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Research Article

Effect of Combination of Calcium Hydroxide and Chlorhexidine Gel 2% as Intracanal Medication in Comparison to Calcium Hydroxide Paste as Intracanal Medication on Postoperative Pain and Bacterial Endotoxin in Necrotic Teeth: A Randomized Controlled Trial

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Abstract

Aim: The aim of this study is to evaluate the effect of calcium hydroxide combined with 2% chlorhexidine gel as intracanal medication compared to calcium hydroxide paste on postoperative pain reduction as the primary outcome and endotoxin reduction as the secondary outcome in a patient with necrotic pulp on two visit treatments.

Materials and Methods: Forty-three patients with necrotic single-rooted tooth with apical periodontitis, aged ranged from 18 to 49 years old with no gender predilection. The participants were randomly assigned into three groups according to the intracanal medication used; (Calcium hydroxide and chlorhexidine 2% gel group I, n = 15), (Calcium hydroxide paste alone group II, n = 15) and (no intracanal medication group III, n = 13). The preoperative pain was recorded using numerical rating scale (NRS). After isolation and disinfection, access cavity was prepared followed by chemomechanical preparation, which was done using rotary Revo-S files with 2.5% NaOCl irrigation. Then, intracanal medication was placed for 14-days. Postoperative pain was recorded at 4, 24, 48 hours and 14-days postoperatively. Paper points collected endotoxin samples before (S1) and after chemomechanical preparation (S2), and after 14-days of intra-canal medication (S3). A sandwich ELISA method was used for the quantification of endotoxin. Patients were randomly assigned to any of these three groups.

Results: Each group resulted in an increase in median pain value from preoperative to 24 hours postoperative record, followed by a gradual decrease from 48 hours to 14-days postoperatively with a statistically significant difference. When comparing to control group (III), Calcium hydroxide combined with chlorhexidine 2% gel group (I) showed lower postoperative pain than that of calcium hydroxide paste group (II) at 24 and 48 hours and 14-days. There was statistically significant difference between the three groups ($p < 0.001$). Regarding endotoxin level, all groups showed marked decrease in endotoxin median values from (S1) to (S3), where the group (I) showed significantly least endotoxin value at (S3) ($p < 0.001$). When compared to group (II) and group (III) groups ($p < 0.0001$).

Conclusion: The use of intracanal medication for 14-days in necrotic teeth with apical periodontitis was more reliable in reducing postoperative pain regardless the type of medication used. However, endotoxin level in the infected root canals was reduced significantly after root canal preparation and further reduced after 14-days of intracanal medication, where calcium hydroxide combined with 2% chlorhexidine gel showed higher endotoxin reduction when compared to calcium hydroxide alone.

Keywords: Calcium Hydroxide Paste; Chlorhexidine 2% Gel; Postoperative Pain (NRS); Endotoxin

Introduction

Postoperative pain after endodontic treatment is a displeasing occasion for the patients and clinicians. Although postoperative pain associated with root canal therapy is a poor indicator of long-term success, it's occurrence and control of clinical interest in endodontics. Certain factors such as preoperative pain, number of

appointments, use of intracanal medications and tooth position may predispose the development of postoperative pain and flare-ups [1]. Therefore, the outcome of the endodontic treatment depends significantly on the elimination of bacterial population from the root canal, or maximum reduction in bacterial count to the levels compatible with the periradicular tissue healing [2].

Apical periodontitis is an inflammatory disease of microbial etiology primarily caused by infection of the root canal system [3]. However, endodontic infections usually develop after pulpal necrosis or in cases in which the pulp was removed for treatment [4]. Therefore, bacteria are the major microorganisms implicated in the etiology of apical periodontitis [5].

The mainly intracanal medications used in clinical practice is the calcium hydroxide. It has initial bactericidal then bacteriostatic effect and characterized by high pH [6]. It has an antimicrobial activity against common endodontic microbes but indeed, [7,8] that it less effective against *E. faecalis* and *C. Albcian's. Calcium hydroxide* has pain preventing properties because of its antimicrobial or tissue-altering effects [9].

