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Comparative evaluation of the antibacterial efficacy of sodium hypochlorite gel and solution at different concentrations against Enterococcus faecalis within the root canals of primary molars: an in vitro study

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Abstract:

Objectives:
The study aimed to evaluate the antibacterial efficacy of sodium hypochlorite (NaOCl) in its solution and gel forms, at different concentrations, as an irrigant against Enterococcus faecalis in primary molars.

Materials and methods:
The sample consisted of 50 upper and lower primary molars, divided into five equal groups: Group 1: 2.25% NaOCl solution. Group 2: 5.25% NaOCl solution. Group 3: 2.25% NaOCl gel. Group 4: 4% NaOCl gel. Group 5 (negative control): 0.9% saline. After irrigation, the teeth were incubated for 24 hours. A sample was then cultured on blood agar plates. The plates were then incubated for 72 hours, and the bacteria were counted.

Results:
NaOCl at all concentrations, both liquid and gel forms, demonstrated antibacterial activity against Enterococcus faecalis, with 2.25% NaOCl gel outperforming 4% ($p < 0.001$).

Conclusions
NaOCl gel at concentrations of 2.25%–4% can be considered an effective alternative against Enterococcus faecalis in the endodontic treatment of primary molars.

Key Words: Naocl Gel, Irrigants, Primary Molars, Enterococcus Faecalis.

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التقييم المقارن للفعالية المضادة للجراثيم لهلام ومحلول هيبوكلوريت الصوديوم بتركيزات مختلفة ضد المكورات المعوية البرازية داخل قنوات جذور الأرحاء المؤقتة: دراسة مخبرية

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الملخص:

الأهداف:

تهدف هذه الدراسة إلى مقارنة الفعالية المضادة للجراثيم لمحلول هيبوكلوريت الصوديوم 2.25٪ و 5.25٪ وهلام هيبكلوريت الصوديوم 2.25٪ و 4٪ ضد المكورات المعوية البرازية في قنوات جذور الأرحاء المؤقتة.

المواد والطرائق:

تألفت العينة من 50 رحى مؤقتة مقسمة إلى خمس مجموعات متساوية: المجموعة 1: محلول 2.25% هيبوكلوريت الصوديوم. المجموعة 2: محلول 5.25% هيبوكلوريت الصوديوم. المجموعة 3: جل 2.25% هيبوكلوريت الصوديوم. المجموعة 4: جل 4% هيبوكلوريت الصوديوم. المجموعة 5 (مجموعة شاهدة سلبية): محلول ملحي 0.9%. حُضنت الأسنان لمدة 24 ساعة، ثم زُرعت العينة على أطباق الآغار الدموي وحُضنت لمدة 72 ساعة، ثم عُدّت المكورات العقدية البرازية.

النتائج:

أظهر هيبوكلوريت الصوديوم بجميع تراكيزه، سواءً المحلول أو الهلامي، نشاطاً مضاداً للجراثيم ضد المكورات العقدية البرازية ، حيث تفوق هلام هيبوكلوريت الصوديوم بتركيز 2.25% على تركيز 4% ($p < 0.001$).

الاستنتاجات:

يُمكن اعتبار هلام هيبوكلوريت الصوديوم بتركيزات تتراوح بين 2.25% و4% بديلاً فعالاً ضد المكورات العقدية البرازية في المعالجة اللبية للأرحاء المؤقتة.

الكلمات المفتاحية: هلام هيبوكلوريت الصوديوم – سوائل الإرواء – الأرحاء المؤقتة – المكورات المعوية البرازية.

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Introduction:

Dental caries is the most common chronic disease among pediatric patients [1] and is a common and increasing health problem, especially in developing countries [2]. The cause may be poor socioeconomic conditions and lifestyle changes. If caries is not treated appropriately, the most common consequences are pain, infection, premature tooth loss, loss of dental arch length, decreased chewing efficiency, impacted permanent teeth, and loss of self-confidence [3]. Endodontic treatment is indicated when caries affects the pulp and if infection has reached the root pulp [4]. The primary goal of root canal treatment is to remove bacteria and their products and infected tissue from the root canal system through disinfection and chemical and mechanical debridement [5].

