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Assessment of Dual Rinse Combined with Sodium Hypochlorite Irrigating Solution on Post-Instrumentation Pain and Bacterial Load Reduction: A Randomized Clinical Trial

Avaliação do Dual Rinse associado à solução irrigadora de hipoclorito de sódio na dor pós-instrumentação e na redução da carga bacteriana: um ensaio clínico randomizado

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ABSTRACT

Objective: The study compared the efficacy of Dual Rinse combined with sodium hypochlorite in comparison to sodium hypochlorite irrigating solution on post instrumentation pain incidence, reduction of the load of intracanal bacteria and periapical MMP-9 expression in patients with pulp necrosis. **Material and Methods:** Thirty-four patients diagnosed with a necrotic mandibular premolar having a single root canal were included. Following confirmation of the diagnosis, patients were randomly allocated into one of two groups of 17 patients each (intervention group; Dual Rinse + 2.5% NaOCl, control group; 2.5% NaOCl irrigation). Standard endodontic treatment was done in two visits. Post-instrumentation pain was assessed at 6, 12, 24 hours and 48 hours using Heft-parker visual analogue scale (Heft parker VAS). The intracanal bacterial levels assessed pre- and post-instrumentation through counting of CFU/mL. Periapical MMP-9 levels were assessed post-instrumentation and pre-obturation by ELISA. All data collected from patients was statistically analyzed. **Results:** Regarding post-instrumentation pain incidence, there was no statistically significant difference detected among tested irrigants at all time intervals ($p>0.05$). There was a significant reduction in the count of intracanal bacterial in both groups after instrumentation, but without any significant difference between tested irrigants ($p>0.05$). Within a week, there was a considerable drop in the periapical MMP-9 levels without any discernible differences between the two irriagnts ($p>0.05$). **Conclusion:** The addition of Dual Rinse to 2.5% NaOCl solution did not result in any apparent increase in the incidence of post-instrumentation pain or in periapical MMP-9 levels. Both irrigating solutions were almost equally successful in lowering the amount of bacteria in primary infected root canals while maintaining the antibacterial activity of the NaOCl.

KEYWORDS

Bacterial load reduction; Dual rinse in sodium hypochlorite; Irrigation; Matrix metalloproteinase (MMP-9); Post-instrumentation pain.

RESUMO

Objetivo: O estudo comparou a eficácia do Dual Rinse associado ao hipoclorito de sódio com a solução irrigadora de hipoclorito de sódio na incidência de dor pós-instrumentação, na redução da carga bacteriana intracanal e na expressão periapical de MMP-9 em pacientes com necrose pulpar. **Material e Métodos:** Trinta e quatro pacientes diagnosticados com necrose pulpar em pré-molar inferior com um único canal radicular foram incluídos. Após a

confirmação do diagnóstico, os pacientes foram alocados aleatoriamente em um dos dois grupos de 17 pacientes cada (grupo de intervenção; Dual Rinse + NaOCl 2,5%, grupo de controle; irrigação com NaOCl 2,5%). O tratamento endodôntico padrão foi realizado em duas visitas. A dor pós-instrumentação foi avaliada em 6, 12, 24 horas e 48 horas usando a escala visual analógica de Heft Parker (EVA de Heft Parker). Os níveis bacterianos intracanais foram avaliados antes e depois da instrumentação por meio da contagem de CFU/mL. Os níveis periapicais de MMP-9 foram avaliados por ELISA pós-instrumentação e pré-obturação. Todos os dados coletados dos pacientes foram analisados estatisticamente. **Resultados:** Com relação à incidência de dor pós-instrumentação, não foi detectada diferença estatisticamente significativa entre os irrigantes testados em todos os intervalos de tempo ($p>0,05$). Houve uma redução significativa na contagem de bactérias intracanais em ambos os grupos após a instrumentação, mas sem nenhuma diferença significativa entre os irrigantes testados ($p>0,05$). Em uma semana, houve uma queda considerável nos níveis periapicais de MMP-9 sem nenhuma diferença perceptível entre os dois irrigantes ($p > 0,05$). **Conclusão:** A adição do Dual Rinse à solução de NaOCl a 2,5% não resultou em nenhum aumento aparente na incidência de dor pós-instrumentação ou nos níveis periapicais de MMP-9. Ambas as soluções irrigadoras foram quase igualmente bem-sucedidas na redução da quantidade de bactérias nos canais radiculares infectados primários, mantendo a atividade antibacteriana do NaOCl.

PALAVRAS-CHAVE

Redução da carga bacteriana, Dual Rinse em hipoclorito de sódio, Irrigação, Matriz metaloproteinase (MMP-9), Dor pós-instrumentação.