Chlorhexidine at low concentrations, low molecular weight substances will leak out resulting bacteriostatic effect and bactericidal effect at high concentration Gomes., *et al*. [10]. The combination of calcium hydroxide and chlorhexidine as intracanal medication has been studied and found to be the efficacious in reducing the postoperative pain [11-13].

This randomized single-blind study was designed to compare the effectiveness of different intracanal medicaments with placebo in reducing postoperative pain and reduction of endotoxin.

Participants and Methods

The protocol and the informed consent forms were approved by the Research Ethics Committee, Faculty of Dentistry, Cairo University, as to their compliance with the regulations followed for conducting human research. A written informed consent was obtained from each patient who kept a copy of it.

Patient selection

Forty-three patients were confined with necrotic single-rooted tooth with apical periodontitis, aged ranged from 18 to 51 years old with no gender predilection, presenting to the department of Endodontics, Faculty of Dentistry, Cairo University for necrotic cause were selected. The diagnosis was performed by negative response to sensitivity pulp tests. Sensitivity pulp test was performed through thermal stimulation with (Endo-Ice spray, Henry Schein, Germany), negatively responded to electric pulp tester (Denjoy DY310 Dental Pulp Tester, Henan, China) and had no periapical radiolucency in periapical radiographs. Further status was confirmed by absence of vital pulp/bleeding during access opening. Clinical and radiographic evidence of apical periodontitis was confirmed by tenderness to percussion and widening of periodontal ligaments space. The patients accepted two visit treatment and criteria for postoperative pain evaluation, the tooth was functional, and the patients was in good general health. Multi-rooted teeth, pulp hyperemia or any conditions other than pulp necrosis-like pulpitis, pregnant women, previously accessed teeth and patients suffering from chronic diseases excluded criteria for our study.

The nature of this study, complications and associated risks were fully explained to the patients and consents were obtained before initial treatment. Patients were assigned to the medication group randomly using computer generated random number table. The operator had no involvement with study outcome. To ensure blinding; neither the operator nor the patient had knowledge about the medication used. Allocation sequence was concealed from researchers who were part of study to reduce bias.

Sampling procedures

All materials, used in this study, were heat sterilized at 250 °C for 30 min, becoming free of endotoxin.

All the patients were anaesthetized with standard infiltration and inferior alveolar nerve block injections by using Articaine forte 4% and Adrenaline 1:100,000 (Carpule 3M™ Espe™ Ubistesin™ forte 3M Germany). Teeth were isolated from the oral cavity with rubber dam, and disinfection of their external surfaces and surrounding areas was carried out by using 3% hydrogen peroxide followed by 2.5% sodium hypochlorite NaOCl. Subsequently, 5% sodium thiosulfate to avoid interference with bacteriologic sampling.

A 2-stage access preparation was performed. The access cavity was made without the use of water spray but under manual irrigation with sterile/endotoxin-free saline and by using sterile/endotoxin-free high-speed diamond bur. This first stage was performed to promote a major removal of the contaminants (microorganisms and caries).

In the second stage, before entering the pulp chamber, the access cavity was disinfected according to the decontamination protocol described above.

The first endotoxin sample (S1) was taken after access cavity preparation by introducing the paper points (Dentsply, Maillefer, Ballaigues, Switzerland) into the full length of the canal confirmed by radiograph and retained in position for 60 seconds. The samples were placed in a pyrogen-free eppendorf tubes and stored at -80C.

After the first sampling, the root canal was explored with a K-file size #10 (Dentsply-Maillefer, Ballaigues, Switzerland), root canal length was determined from the preoperative radiograph and confirmed by the apex locator (iPex II NSK, Tochigi, Japan). The root canal was done by crown down technique using Revo-S instruments (Micro Mega, Besancon Cedex, France) The sequence of the root canal preparation started with SC1 Then SC2. Finishing files started with SU, AS 30/06, AS 35/06, AS 40/06 apical finishing, using an endodontic motor (Endo-Mate TC2, NSK, Tochigi, Japan.) adjusted according to the manufacturer's instructions. Before the use of each instrument, irrigation was performed with a syringe (side vented needle of 30-Gauge) containing 3-ml of 2.5% sodium hypochlorite solution, subsequently, 5 mL saline solution was used to wash the canal.