Bacteria play a major role in the initiation and progression of endodontic and periapical diseases [6]. Mechanical debridement of the root canals alone cannot eliminate bacteria from the primary root canals due to their complex anatomy [7]. Therefore, reliance on irrigants becomes even more important for removing bacteria in the primary canals of teeth with their thin root dentin walls, complex internal anatomy, and irregular root canal system [8].

Sodium hypochlorite (NaOCl) solution is the most widely used irrigant. Despite its unpleasant taste and pungent odor, it has a broad spectrum of bactericidal activity [9]. NaOCl solution is used in concentrations ranging from 0.5% to 6% [10]. It is good at dissolving pulpal debris, collagen fibers, and dead tissue. It also provides complete debridement of the root canals, lubrication is easy to maintain, and has a long shelf life [11].

One of its major drawbacks is its high toxicity to living tissue, especially if the irrigant escapes from the apex, which can cause significant damage to periapical tissues or trigger an allergic reaction [12]. Applying NaOCl in gel form offers better control over application and reduces the risk of over-apexing, especially in children with young and primary teeth. The gel also has a more pleasant odor and taste than the liquid [13, 14]. This study aims to compare the antibacterial efficacy of 2.25% and 5.25% NaOCl Solution and 2.25% and 4% NaOCl gel against Enterococcus faecalis in the root canals of primary molars. The null hypothesis is that no statistically significant difference would be noted between NaOCl solution and gel at different concentrations in the antibacterial efficacy against Enterococcus faecalis.

Materials And Methods:

Study design and ethics:

It was an in vitro study conducted at the Pediatric Dentistry Department of the Faculty of Dentistry at Damascus University in September 2024. The Local Ethics Committee at Damascus University approved the study under N/2024, and the research adhered to the Checklist for Reporting In-Vitro Studies (CRIS) guidelines [15]. Written informed consent for tooth donation was obtained from the legal guardians of the patients, and the extraction of human primary molars was carried out for pathological or orthodontic purposes.

Sample size calculation and tooth selection:

The criteria for determining the sample size included an effect size of 0.51 (effect size $f = 0.51$), a two-tailed significance level of 5% ($\alpha = 0.05$), a 95% confidence interval, a statistical power of 80% (1-β err prob = 0.80), and five experimental groups. Fifty specimens were gathered for the sample size, and the effect size calculation was derived from an initial study [16]. Teeth exhibiting root resorption greater than one-third of the root length, those that have had prior endodontic treatment, roots with calcified canals, and teeth presenting fractures or fissures were excluded. All teeth were subjected to disinfection by being immersed in a 5% NaOCl solution (Carmel®; AkkaBrothers Co., Carmel Detergent, Damascus, Syria) for one hour. Following the disinfection process, the teeth were thoroughly rinsed with tap water and preserved in normal saline (SODIUM CHLORIDE 0.9% MIAMED, Miamed Pharmaceutical Industry, Damascus, Syria) until utilized for the study [17]. The fifty human primary molars were randomly allocated into five groups (n = 10):

- Group 1: irrigation with 2.25% NaOCl solution (Hypo Chlorite, Noura, Damascus, Syria).
- Group 2: irrigation with 5.25% NaOCl solution.
- Group 3: irrigation with 2.25% NaOCl gel (BLEACH gel, WC NET, Milano, Italy).

- Group 4: irrigation with 4% NaOCl gel (LET'S CLEAN Concentrated Chlorine, DTIC®, Damascus, Syria).
- Group 5 (negative control): irrigation with 0.9% saline.