INTRODUCTION

Root canal procedures are commonly associated with pain during the first 24 hours. The incidence of postoperative discomfort is reported to be up to 40% and then gradually declines [1]. Many factors affect the occurrence of postoperative pain such as: mechanical, chemical and microbial factors which are responsible for periradicular irritation [1,2]. The primary objective of endodontic therapy is to totally eradicate bacteria along with their byproducts from root canals. It is considered as a difficult task since the root canal anatomy is complex, which makes the entire removal of microorganisms challenging. All tissue remnants and debris cannot be removed using only root canal instruments. Thus, to achieve proper disinfection of the root canals, irrigants play an important role to overcome the limitations of instrumentation [3]. Sodium hypochlorite (NaOCl) is the most commonly utilized irrigant for root canal disinfection due to its excellent antibacterial action on gram positive, gram negative planktonic bacteria and its ability to effectively remove the bacterial biofilm. In addition to its proteolytic action with organic tissue dissolving ability [4]. However, the smear layer's inorganic component and accumulated hard tissue debris created during instrumentation cannot be removed by NaOCl solution alone. As a result, it is usually recommended to apply ethylenediaminetetraacetic acid (EDTA) and NaOCl in an alternating manner [5]. However, combination of EDTA with NaOCl was found to eliminate the active chlorine in the NaOCl, thus impairing its antibacterial action, and the excessive use of EDTA leads to dentine erosion [6]. Therefore, using EDTA as a final irrigant prior to obturation is advised.

Etidronic acid, also known as HEDP or 1-hydroxyethane 1,1-diphosphonic acid is biocompatible with NaOCl and has a mild chelating action [7] hence the idea of "continuous chelation" was introduced [8], which involved the use of NaOCl in combination with 9% Etridonic acid. The main clinical advantage of mixing HEDP and NaOCl during irrigation, is the application's ease and time saving over flushing root canals with two irrigating agents alternatively. The chemistry of Dual Rinse HEDP is based on sodium etidronate, the HEDP salt is added to the NaOCl for clinical use just before the treatment begins, in order to provide in order to provide single root canal irrigant that combines proteolytic and chelating properties. It is considered to be the first HEDP material advocated to be used in root canal treatment. It comes in the form of capsules with 0.9 grams of powdered etidronate, which needs to be combined with 10 mL of NaOCl prior to use. As a result, an irrigant mixture containing about 9% HEDP and active chlorine is created [9].

Several in vitro studies [10-16] and scoping review of laboratory studies [17] have shown promising findings when HEDP was mixed with NaOCl. The combination of HEDP with NaOCl was not found to impair the antibacterial

action of NaOCl in terms of eradication of *Enterococcus faecalis* within biofilms and in the dentinal tubules [10-12], this antibacterial efficacy was not diminished even in the presence of the smear layer [13]. The same combination of irrigants during canal instrumentation were associated with significantly less hard-tissue debris deposition [14,15]. The use of this irrigant mix as a final rinse did not decalcify the canal walls and was capable of adequate smear layer removal [16].

Chelating agents such as HEDP when combined with NaOCl have shown to significantly reduce debris deposition, but might have potential undesirable effects as they may excessively erode dentine in the apical portion of the canal, thus widening the apical foramen and causing irrigant extrusion, thereby increasing postoperative discomfort and/or inflammatory changes within the periapical tissues [18]. Furthermore, it is possible that HEDP reacts slowly when combined with NaOCl and limits its antibacterial action [9].

After thorough systematic online search, articles published on the use of HEDP in combination with NaOCl are mainly in-vitro studies and only one clinical trial was found measuring postoperative pain and bacterial reduction after using HEDP in combination with NaOCl versus NaOCl alone [19]. Since clinical studies are the gold standard of interventional trials as they produce the highest level of evidence, effectively aid in clinical decision-making regarding the best intervention for the patient's condition, and provide the most successful clinical outcomes for the patient's satisfaction.

Thus, the aim of this study was to compare the efficacy of Dual Rinse combined with sodium hypochlorite in comparison to sodium hypochlorite irrigating solution on post instrumentation pain incidence, reduction of the load of intracanal bacteria and periapical MMP-9 expression in patients with pulp necrosis. The null hypothesis presumed that there was no difference in postinstrumentation pain, bacterial reduction and periapical MMP-9 expression after irrigation using Dual Rinse combined with sodium hypochlorite versus sodium hypochlorite.

MATERIAL & METHODS

Ethical approval and protocol registration

The study proposal was approved by the Research Ethics Committee of the Faculty of Dentistry in Cairo university with approval number (REF No:19-7-61) and registered in Clinical Trials Registry of the US (ClinicalTrials.gov NCT04035330). The reporting of this study was done in accordance with the CONSORT guidelines.

Trial design and study settings

The study was a prospective randomized controlled blinded clinical trial, with two parallel groups and an allocation ratio 1:1. The research was carried out in compliance with the 2013 Helsinki Declaration guidelines. Each patient gave their written informed consent and were given a copy of it. This study was conducted in the outpatient endodontic department clinic inside Faculty of Dentistry, Cairo University, during the period from May November 2020 to March 2022 by the same operator.

Sample size calculation

According to results of a previous investigation [20], the anticipated mean difference in pain score at 48 hours using Heft parker VAS scale between the two groups was 20. Using power 80% and 5% significance level a total of 15 patients in each group (total 30 patients) in the two groups were studied. To compensate for losses during follow-up, the number was raised once more to a total sample size of 17 per group (drop-out rate 15%). PS: Power and Sample Size Calculation Software Version 3.1.2 was used to calculate the sample size (Vanderbilt University, Nashville, Tennessee, USA).