The Second endotoxin sample (S2) was taken just after chemo mechanical instrumentation. Then, the canals were dried with sterile paper points and filled with intracanal medication plugged into the canal by using Lentulo spiral (Dentsply Maillefer) according to the randomly selected groups: group (I) CHX (Gluco-CHeX 2% gel, Cerkamed Medical Co. Poland) in equal parts (w/w) mixed with $Ca(OH)_2$ (CalciPaste, Cerkamed Medical Co. Poland), group (II) $Ca(OH)_2$ (CalciPaste, Cerkamed Medical Co. Poland) paste alone and group (III) received cotton pellet (placebo). The access cavities were properly filled with Glass ionomer (Medifil, Promedica, Germany) filling to ensure proper sealing with no leakage of any oral fluids inside the root canal, which might disturb the action of the intracanal medication.

The third endotoxin sample (S3) was taken after 14-days of intracanal medication. The canals were accessed under rubber dam isolation by using the protocol of disinfection described previously. The intracanal medication was removed by irrigating canal with 5ml saline and filing the canal with the master apical file, all patients returned to the clinic with their recorded postoperative pain charts. Then, CHX antimicrobial activity was inactivated with 5 mL of a solution [5% Tween 80 and 0.07% (w/v) lecithin] during 1 min, which was then flushed out with 5 mL of sterile/endotoxin-free saline solution [14]. Before the second sampling procedure (S2), a continuous rinse with 5 mL of 17% EDTA solution for 3 min followed by a final rinse with 5 mL of sterile/endotoxin-free saline solution was performed.

Recording postoperative pain

Patients were told to evaluate pain experienced 4 hrs. after treatment and daily for additional 24, 48 hrs. and 14-days according to the numerical rating scale (NRS).

Sandwich-ELISA method (Quantification of endotoxin)

Quantification of endotoxin was done using the Sandwich-ELISA method at predetermined time intervals (after access, after instrumentation and after 14-days days of intracanal medication). The Micro-elisa strip plate provided in the ELISA kit was pre-coated with an antibody specific to ET. Samples were added to the appropriate Micro-elisa strip plate wells and combined to the specific antibody. Then, a Horseradish Peroxidase (HRP)-conjugated antibody specific for ET was added to each Micro-elisa strip plate well and incubated. Free components were washed away. The TMB substrate solution was added to each well. Only those wells that contain ET and HRP conjugated ET antibody appeared blue in color and then turned yellow after the addition of the stop solution. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value was proportional to the concentration of ET. The concentration of ET in the samples was calculated by comparing the OD of the samples to the standard curve. Sandwich-ELISA method was chosen to quantify endotoxin levels in post-treatment apical periodontitis because of its extreme sensitivity for the detection of minute quantities of endotoxin.

Results

Clinical data of pain intensity after 4 hours, the median and range of the NRS scores for Group I: was 2(0-6), for Group II: 1(0-5) and for Group III: 1(0-7) with no statistically significant difference between three groups ($p = 0.27$). After 48hours, the median and range of the NRS scores for Group I: was 0(0-3), for Group II: 0(0-2) and for Group III: 1(0-6) that showed statistically significant more pain compared to other groups ($p = 0.012$). After 14-days, the median and range of the NRS scores for Group I: was 0(0-3), for Group II: 0(0-2) and for Group III: 0(0-6) that showed statistically significant more pain compared to other groups ($p = 0.026$). Comparing median pain score over time in each single group was statistically significant ($p < 0.001$) (Table 1). During the study, no complication

	Group I			Group II			Group III			P value1
	Median	Min.	Max.	Median	Min.	Max	Median	Min.	Max.	
Different times										
4 Hours	2	0	6	1	0	5	1	0	7	0.27
24 Hours	1	0	4	0	0	3	1	0	6	0.095
48 Hours	0	0	3	0	0	2	1	6	6	0.012
14 Days	0	0	3	0	0	2	0	0	6	0.026
P value 2	<0.001			<0.001			<0.001			

Table 1: Median and range of NRS score at different time points in the tested groups and overtime in each Group.

associated with any of the intracanal medications was detected or reported by any patient.