Randomization and blinding

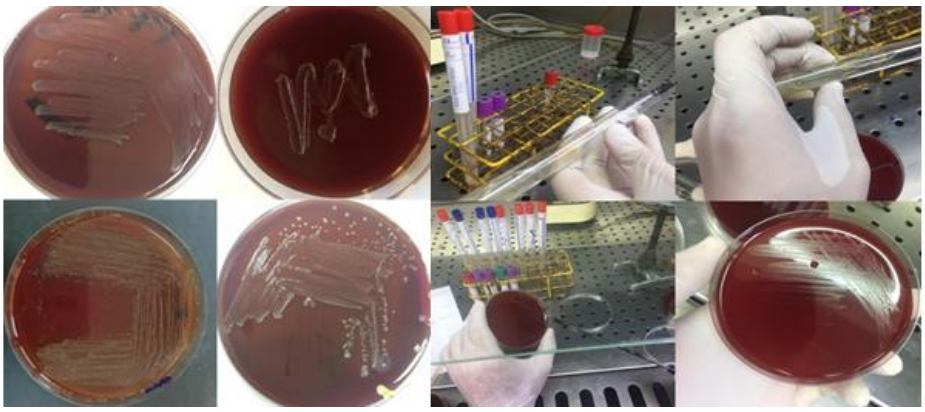
The sample was randomly assigned using a lottery method, with numbers from 1 to 50 written on paper cards. The study was double-blinded, where the outcome assessor and the statistician were unaware of the groups.

Procedure

The access cavity was performed. The working length was determined using a 15 K-file size (K File, FANTA DENTA©, Shanghai, China) until the file penetrated and retreated 1 mm. A 20 K-file size 4% taper (SC Niti File, SOCO PRECISION INSTRUMENT CO. LTD., Guangdong, China) was used, followed by a 1 mL irrigation with saline (SODIUM CHLORIDE 0.9% MIAMED, Miamed Pharmaceutical Industry, Damascus, Syria). Next, A 25 K-file size 4% taper was used, followed by a 1 mL irrigation with 5% NaOCl solution for 3 min, followed by a final irrigation with saline. Teeth were placed in acrylic cubes for ease of handling. The canals were dried, then moist-heat sterilized at 121°C for 20 min (Figure 1) [18].

Figure(1): Study sample within moist heat sterilization bags.

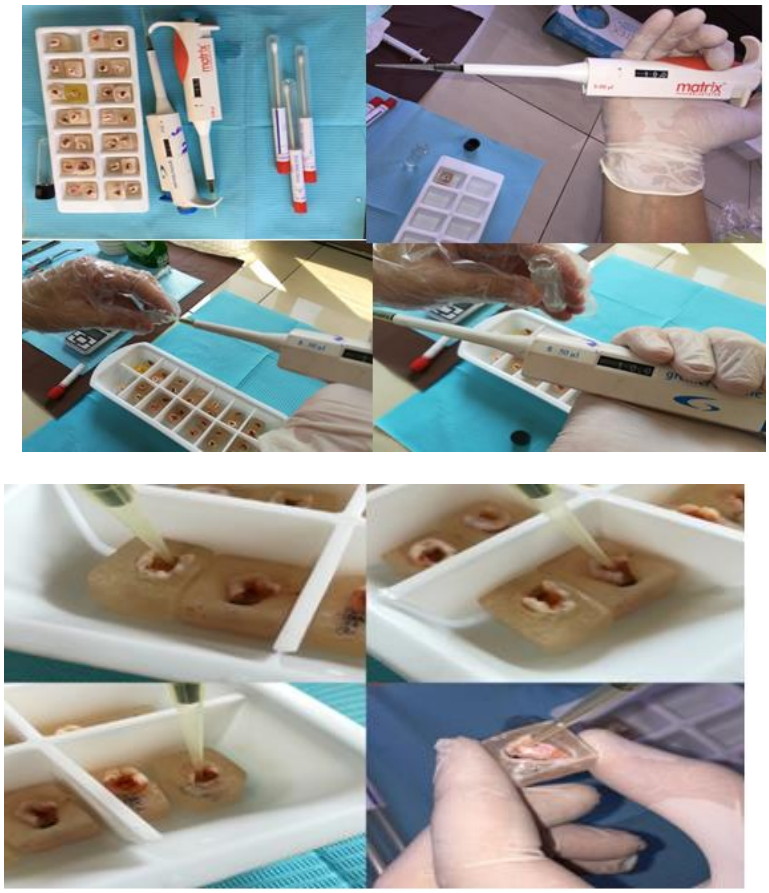
Enterococcus faecalis was cultured on blood agar plates every few days to ensure fresh bacteria were used (Figure 2). A suspension containing Enterococcus faecalis at a concentration of 1.5 x 10^8 CFU/mL, equivalent to 0.5 on the McFarland scale, was prepared using a turbidimeter (TB1 Turbidimeter, A-MATRIX©, Lagos State, Nigeria). The sample was shaken on a shaker to ensure the solution was homogeneous. The glass tube was then placed in the turbidimeter [19].