Eligibility criteria

ASA I or II healthy male or female patients, 18–50 years old, with necrotic permanent mandibular premolar teeth were enrolled in this trial. Clinically the diagnosis was established according to following criteria; lack of spontaneous pain and radiographically through widening of the periodontal membrane space or a periapical radiolucency within range of 2 mm. Patients with compromised medical conditions, pregnant women, history of intolerance to any medication, and those who received analgesics or antibiotics during the last 24 hours were excluded. Other exclusion criteria included teeth associated with any acute pain and/or swelling, pocket depth more than 5mm, mobility more than grade I, previously treated teeth, non-restorable teeth.

Incompletely formed roots, signs of vertical root fracture, external or internal root resorption, calcification or perforation were also excluded.

Clinical diagnosis

All patient information, including their name, phone number, address, their medical and dental history along with history of chief complaint was recorded. Extraoral and intraoral clinical examination of the suspected tooth by percussion, palpation, pulp testing and radiographic examination were precisely performed. The diagnosis of necrotic mandibular premolar teeth was confirmed by the chief complaint's history of reporting no discomfort in response to heat or cold, negative response to the electric pulp testing, where the contralateral tooth served as a control to verify the previous diagnostic tests, and radiographic assessment using intra-oral sensor plate and Digora software (Soredex, Helsinki, Finland) showed lower premolar with slight widening in the periodontal membrane space or a periapical radiolucency within 2 mm.

Randomization, allocation concealment and blinding

Participants included thirty-four patients who met the inclusion criteria. They were randomly assigned to one of two different irrigation regimens. Software (http://www.random.org/) was used to create a computerized random sequence. For the allocation concealment, the operator placed eight folded numbered papers in sealed envelopes and these papers were later dragged by the patients. The investigator, the participants, and the assessors were blinded. The assistant prepared 25 mL of the irrigant in an amber bottle and gave them to the investigator who was carrying out the root canal treatment, such that the irrigants cannot be distinguished from one another.

Root canal clinical procedures and sampling

First visit

The teeth were anaesthetized by 2% mepivacaine hydrochloride with epinephrine 1:100,000 (Mepecaine-L; Alexandria Company for Pharmaceuticals, Alexandria, Egypt) then isolated using rubber dam (Dental Dam; Sanctuary Dental, UK). The field was swabbed with 30% hydrogen peroxide and 5.25% NaOCl to thoroughly disinfect all surfaces. Access cavity was prepared by a sterile round and an Endo Z bur (Dentsply Maillefer, Ballaigues, Switzerland) under rubber dam isolation. After completion of the access, the pulp chamber and the operational field were cleansed and sanitized once more in the manner described above. Sodium 5% thiosulfate was then used to neutralize the NaOCl. Confirmation of canal patency was carried out with hand K-files (MANI Inc; Utsunomiya, Japan) size #10 or #15. Using an electronic apex locator (Root ZX; J. Morita, Irvine, CA, USA), the working length was determined then radiographically verified at 1 mm short of the radiographic apex. The canals were enlarged to size #20. Collection of the *pre-instrumentation bacterial sample S-1* was done by flushing the canal with saline and a sterile paper point #15 or #20 (Meta Biomed Co., Ltd, Korea) was then inserted for at least one minute to absorb the fluid inside the canal within 1 mm from the radiographic apex. This was done 3-4 times. Then, paper points were aseptically transferred to tubes that contain 2 mL of sterile thioglycollate broth (Merck, Darmstadt, Germany). The patients were randomly divided into 2 groups as follows; control group (2.5% NaOCl) and intervention group (Dual Rinse + 2.5% NaOCl).

Preparation of irrigation

25 mL of 2.5% NaOCl irrigation was prepared for the control group by mixing 12.5 mL of 5% NaOCl with 12.5 mL of distilled water. In the intervention group, preparation of 25 mL of 2.5% NaOCl in a similar manner as in control group and to prepare 9% Dual Rinse HEDP directly before treatment; 10 mL NaOCl was added with each Dual Rinse HEDP capsule containing 0.9 grams of the etidronate powder to create a combined irrigant. A total of 2.5 Dual Rinse HEDP capsules were used to prepare a total volume of 25 mL of the allocated irrigant. Mechanical preparation was done according to the manufacturer's recommendations utilizing M Pro rotary files sizes 18/.09, 20/.04, 25/.06, 35/.04 (Innovative materials and Devices; Shanghai, China) and an endodontic X smart motor followed by using #40 hand K-files for apical gauging. Irrigation was done with a disposable plastic syringe and a 30 gauge side vented needle (Steri irrigation tips; Diadent, Chungcheongbuk-do, Korea) that reached 1 mm less than the working length,

