, the snap valve was flexed forward and backward, allowing the liquid to flow down the tube. The sample collection tip was immersed in the solution, gently shaken for 5–10 s, and then the ultra-snap tube containing the sample was inserted into the luminometer to obtain readings (Fig. 4).

2.2.2. Post-operative sample collection

In all cases, chemo-mechanical preparation was performed during the same session. Kedo S Plus rotary files were used to prepare the canal to the working length for all the particiapants. A total of 2 ml of either 1 % or 3 % NaOCl was used for irrigation, depending on the assigned group. Subsequently, postoperative microbiological samples were collected from the same canal using paper points, which were then transferred to ultra-snap tubes for analysis, and the results were obtained.

2.3. Statistical analysis

The data were analyzed using SPSS Software version 23.0. To

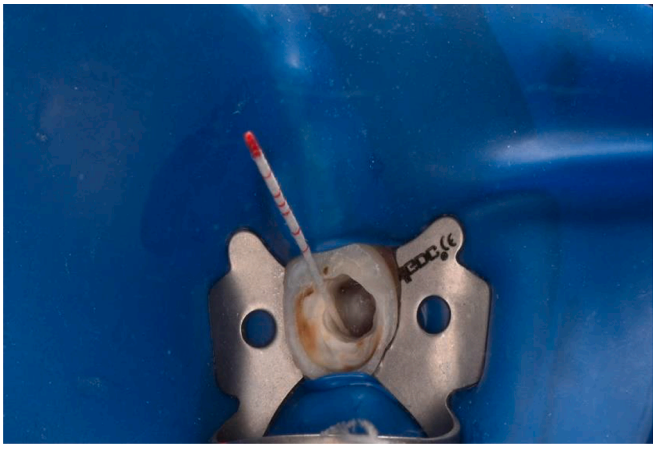

Fig. 2. Microbial sample being collected from the canal using paper point.

Fig. 3. The ultra-snap tube to which the sample was transferred.

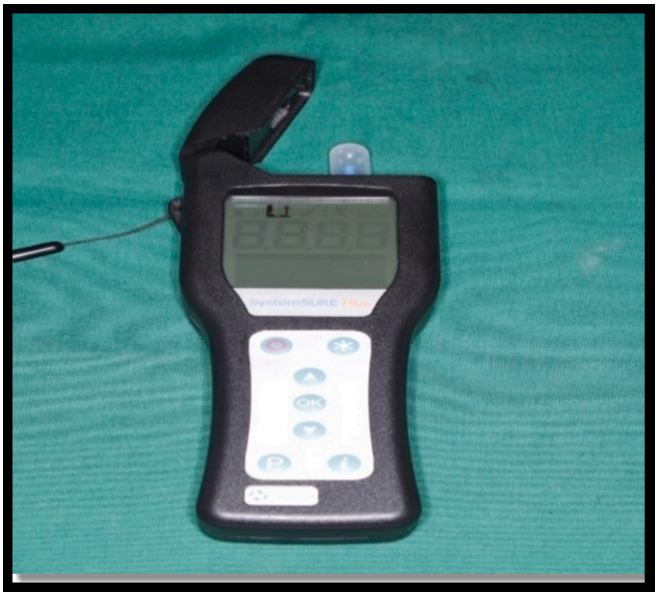

Fig. 4. The ultra-snap tube containing the sample being placed in the luminometer.

compare the groups, an independent *t*-test was conducted to assess the mean difference between pre-operative and postoperative bacterial counts for 1 % and 3 % NaOCl groups. Additionally, a paired *t*-test was used to evaluate the difference in bacterial count within each group. The significance level was set at $p < 0.05$ with a 95 % confidence interval.

3. Results

In this study, 40 samples were analyzed for bacterial count. The mean colony count of the samples decreased after irrigation compared to that before irrigation. A paired *t*-test demonstrated a significant reduction in colony count post irrigation in both groups (Table 1).

However, intergroup comparison using independent t-tests revealed no significant difference, with a p-value > 0.005 (Table 2)

4. Discussion

The main objective of endodontic treatment is the complete elimination of bacteria from the root canal, achievable through mechanical instruments and chemical irrigation. NaOCl has long been the main irrigating fluid for endodontic treatment in primary teeth. While NaOCl is highly effective against bacteria, its contact with periapical tissues can lead to adverse effects, compounded by its unpleasant odor and taste (Siqueira et al., 1997). Spencer et al., (2007) extensively discussed the negative repercussions of NaOCl use in the periapical region. Recent studies suggest that low concentrations of NaOCl may mitigate its adverse effects on surrounding tissues, rendering it a safer clinical option (Vianna et al., 2006). However, supporting clinical trials are limited, requiring further investigation. Therefore, this study was conducted to assess the microbial count reduction achieved using a low concentration of NaOCl solution, employing a Bioluminometer for analysis. Colony count analysis was chosen due to its high reliability in microbiology, offering a direct measurement of viable microorganisms.