Endotoxin were detected in 100% of the all samples (S1). Group I: was 23.05 (1.35-661.4), for Group II: 36.15 (1.76-1280.1) and for Group III: 110.2 (4.73-689.76) with no statistically significant difference between the three groups (p = 0.26) (Figure 1). Higher levels of endotoxin in the first samples (with a median value of 175 EU/mL, ranging from 93–277) than in paired RCs (with a median value of 41.5 EU/mL, ranging from 7.26–63; P = .005) (Figure 1). After chemomechanical preparation using rotary instrumentation (Revo-S) and Irrigating solution (S2), the median and range of the bacterial endotoxin for Group I: was 4.63(0.13-459.3), for Group II: 9.1(1.35-1070) and for Group III: 86.6(4.26-389.44) with statistically significant more bacterial endotoxin in group III compared to other groups (p = 0.032) (Figure 1). Subsequently, after the use of intracanal medication (S3), the median and range of the bacterial endotoxin were for Group I: 1.35 (0.01-15.13), for Group II: 5.4 (0.95-812) and for Group III: 64.6 (2.9-217.09) with statistically significant more bacterial endotoxin in group III compared to other groups (p < 0.0001).

The flow of the patients through the study is presented in the CONSORT flow diagram in figure 2. The baseline characteristics, preoperative pain, CHX mix $Ca(OH)_2$, $Ca(OH)_2$ only and placebo groups are presented in Table (1). Forty-three patients with apical periodontitis participated in this study (24 males, 19 females). They were randomly divided into three equal groups. Two patients one from placebo group were excluded after receiving the intervention as they did not meet the confirmatory inclusion criteria. There was no statistically significant difference between three groups for a tooth type, age and gender distribution, preoperative pain.

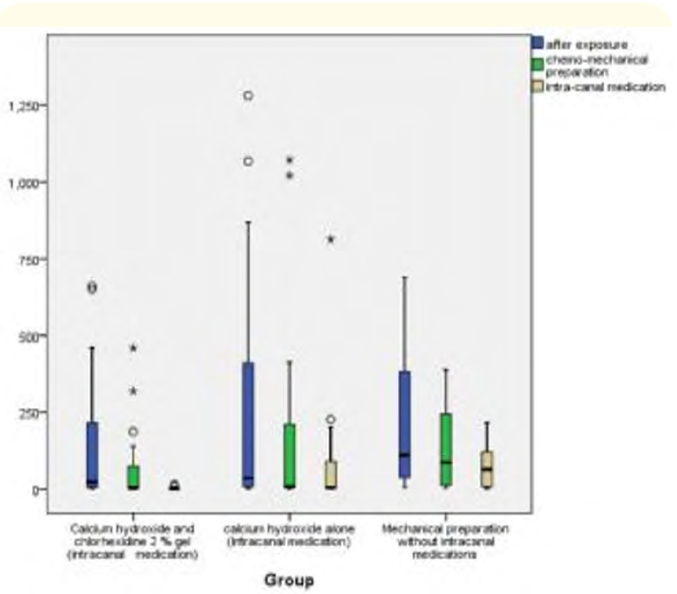

Figure 1: Box plot showing the median and range of bacterial endotoxin reduction in the tested groups.

Discussion

Cases with apical periodontitis were selected for this study. Therefore, certain factors affecting the treatment outcome were eliminated. Occlusal reduction was done in the first visit and apical patency was determined. Apical patency was not maintained during root canal preparation, although Arias., *et al*. [15] reported that there was significantly less postoperative pain when apical patency was maintained in non-vital teeth. However, this concept is controversial and not established yet. Moreover, Arias., *et al.* [15] also suggested that patient with preclinical symptoms results in longer duration of pain when apical patency was maintained. The results of the present study revealed significant information on intracanal medications in controlling postoperative pain. There was