the canals were irrigated thoroughly using the appropriate irrigating solution at a rate of 5 mL for 1 min. Equal volume of irrigation was applied to all root canals (5 mL prior to instrumentation, 5 mL between each subsequent file, and 5 mL as the last flush after root canal instrumentation, for a total volume of 25 mL). Sterile paper points were used to dry the canal and 5 mL of 5% sodium thiosulfate solution was flushed into the canal, followed by addition of 5 mL distilled water for inactivation of NaOCl prior to obtaining the post-instrumentation bacterial sample (S-2) which was collected in a similar way as S-1 sample. The first periapical sample (post-instrumentation) (PS-1) was collected after instrumentation, by inserting sterile #20 paper point for one minute, past the apical foramen by 2mm and this process was repeated 3-4 times. Then, the paper points were put into a sterile 2 mL microcentrifugation tube that contained sterile saline solution before being quickly transported to an 80 °C freezer for further use. The access cavities were sealed by glass ionomer (Medifil, Promedica) without the use of intracanal medication. The patients were scheduled for recall after 1 week. Treatment of all participants was completed in two visits and the patients recorded their degree of post-instrumentation pain on Heft parker VAS at 6,12, 24 and 48 hours. Ibuprofen 400 mg (Novartis Pharma S.A.E., Pathion Inc., Ontario, Canada) was prescribed in case of severe pain.

Second visit

The canals were re-entered and flushed with saline under rubber dam isolation. To obtain the second periapical sample (pre-obturation) (PS-2), canals were disinfected in a sample collected in a similar manner to PS-1. Following the sampling, the final flushing of the canals was done in the control and intervention groups with 5 mL of (2.5% NaOCl- 17% EDTA), (2.5% NaOCl- 9% Dual Rinse HEDP) respectively. Passive ultrasonic irrigant activation was done for 60 seconds using a P5 Satalec ultrasonic device with an Irrisafe tip #25, zero taper. Gutta-percha master cones with a #40, 0.04 taper were fitted to the working length which was verified with a radiograph. Obturation of the canals was done with the modified single cone technique and a resin-based sealer (ADseal, Meta Biomed Co. Ltd, Korea) using the 0.04 taper gutta percha cones (Meta Biomed Co. Ltd, Korea) with auxillaries. Access cavities were sealed with composite resin (3M, ESPE, Filtek).

Post-instrumentation pain assessment

The patients recorded their degree of post-instrumentation pain on Heft parker VAS at 6,12, 24 and 48 hours. Patients were asked to place a mark anywhere on the horizontal VAS that is 170mm long. The distance between point zero and the patient's mark was measured by the operator using a ruler so that their pain level was assigned as the following: No pain; = 0mm, mild pain; >0 mm and ≤ 54 mm, moderate pain; > 54 mm and <114 mm and severe pain (≥114 mm).

Microbiological analysis

Isolation and identification of aerobic and anaerobic bacteria): Pre and post treatment root canal samples in both groups were cultured for qualitative and quantitative assessment of microbial infection. For isolation of anaerobic bacteria, the samples were streaked with a metronidazole disc (5 μg, Oxoid, Basingstoke, UK) on neomycin blood agar, blood agar as well as phenyl ethyl alcohol agar. Anaerobic incubation of the cultured plates was done in a chamber at 37 °C for 3 days with an automatic, microprocessor-controlled system for the cultivation of anaerobic bacteria. In parallel, specimens were plated aerobically on blood agar, chocolate and MacConkey agar (BD, Becton Dickinson, Heidelberg, Germany) and incubated aerobically at 37 °C for 48 hours. 10-fold serial dilutions up to $1/10^4$ were made in thioglycollate broth in order to quantify the number of bacteria. The main bacterial species isolated in culture were identified according to standard microbiological procedures on the basis of colony morphology, biochemical properties and Gram stain. The use of matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) analysis allowed for the identification of bacterial morphotypes that could not be detected using standard techniques.

Analysis of the periapical MMP-9 levels

Quantikine ELISA (Human MMP-9 Immunoassay USA & Canada | R&D Systems, Inc.Catalog Number SMP900) to quantitatively determine the concentration of both pro- (92 kDa) and active (82 kDa) human MMP-9.

Sample preparation

The extraction of MMP-9 form the paper points was done by adding 1 mL PBS then

vortexing for 15 seconds, and then centrifugation for 5 minutes at 3000xg. The supernatant was used for quantitative measurement of concentration of human active (82kDa) and Pro-(92kDa) Matrix Metalloproteinase 9 (MMP-9).

Principle of the assay

It is a quantitative technique that used the sandwich enzyme immunoassay. A microplate was precoated with a monoclonal antibody that was selectively directed against human MMP-9. Standards and samples were pipetted inside the wells, followed by binding of immobilised antibody to MMP-9. Following removal of any unbound material by washing, the human MMP-9 polyclonal antibody linked to an enzyme was added to the wells. The wells were next filled with a substrate solution, and a colour developed proportional to the amount of MMP-9 initially bounded. This was done after a wash to remove unbound antibody-enzyme reagent. The colour development was stopped, and the colour intensity was measured.