Table 1
Paired *t* test showing the mean differences of colony count within the group between the timelines.

Paired *t* test	Mean ± SD	P value
1% sodium hypochlorite pre and post op	3328.55 ± 212.84	0.000*
3% sodium hypochlorite pre and post op	3373.85 ± 214.64	0.000*

Table 2
Independent *t*-test showing the mean differences in colony count between the groups before and after irrigation.

Independent *t* test			
	Groups	Mean ± SD	P value
Pre –OP	1 % Sodium Hypochlorite	3596.15 ± 216.65	0.680
	3 % Sodium Hypochlorite	3631.90 ± 318.32	
Post OP	1 % Sodium Hypochlorite	267.60 ± 30.56	0.314
	3 % Sodium Hypochlorite	258.05 ± 28.61	

The total bacterial count was assessed using a Bioluminometer (SystemSURE Plus by Hygenia, USA). This method offers rapid microbial detection by measuring ATP levels. The ATP bioluminescence method involves using a swab to collect a sample from a standardized area. This sample is then placed in a tool that utilizes the firefly enzyme "luciferase" to catalyze the conversion of ATP into Adenosine Monophosphate (AMP). As a result of this reaction, light is emitted and detected by a bioluminometer, which quantifies it in Relative Light Units (RLU). ATP bioluminescence efficiently identifies microorganisms by detecting intracellular ATP. Microbial cells utilize ATP as a molecular energy carrier. Luciferase and luciferin in the liquid in the ultra-snap tube react with microbial ATP, generating bioluminescence and thereby facilitating the detection of microorganism levels. Grossman et al. recommended using sterile paper points for root canal sampling. In line with this recommendation, microbial samples were collected from the root canal using absorbent paper points (Grossman et al., 1988).

Comparing the antibacterial efficacy of different concentrations of NaOCl specifically 1 % and 3 %, no significant difference was observed between them following irrigation. This study showed that a 1 % NaOCl solution effectively reduced bacterial counts post irrigation. These findings align with the study by D'Arcangelo et al. (1999), who examined the efficacy of various NaOCl concentrations on both facultative and strictly anaerobic bacteria. Their research revealed that 0.5 %, 1 %, 3 %, and 5 % NaOCl concentrations demonstrated similar antibacterial properties.

In this study, both groups exhibited a statistically significant reduction in bacterial count post irrigation. Thus, it can be concluded that both 3 % and 1 % NaOCl solutions demonstrated significant efficacy in reducing bacterial count. However, no significant difference was observed when comparing the overall mean bacterial count between the groups (p-value > 0.05). This finding aligns with a study by Siqueira et al., who reported that various concentrations of NaOCl showed no significant difference in reducing microbial count within root canals after irrigation (Siqueira et al., 2000). Additionally, none of the treatment groups investigated in our study achieved complete bacterial elimination within the root canal system. In contrast, Du et al., (2014) and Zand et al.,(2016) reported that high NaOCl concentrations were necessary to significantly decrease bacterial count within the root canal.

Based on the results, it is evident that 1 % NaOCl solutions exhibit comparable effectiveness in reducing bacterial counts. This finding aligns with a study by Walia et al., who concluded that 1 % NaOCl was as effective as laser and chlorhexidine irrigation in reducing microorganism numbers in the root canal (Walia et al., 2019). Similarly, in another study, Valdez-Gonzalez et al (2012) determined that 1 % NaOCl was equally effective as oxidative potential water in reducing bacterial counts in root canals. Furthermore, the reduced toxicity of 1 % NaOCl compared to that of 3 % NaOCl is a crucial consideration in clinical applications. High concentrations of NaOCl have been linked to increased tissue irritation and cytotoxic effects. Therefore, using 1 % NaOCl as an irrigant is a promising alternative because it demonstrates comparable efficacy while potentially minimizing adverse effects on surrounding tissues.

This study has certain limitations. Conducting further research with a larger sample size for each treatment group could enhance the validity of the findings. Additionally, future studies could compare the efficacy

of different irrigation solutions with low-concentration NaOCl solutions, providing a more comprehensive understanding of their respective benefits.

5. Conclusion

In conclusion, this study confirms that 1 % NaOCl is equally effective as 3 % NaOCl in reducing bacterial count in the root canal. Consequently, considering its comparable efficacy, using 1 % NaOCl as an irrigant warrants consideration in clinical practice. Its lower toxicity profile compared to that of high NaOCl concentrations renders it a viable alternative, potentially minimizing tissue damage while ensuring effective bacterial elimination.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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