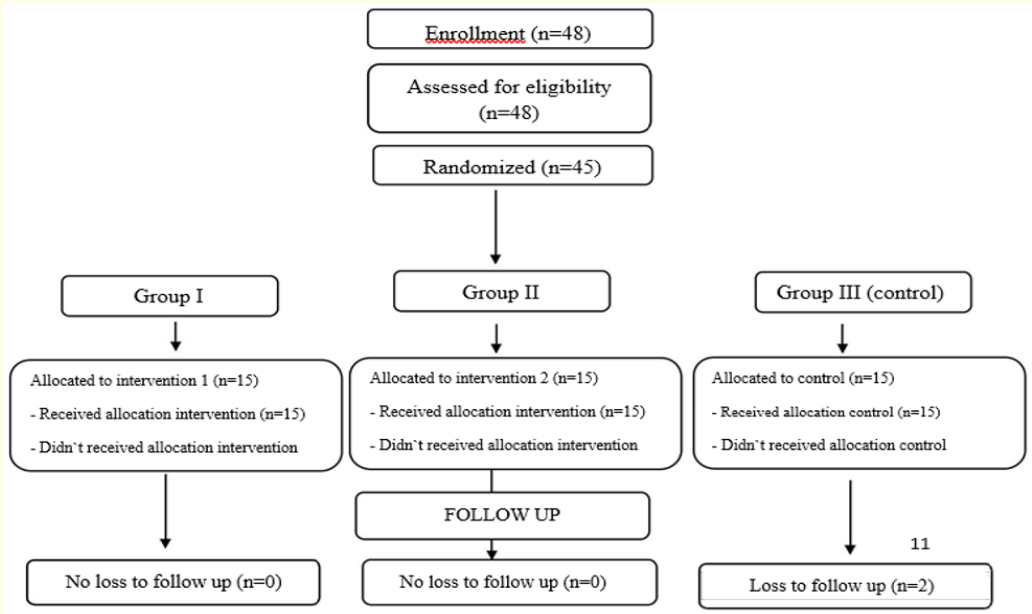

Figure 2: CONSORT flow diagram of the study.

	Group I			Group II			Group III			P value1
Different times	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	
After exposure	23.05	1.35	661.4	36.15	1.76	1280.1	110.2	4.73	689.76	0.26
After chemomechanical preparation	4.63	0.13	459.3	9.1	1.35	1070	86.6	4.26	389.44	0.032
After intracanal medication	1.35	0.01	15.13	5.4	0.95	812	64.6	2.9	217.09	<0.0001

Table 2: Median and range of bacterial endotoxin reduction in the tested groups.

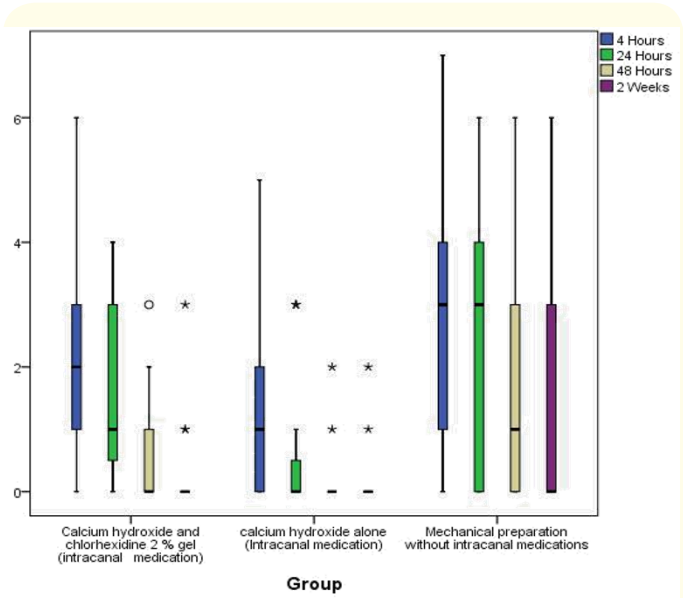

Figure 3: Box plot showing the median NRS score in the tested groups.

greatest reduction in the pain values with chlorhexidine containing medications. It became apparent that the greatest reduction in pain took place when chlorhexidine alone or in combination with calcium hydroxide was used, with the greatest effect occurring during the first four hours after treatment followed by gradual decrease during subsequent days. Although the initial pain scores in cases treated with chlorhexidine [16] was more than that for the other drugs [17,18], it had moved well below the other two from the 4th h postoperatively; the pain value of chlorhexidine remained well below that of other two over the next four days.