Statistical analysis

Categorical data were reported as frequency and percentage values and Fisher's exact test was used for comparisons between the groups. For comparisons within the same group, pairwise comparisons using repeated McNemar's tests with Bonferroni correction were conducted after the Cochran q test. Numerical data values were presented in the form of mean and standard deviation (SD), they were checked for normality using Shapiro-Wilk test. VAS data showed non-parametric distribution so they were analyzed using Mann-Whitney U test for comparisons between the groups and Friedman's test followed by Nemenyi post hoc test for comparisons within the same group. Bacterial count data were log transformed to correct for positive skewness and with other numerical data they were found to be normally distributed. For intergroup comparisons, the independent t-test was employed, and for intragroup comparisons, repeated ANOVA measures were conducted followed by the Bonferroni post hoc test. All tests were conducted with a significance level of $p<0.05$. R statistical analysis software, windows version 4.1.3 was used to conduct the statistical analysis [21].

RESULTS

Out of 50 patients meeting the eligibility criteria, a total of 34 patients met the inclusion criteria and were enrolled, patients were randomized into one of two groups (17 patients each). All patients were included in the analysis as shown in (Figure 1). No statistically significant difference was detected among the tested groups regarding age, gender, tooth type (first or second mandibular premolar) as shown in (Table I).

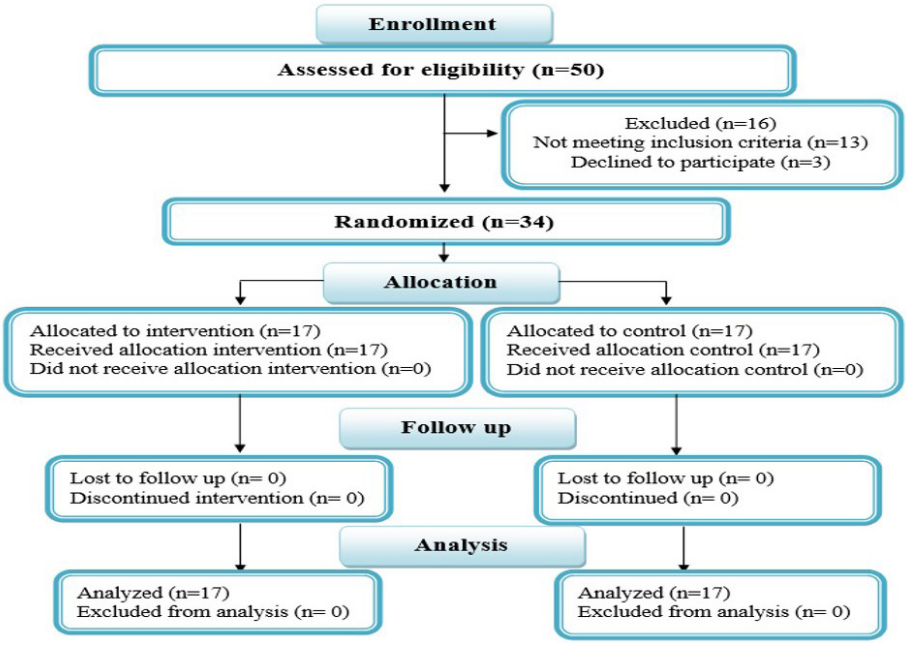

Figure 1 - CONSORT 2010 Flow diagram of the trial design.

Post-instrumentation pain

At 6 hours, a higher incidence of post-instrumentation pain was recorded in the Dual Rinse + NaOCl group (5.9%) compared to no pain reported in the NaOCl group. At 12 hours, both groups showed a similar incidence of post-instrumentation pain (23.5%), while at 24 hours, a higher incidence was reported in the NaOCl group (23.5%) compared to the Dual Rinse + NaOCl group (17.6%). Finally, at 48 hours, the incidence of pain was higher in the Dual Rinse + NaOCl group (5.9%) compared to no pain reported in the NaOCl group. The only pain category reported among participants at any time intervals was the mild category. There was no statistically significant difference detected among the tested groups at any time interval ($p>0.05$).

Microbiological analysis

Incidence of bacterial growth

All pre-instrumentation root canal samples at S-1 showed positive microbial growth in both groups. Irrigation with Dual Rinse + NaOCl rendered 47.1% of canals free from bacteria (8 out of 17 cases), while irrigation with NaOCl rendered 41.2% of canals free from bacteria (7 out of 17 cases). There was no significant difference between both groups regarding incidence of aerobic, anaerobic and total bacterial growth at S-1 and S-2 time periods ($p>0.05$) (Table II).

Percent % of bacterial load reduction

The mean % reduction for the total bacterial count was higher in Dual Rinse + NaOCl group (72.35±40.97) than in the NaOCl group (68.92±41.31) with no statistically significant difference between the two groups ($p=0.759$) (Figure 2).

Bacterial species

As determined by MALDI-TOF, the isolated aerobes were found to belong to four species, predominated by *Streptococcus mitis* and *Enterococcus faecalis*. The identification of anaerobic isolates by MALDI-TOF revealed nine species with the highest frequencies shown for *Fusobacterium, Parvimonas, and Eikenella*. There was no significant difference between both groups in terms of the presence of different

Table I - Mean, standard deviation (SD), frequencies (n), and percentages (%) for comparison of demographic data between the two groups

Parameter			NaOCl	Dual Rinse + NaOCl	p-value
Gender	Male	n	6	5	1 ns
		%	35.3%	29.4%	
	Female	n	11	12	
		%	64.7%	70.6%	
Age	Mean ± SD*		32.94±9.40	35.82±7.02	0.319 ns
Tooth type	Mandibular 1st Premolar	n	7	6	1 ns
		%	41.1%	35.3%	
	Mandibular 2nd Premolar	n	10	11	
		%	58.9%	64.7%	

Significant ($p \leq 0.05$). ns: non-significant ($p>0.05$).