Figure(2): Cultivation of Enterococcus faecalis and use of fresh bacteria.

The experiment was repeated until a concentration of 0.5 McFarland was reached. Ten µL of the bacterial suspension was taken using a micropipette (Micropipette 100, Matrix Healthcare Pvt. Ltd., New Delhi, India) and placed in the root canals (Figure 3). The samples were then incubated in an anaerobic incubator for 24 hours. Root canals were irrigated with 5 mL of each irrigant for 5 minutes using a 31-gauge side-vented irrigating needle. They were then flushed with 5 ml of sterile saline. The root canals were dried along their entire length, filled with sterile saline, and circumferentially filed with an H-file for 15 seconds. Bacterial swabs were then collected using paper cones. The paper cone was inserted and left for 60 seconds before being transferred into an Eppendorf tube containing 2 mL

of sterile saline. The swabs were repeated three times to transfer the bacterial count, and the tube containing the paper cones was shaken for 1 min to ensure solution homogeneity. Ten µL of the solution was taken using a micropipette and cultured on pre-prepared Petri plates, and placed in an incubator at 37°C. After 72 hours, the plates were removed from the incubator, and the bacterial colonies were counted. The units were converted to logarithmic numbers to facilitate statistical analysis.



Figure(3): Contamination of the root canals with Enterococcus faecalis.

Statistical analysis

IBM SPSS software version 26 (IBM Corp., Armonk, NY, USA) was utilized for statistical analysis. One-way ANOVA test was used to compare the mean differences in mean logarithmic reduction between study groups, and the Bonferroni test was used to study pairwise differences between groups. The logarithmic reduction of bacterial colony counts was calculated according to the following equation:

Log reduction = $\log_{10}(A) - \log_{10}(B)$

A: The bacterial count before irrigation.

B: The bacterial count after irrigation.

Results:

Descriptive statistics of the decimal logarithm of bacterial count after irrigation is listed are Table 1.

Table 2 shows a statistically significant difference in the mean logarithmic reduction between the study groups ($p < 0.001$). Therefore, the null hypothesis was rejected. Table 3 shows a statistically significant difference between the 0.9% saline group and other study groups ($p < 0.001$) and between the 4% NaOCl gel group and 5.25% NaOCl solution and 2.25% NaOCl gel groups ($p < 0.001$) (Figure 4 and Figure 5).

Table(1): Descriptive statistics of the decimal logarithm of bacterial count after irrigation.