The fast and continuous action of chlorhexidine in controlling postoperative pain is striking. Its effect was measurable in 4 h after placement, even though medication had to diffuse into the dentinal tubules and the periapical tissues. It corroborates with the findings of previous studies [19,20], that found that chlorhexidine gel provided 100% inhibition of microorganisms at the depth of 200 µm as well as 400 µm from the day 1 and thus demonstrating its high infusibility. Moreover, the effectiveness of chlorhexidine as

intracanal medication in controlling the postoperative pain might be because of its ability to reduce or eliminate the endotoxin associated with the development of spontaneous pain. However, Gomes., *et al*. [21] evaluated that 2% chlorhexidine gel was not effective in eliminating endotoxin from the primary infected root canals. However, in their study chlorhexidine was used as an irritant and not as an intracanal medication. Furthermore, the present study is not in concurrence with that of Gama., *et al*. [22], who reported that intracanal dressings with 0.2% chlorhexidine gluconate or calcium hydroxide in combination with CPCM were equally effective in reducing the postoperative pain. The difference in results could be because of lower percentage of chlorhexidine used. Apart from the positive antimicrobial efficacy of chlorhexidine, it is important that 0.1% to 2% chlorhexidine preparations were considered as toxicologically safe [23].

In the present study, calcium hydroxide was effective medication in reducing the postoperative pain also. Despite good antibacterial properties shown by previous studies, many later studies have demonstrated the inability of calcium hydroxide to eliminate bacteria commonly found in the root canals [24] and those penetrated in dentinal tubules [25]. The limited action of calcium hydroxide could be because of the buffer effect that dentin exerts over calcium hydroxide, reducing its antimicrobial action [26]. In addition, few studies [24,25] found that certain bacteria present in root canal system were resistant to high pH of calcium hydroxide. In this study, the combination of calcium hydroxide and chlorhexidine was found to be the most effective in reducing the postoperative pain. Yoldas., *et al*. [27] reported that two-visit endodontic treatment with intracanal medication with calcium hydroxide in combination with chlorhexidine decreased the postoperative pain in retreatment cases. It might be due to its high pH suggesting an increase of the ionized capacity of the chlorhexidine molecule [28,29]. Moreover, Martinho., *et al*. [30] concluded that there all the 7- and 14-day intracanal medications were effective in reducing bacteria and endotoxin as well as in lowering the levels of inflammatory cytokines, with CHX showing limited effectiveness against endotoxin. The addition of chlorhexidine to calcium hydroxide lowers its contact angle and improves the wettability of the medication on the root canal [31]. This could also be because of synergistic effect of calcium hydroxide and chlorhexidine on liposaccharides/ endotoxin produced by gram negative bacteria. Hence, both the intracanal medications complement their actions. This corroborates the findings of another study [14], which found that the antimicrobial efficacy of calcium hydroxide is increased when used in combination with chlorhexidine. However, according to Schafer and Bossmann [31], chlorhexidine alone was a more effective antimicrobial than its combination with calcium hydroxide whereas Delgado., *et al*. [32] concluded that there was no difference between the antimicrobial activity of chlorhexidine with or without calcium hydroxide. The use of intracanal medications inhibits the growth of bacteria resulting in the reduction of microbial factors responsible for pain and inflammation as well as endotoxin reduction. Under the conditions of the present study, it was concluded that pain associated with teeth having necrotic pulps, which were dressed with calcium hydroxide alone or in combination with chlorohexidine experienced less pain after the first appointment than patients whose teeth had been dressed with no intracanal dressing.

Conclusion

The use of intracanal medication for 14-days in necrotic teeth with apical periodontitis was more reliable in reducing postoperative pain regardless the type of medication used. However, endotoxin level in the infected root canals was reduced significantly after root canal preparation and further reduced after 14-days of intracanal medication, where calcium hydroxide combined with 2% chlorhexidine gel showed higher endotoxin reduction when compared to calcium hydroxide alone.

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