Table II - Frequency and percentage values for incidence of bacterial growth between two groups at S-1 and S-2

Bacterial type	Incidence of Bacterial growth at time intervals		NaOCl	Dual Rinse +NaOCl	p-value
Total	**S-1**	n (%)	17 (100.0%)	17 (100.0%)	NA
	S-2	n (%)	10 (58.8%)	9 (52.9%)	1 ns
Aerobic	**S-1**	n (%)	14 (82.4%)	13 (76.5%)	1 ns
	S-2	n (%)	6 (35.3%)	2 (11.8%)	0.225 ns
Anaerobic	**S-1**	n (%)	13 (76.5%)	15 (88.2%)	0.653 ns
	S-2	n (%)	4 (23.5%)	7 (41.2%)	0.463 ns

Significant ($p \leq 0.05$). ns: non-significant ($p>0.05$). N/A: means not applicable

types of bacteria at both time intervals and there was no clear bacterial species selection by either treatment groups, and similar taxa prevalence across the two groups ($p>0.05$) (Table III, Table IV, Figure 3, Figure 4).

Periapical MMP-9 levels

At the first treatment visit, mean values of MMP-9 were (217.82±82.94) in the NaOCl group and (177.87±39.63) in the Dual Rinse + NaOCl group. These values dropped significantly in the second visit reaching mean values of (92.45±14.66) in the NaOCl group and (85.88±18.46) in the Dual Rinse + NaOCl group. There was no statistically significant difference in the ability of any of both irrigants to lower periapical MMP-9 levels ($p>0.05$).

DISCUSSION

One of the fundamental goals of endodontic therapy is controlling and reducing pain during and particularly after root canal treatment.

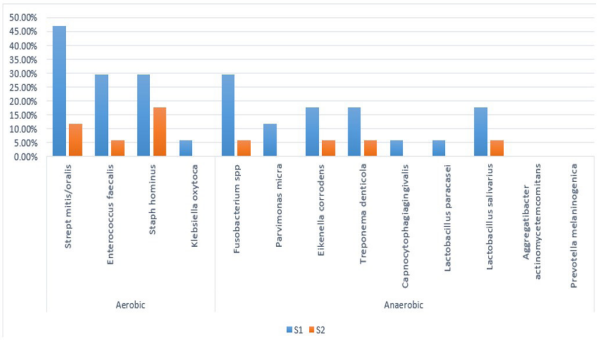

Figure 3 - Bar chart showing percentage of bacterial species at S1 and S2 in NaOCl group.

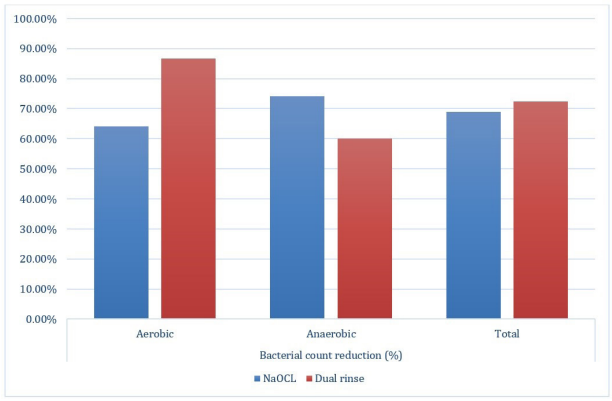

Figure 2 - Mean values of percent % reduction of aerobic, anaerobic and total bacterial count for the two groups.

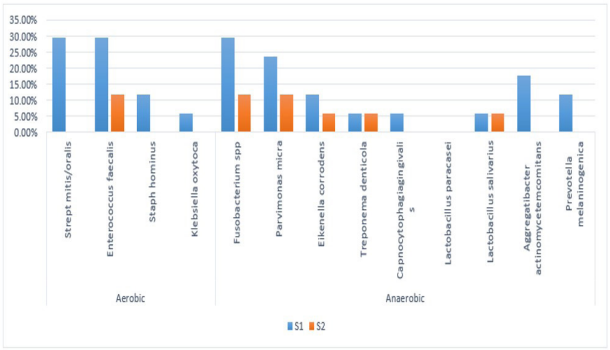

Figure 4 - Bar chart showing percentage of bacterial species at S1 and S2 in Dual rinse + NaOCl group.