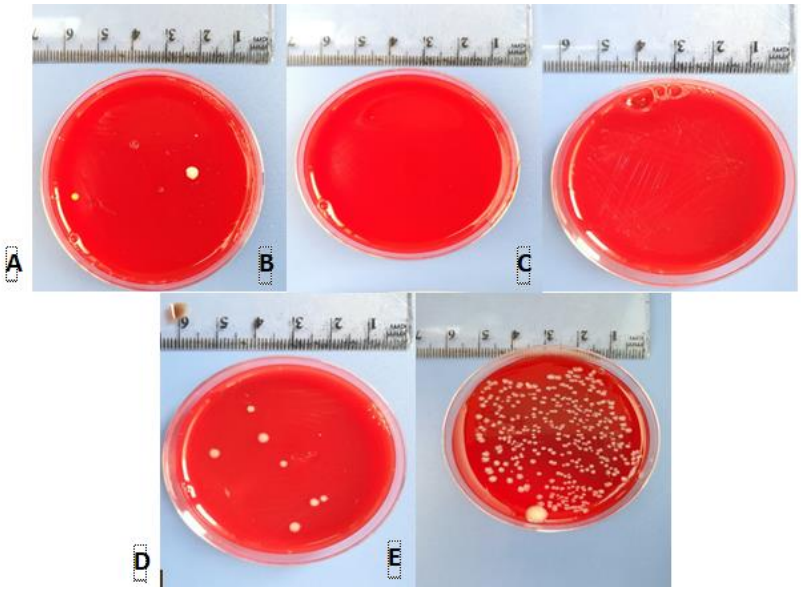
Groups	Mean	SD	Min	Max
2.25% NaOCl solution	1.04	1.15	0	1.66
5.25% NaOCl solution	0.30	0.48	0	0.95
2.25% NaOCl gel	0.30	0.48	0	0.90
4% NaOCl gel	1.85	1.93	0.48	2.43
0.9% saline	4.60	4.32	3.61	4.84

Table(2): One-way Anova test results for logarithmic reduction of bacterial counts.

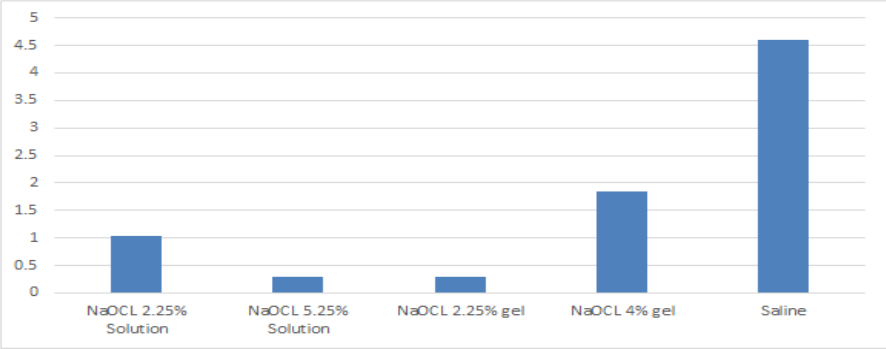
Groups	*p*-value
2.25% NaOCl solution	< 0.001*
5.25% NaOCl solution	
2.25% NaOCl gel	
4% NaOCl gel	
0.9% saline	

Table(3): Bonferroni test results for pairwise comparisons of logarithmic decimal reduction of bacterial counts.

Pairwise comparisons		*p*-value
2.25% NaOCl solution	5.25% NaOCl solution	0.104
	2.25% NaOCl gel	0.158
	4% NaOCl gel	0.167
	0.9% saline	0.001*
5.25% NaOCl solution	2.25% NaOCl gel	0.830
	4% NaOCl gel	0.003*
	0.9% saline	< 0.001*
2.25% NaOCl gel	4% NaOCl gel	0.004*
	0.9% saline	< 0.001*
4% NaOCl gel	0.9% saline	0.043*



Figure(4): The bacterial count remaining on blood agar plates after irrigation. (A) 2.25% NaOCl solution. (B) 5.25% NaOCl solution. (C) 2.25% NaOCl gel. (D) 4% NaOCl gel. (E) 0.9% saline.



Figure(5): The decimal logarithm of bacterial count after irrigation

Discussion:

Necrotic primary teeth contain numerous microorganisms, particularly anaerobic bacteria, which reside deep within the dentin canals and cementum around the apical region. These microorganisms persist even after mechanical preparation of the root canals. Mechanical preparation alone cannot effectively eliminate bacteria in the canals of primary teeth due to their anatomical complexity [20]. Therefore, reliance on irrigation has become increasingly important, especially in primary teeth due to their thin walls and irregular root canal anatomy [8]. NaOCl solution is considered the best irrigating solution for root canals. It possesses antibacterial activity by releasing hypochlorous acid (HClO), which exerts an oxidative action on the sulfhydryl groups of bacterial enzymes, disrupting bacterial cell metabolism [21]. The current study compares the antibacterial activity of 5.25% and 2.25% NaOCl solution and 4% and 2.25% NaOCl gel against Enterococcus faecalis in vitro on maxillary and mandibular primary molars.