Table III - Frequency (n) and % values for bacterial species at S1and S2 in NaOCl group

Group	Bacterial species	S1	S2	p-value
Aerobic	*Strept mitis/oralis*	8 (47.1%)	2 (11.8%)	0.710ns
	Enterococcus faecalis	5 (29.4%)	1 (5.9%)	
	Staph hominus	5 (29.4%)	3 (17.6%)	
	Klebsiella oxytoca	1 (5.9%)	0 (0.0%)	
Anaerobic	*Fusobacterium spp*	5 (29.4%)	1 (5.9%)	0.973ns
	Parvimonas micra	2 (11.8%)	0 (0.0%)	
	Eikenella corrodens	3 (17.6%)	1 (5.9%)	
	Treponema denticola	3 (17.6%)	1 (5.9%)	
	Capnocytophagiagingivalis	1 (5.9%)	0 (0.0%)	
	Lactobacillus paracasei	1 (5.9%)	0 (0.0%)	
	Lactobacillus salivarius	3 (17.6%)	1 (5.9%)	
	Aggregatibacter actinomycetemcomitans	0 (0%)	0 (0%)	
	Prevotella melaninogenica	0 (0%)	0 (0%)	

Table IV - Frequency and % values for bacterial species at S1and S2 in Dual rinse+ NaOCl group

Group	Bacterial species	S1	S2	p-value
Aerobic	*Strept mitis/oralis*	5 (29.4%)	0 (0%)	0.451 ns
	Enterococcus faecalis	5 (29.4%)	2 (11.8%)	
	Staph hominus	2 (11.8%)	0 (0.0%)	
	Klebsiella oxytoca	1 (5.9%)	0 (0.0%)	
Anaerobic	*Fusobacterium spp*	5 (29.4%)	2 (11.8%)	0.836 ns
	Parvimonas micra	4 (23.5%)	2 (11.8%)	
	Eikenella corrodens	2 (11.8%)	1 (5.9%)	
	Treponema denticola	1 (5.9%)	1 (5.9%)	
	Capnocytophagiagingivalis	1 (5.9%)	0 (0.0%)	
	Lactobacillus paracasei	0 (0.0%)	0 (0.0%)	
	Lactobacillus salivarius	1 (5.9%)	1 (5.9%)	
	Aggregatibacter actinomycetemcomitans	3 (17.6%)	0 (0%)	
	Prevotella melaninogenica	2 (11.8%)	0 (0%)	

Significant ($p \leq 0.05$) ns: non-significant (p>0.05).

Although the exact cause of postoperative pain is unknown, it has been linked to various causes, of which the microbiological aspect is one of most important factors. Periapical inflammation is one of the most prevalent causes of pain experienced after RCT as a result of microorganisms remaining within the root canal or debris extruded apically in the periapical tissues that cause irritation [22].

The complete eradication of bacteria from infected root canals through effective irrigation and instrumentation is a prerequisite for effective endodontic therapy [23]. Several antimicrobial agents have been used in endodontics to remove debris, smear layer, necrotic pulp tissue and to ensure proper eradication of microorganisms. Thus, for proper disinfection and smear layer removal, the combination of NaOCl with a chelating agent such as EDTA represented the gold standard. To counteract the problems with the use of EDTA, it has been suggested to use the mild chelator HEDP, which has short-term compatibility with NaOCl [7].

Several in-vitro investigatons [10-16] on the usage of NaOCl and HEDP together as a root canal irrigant had promising results. However, it should be considered that most of these studies are in-vitro and that those based on evidence are still lacking.

The present study included thirty-four participants with necrotic pulp and slight widening or small periapical radiolucency within 2mm. Large periapical radiolucencies were not included in the study because they may suggest a longer-lasting root canal infection and may also be a sign of extra-radicular infection or cystic transformation, both of which have the potential to negatively affect the success rate [24,25].

2.5% NaOCl was used in the current investigation because it is less cytotoxic than 5.25% NaOCl. Lower NaOCl concentrations were related to less frequent post-endodontic discomfort than higher concentrations used in previous randomized clinical studies treating nonvital pulps and both concentrations had similar antibacterial effectiveness [26-29].

In order to prevent the possibility that intracanal medicament to increase the likelihood of post-instrumentation pain, bacterial load reduction and MMP-9 expression, no intracanal medication was used. Several randomized clinical trials investigated the effects of irrigants and irrigation devices without the use of intracanal medications to avoid their chemical effects, particularly when extruded [27,30,31].

The results of this study revealed that the demographic information (age, gender and tooth type of participants) of the two groups did not significantly differ between them. Thus, provided that the two groups had similar baseline characteristics which indicate a successful randomization, removing any confounding factors and enabling adequate comparison of the intervention's impact on the two groups.

Pain assessment by the patients was done at 6, 12, 24 and 48 hours. In order to be sure that the effects of the local anesthetic worn off, pain

assessment began six hours postoperatively [32]. According to Pak and White [33] the incidence and degree of postoperative pain reported by patients is significant in the first day and diminishes significantly to low levels throughout the course of the first two days, thus a maximum of 48 hours was chosen.

The majority of the patients showed no postoperative pain after Dual Rinse + NaOCl or NaOCl irrigation alone, and if pain occurred, it was in the mild pain category with no significant difference between both irrigants. This came in agreement with a prior randomized clinical trial by Ballal et al. [19].

Regarding the effectiveness of the irrigating solution on intracanal bacterial growth, both NaOCl and Dual Rinse + NaOCl groups revealed a considerable decrease in incidence of bacterial growth after instrumentation in comparison to pre-instrumentation values. This is consistent with results from prior clinical trials that investigated the effectiveness of irrigation and instrumentation for disinfecting and reducing intracanal bacteria [34-37].

Dual Rinse + NaOCl irrigation rendered 47% of the canals free from bacterial growth, while NaOCl irrigation rendered 41% of canals free from bacteria, with no significant difference between the two irrigating solutions. These results show that when both irrigants are combined fresh, HEDP does not reduce the antibacterial activity of NaOCl, which is highly advantageous in clinical settings.

The results of our study are in agreement with a randomized clinical trial by Ballal et al. [19], which previously showed that irrigation with a mixture of Dual Rinse HEDP and 2.5% NaOCl irrigant resulted in the lack of culturable bacteria in half of the sampled canals, as opposed to 40% when 2.5% NaOCl was only used. Our study results are also supported by findings of previous clinical trials [35,38] in which irrigation with 2.5% NaOCl rendered 40-60% of the root canals free from cultivable bacteria.

The rationale for the antibacterial efficacy obtained with the Dual Rinse could be explained by the action of continuous chelation that prevents the formation of a smear layer, allowing greater NaOCl penetration into the dentinal tubules, promoting bacterial reduction. This rationale is in alignment with previous findings from other invitro studies [4,15,16]. Another possible rationale that the continuous chelation approach was able to allow for the irrigant to penetrate and disturb the bacterial biofilm matrix, allowing for improved NaOCl contact with the bacteria, increasing its antibacterial efficacy. This has been reported in previous in-vitro studies [10,11].

Percent reduction of intracanal bacteria for Dual Rinse + NaOCl irrigation was more than NaOCl group but with no significant difference between the two irrigating solutions. The lack of a significant difference between the two irrigating solutions in a clinical situation could be linked to the regular and abundant irrigation volume [39]. This can enhance the benefits of mechanical preparation and eradication of the bacteria regardless of the irrigant type utilized.

In this study, there was no evident selection of either aerobic or anaerobic taxa in any group. The microorganisms detected inside the root canals are consistent with findings from previously published studies about teeth with primary asymptomatic apical periodontitis [19,35,40,41] where the Streptococcus mitis and *Enterococcus faecalis* were found as the main aerobic taxa. *Staphylococcus* was also identified but less frequently. The main anaerobic taxa identified was *Fusobacterium* species, while other anaerobic taxa identified but less frequent than *Fusobacterium* were *Parvimonas micra, Treponema denticola, Capnocytophagiagingivalis*, *Prevotella melaninogenica*. The microbial composition observed in our study aligns with previous research findings, which have consistently reported the prevalence of Gram-negative anaerobic bacteria in primary untreated root canal infections with necrotic pulp [19,42,43].

The periapical MMP-9 expression was measured post-instrumentation and pre-obturation using periapical fluid analysis. MMP-9 was chosen as it was demonstrated to be crucial in apical periodontitis through the initiation of osteoclastic bone resorption- [44]. MMP-9 is also crucial in inducing pain through proinflammatory cytokines that modulate and increase expression of MMP-9 by nerve cells following peripheral nerve injury [45].

The results of periapical levels MMP-9 came in agreement with a previous study [19] which showed no statistically significant difference between both irrigants at both time intervals on the periapical MMP-9 levels.

There was a statistically significant reduction in MMP-9 levels from post-instrumentation levels to the pre-obturation levels measured one week after the first visit in both groups. This result is consistent with earlier research that found a substantial decrease in periapical MMP-9 levels between the two visits [46,47] and in another study [19] in which Dual Rinse + NaOCl as well as NaOCl irrigation alone significantly reduced the MMP-9 expression from baseline levels when teeth were sampled 1 week after the cleaning and shaping procedure. The antimicrobial activity of the irrigants may be responsible for the decrease in MMP-9 levels, which was confirmed by microbiological analysis.

The clinical significance of the present work showed that Dual Rinse seems to be a promising root canal irrigant with no fear of loss of antibacterial efficacy of NaOCl and no apparent increase in post-operative pain at clinical levels or histological levels as determined by levels of inflammatory marker MMP-9.

CONCLUSION

The present study accepted the null hypothesis. There was no increase in the incidence of post-instrumentation pain by the addition of Dual Rinse to 2.5% NaOCl. The antimicrobial efficacy of the NaOCl was not affected, with no increase in periapical MMP-9 and no obvious periapical inflammation in the form of postoperative pain. Both irrigating solutions successfully reduced bacterial counts from primary infected root canals, without any significant difference between them.

Author's Contributions

LZIAH: Methodology, Investigation, Writing – Original Draft Preparation. RMEB: Conceptualization, Writing – Review & Editing, Supervision. SIG: Conceptualization, Methodology, Writing – Review & Editing. STS: Investigation, Writing – Review & Editing. NSS: Investigation, Writing – Original Draft Preparation.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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Regulatory Statement

Every procedure used in the study complied with the 1964 Helsinki Declaration and its later 2013 revisions, as well as the ethical guidelines established by Cairo University's institutional research council approval number (REF No:19-7-61).

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