Enterococcus faecalis was chosen because it is one of the most resistant intracanal bacteria and the most common bacteria isolated from periapical infections. Enterococcus faecalis is the most dominant bacterium in failed root canal treatments, accounting for 50-70% of these cases. It is due to its high survival capacity, resistance to antiseptics used in root canals, and ability to survive in low-nutrient environments. The concentration of the Enterococcus faecalis suspension was selected at 1.5×108 CFU/mL, according to the study of Öter et al. [19]. The suspension was introduced using a micropipette with atomized tips to ensure the bacterial sample was not contaminated with other bacteria.

The study sample consisted of 50 maxillary and mandibular primary molars. Considering that the anatomy and resorption of the root canals can affect the bacterial load, the palatal roots of the maxillary second molars and the distal roots of the mandibular second molars were used to standardize the samples. Furthermore, these roots have a higher curvature than the maxillary and mesial canals [22]. The irrigation tip was 1 mm away from the full root length, according to a study of Pinker et al. [23]. The irrigation protocol was standardized in terms of time and volume of irrigant. Irrigation time ranged from 30 seconds to 10 min, while the volume of irrigant ranged from 0.05 mL to 15 mL. Irrigation was performed with 5 mL of the irrigant used in this study. Due to the different viscosities of the irrigant fluids and the pressure applied to the syringe, the irrigation speed was standardized across all groups at 1 mm per minute [24].

A vibrating device was used to shake the tubes for one min to ensure homogeneity of the suspension before transferring them to the blood agar plate. Ten µL of the suspension were then withdrawn using a micropipette with a plastic tip for each sample to ensure no contamination. The suspension was spread on the plate using a culture tube so that it was distributed over the entire surface of the plate.

NaOCl was used in this study because it is considered the gold standard irrigant. It can dissolve dead pulp tissue and collagen, and is readily available and inexpensive. The 5.25% NaOCl solution concentration was chosen for this study because it is the most common concentration used in endodontic treatments. The 2.25% NaOCl solution concentration was chosen to resemble the gel concentration used in this study. It is close to the 2.5% concentration, which eliminates toxins produced, and because of its ability to dissolve organic tissue [9-11]. Vaziri et

al. [25] also demonstrated that a 2.5% NaOCl solution was effective against Enterococcus faecalis upon direct contact after two min.

The results of this study are consistent with the study by Abu Hasna et al. [26], who evaluated the effectiveness of 2.5% NaOCl solution and 3% NaOCl gel against Enterococcus faecalis. The results demonstrated no statistically significant differences in the antibacterial effect of the solution and gel forms. They are also consistent with the study by Zand et al. [27], who indicated that both the 2.25% gel and the 2.25% and 5.25% solutions were able to eliminate Enterococcus faecalis, but the gel demonstrated a lower efficacy compared to the solution at both concentrations. The lower antibacterial efficacy of the gel compared to the solution is likely due to differences in the manufacturers of the gels used in their study. The results of this study were consistent with the study Sahebi et al. [6], as there was no statistically significant difference between the 2.25% NaOCl gel and liquid, and both were effective in eliminating bacteria. All concentrations of NaOCl gel and lsolution showed antibacterial activity, with a statistically significant difference compared to the saline group, which did not show any antibacterial activity but had a rinsing effect that contributed to reducing the number of bacteria.

Conclusions:

Within the limitations of this study, we recommend the use of NaOCl gel at concentrations of 2.25% (WC NET) and 4% (LETS CLEAN) for 5 min in endodontic treatment of primary molars in children. It is considered a safe and effective alternative to solution against Enterococcus faecalis, with preference given to the 2.25% NaOCl WC NET gel.

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