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## Effect of Combination of Calcium Hydroxide and 2% Chlorhexidine Gel as Intracanal Medications in Comparison to Calcium Hydroxide Paste on Postoperative Pain and Bacterial Endotoxins in Necrotic Teeth: Randomized Clinical Trial

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**Aim:** Assessment of post-operative pain and quantification of bacterial endotoxins in adult patients suffering from necrotic pulp condition with chronic periapical periodontitis by using calcium hydroxide paste intracanal medication with 2% chlorhexidine gel, Calcium hydroxide paste intracanal medication only and with mechanical preparation without intracanal medications.

**Materials and methods:** Forty five patients with single rooted teeth suffering from necrotic pulp status were divided into 3 groups (n=15), the first group received mechanical preparation only, the second group received calcium hydroxide intracanal medication only, while the third group received mixture of calcium hydroxide and 2 % chlorhexidine gel intracanal medication. Numerical rating scale was used to assess the pain experience post operatively while chromogenic end point Limulus Amebocyte Lysate assay (LONZA) was used to quantify the amount of endotoxins.

**Results:** Calcium hydroxide was superior in decreasing the postoperative pain at time interval 4 hours and 24 hours in comparison to the mixture of calcium hydroxide paste and 2 % chlorhexidine gel significantly, while the mixture was better to reduce the endotoxins with statistically insignificant value.

**Conclusion:** Using one of the intracanal medications for 2-week time interval in necrotic teeth can help to allivate postoperative pain and reduce endotoxins.

**Key words:** Intracanal medications, calcium hydroxide, 2% chlorhexidine gel, bacterial endotoxins.

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## Introduction

Bacteria play an essential role in the development and necrosis of pulp and periapical diseases. Elimination of microorganisms from infected root canal systems is a complicated task. Numerous measures have been described to reduce the number of micro-organisms from the root canal system, including the use of various mechanical instrumentation techniques, irrigation regimes and intra-canal medicaments. There is no definitive evidence in literature to show that mechanical instrumentation alone will predictably result in bacteria-free root canal system.<sup>1</sup>

Bacteria and their byproducts play a primary etiologic role in the development of apical periodontitis; the bacteria involved in the primary endodontic infections are predominantly gram-negative anaerobic species that present lipopolysaccharide (LPS) in the outer layers of their cell walls, which functions as endotoxin in the host organism. LPS (virulence factor), generally referred to as endotoxin released during disintegration of bacteria after multiplication and death, has been detected in pulp necrosis. Strong evidence correlates its presence in the root canals with inflammatory reactions and bone resorption of the periradicular tissues. A high content of endotoxins in the root canals has been associated with endodontic signs and symptoms such as spontaneous pain, pain on palpation, and tenderness to percussion. Furthermore, its egression through the apical foramen into periapex can even perpetuate an apical periodontitis.<sup>2</sup>

Medicaments are antimicrobial agents that are placed inside the root canals between treatment appointments in an attempt to destroy remaining microorganisms and prevent reinfection.<sup>3</sup>

The antimicrobial activity of calcium hydroxide  $\text{Ca}(\text{OH})_2$  is related to the release of hydroxyl ions in an aqueous environment<sup>4</sup> leading to damage in the bacterial cytoplasmic

membrane, protein denaturation and DNA damage.<sup>5</sup>

Chlorhexidine is widely used as both intracanal medicament and irrigating solution, this is attributed to its penetration to cell wall or outer membrane of Gram negative cells and attacks the cytoplasmic membrane leading to coagulation of the intracellular components.<sup>6</sup>

## Materials and methods

The interventions was done in the 1<sup>st</sup> appointment where all the procedure was explained to the patient then signing the informed consent. Forty five patients were divided randomly into 3 groups each of 15 patients. At first pulp vitality test was done by thermal pulp testing at the facial aspect of the tooth structure when it is dry then preoperative periapical x- ray film was done to detect any periapical changes. Administration of local anesthesia (Mepivacain HCl2%- Levonordefrien 1:20000 Alexandria, Egypt) to the tooth required to be treated then isolation with rubber dam.

- Disinfecting the tooth surface by 30% H<sub>2</sub>O<sub>2</sub> (Perfect Medical, Egypt) followed by 2.5% NaOCl (CLOROX) inactivated with sodium thiosulfate (El Nasr CO. for pharmaceuticals and chemicals).

- Sterile burs sterilized by the plasma sterilizer (HMTS-142) for caries removal and another one for pulp chamber penetration.

- Working length determination was done using MORITA Root ZX Mini apex locator (Japan) for accuracy.

- The first sample of endotoxins (S1) was taken by a sterile paper points size 20 or 25 sterilized in the plasma sterilizer (HMTS-142) and introduced in the root canal for the working length for a period of 60 seconds.

- The sample is then placed in a pyrogen free glass tube and frozen to negative 200°C for endotoxin determination by Limulus Amebocyte Lysate (LAL) test.

- Cleaning and shaping procedure was established by Revo-S rotary system (Micro Mega, France) and gates glidden sizes 3 and 4 (Mani, Japan) to ensure adequate space for placement of the intracanal medicaments and proper removal of bacteria and debris, till size SU file then the canal will be enlarged manually till size 35 k-file to ensure adequate space for placement of the intracanal medicaments and proper removal of bacteria and debris.

- Another paper point sample (S2) was taken after the cleaning and shaping and before placing the intracanal medications for endotoxin determination.

- The irrigant solution was NaOCl with concentration 2.5% for its tissue dissolving properties.

- Placement of the intracanal medications by lentuolo spiral by low speed hand piece and condensed with paper points, the first group (n=15) was the control without receiving any intracanal medicaments, while the second group (n=15) received calcium hydroxide ready-made paste only (Promedica, Germany), then the third group (n=15) received combination of calcium hydroxide paste (Promedica, Germany) mixed with chlorhexidine gel 2% (Gluco-Chex 2%, Cerkamed, Poland) by equal ratios mixed on a glass slab.

- Temporary filling was done by resin modified glass ionomer to ensure adequate sealing and preventing micro leakage.

- Pain experience assessed by numerical pain rating scale the after the treatment by 4 hours, 24 hours, and 48 hours and after 14 days in a follow up card introduced to the patient.

- After 2 weeks (second appointment) rubber dam was applied, local anesthesia was received then removal of the temporary dressing by following the previous infection control protocol, then a sterile paper point introduced into the canals after irrigation and removal of the remnants of the intracanal

medicament with the saline solution for 60 seconds.

- After saline irrigation, neutralizing agents must be used to deactivate the effect of the intracanal medication to avoid false negative results in what is called carry over effect due to the bacteriostatic effect of the medication leading to misleading results.<sup>7</sup>

- Neutralization of the chlorhexidine was done by 5% tween 80.<sup>8,9</sup>

- Then saline irrigation to remove the effect of the deactivating agents.

- Insertion of the third sample paper point (S3) to determine the endotoxin concentration after 14 days of intracanal medication.

- Preservation of the paper point samples in the sterile test tubes and stored at -20°C.

- Obturation accomplished by lateral condensation technique with gutta percha size and with resin sealer and auxiliary cones size 25 or 30 according to the space beside the master cone then final restoration.

#### **LAL QCL 1000 endotoxin kit (LONZA, Belgium) preparation and setting:**

1- Remove the kit from the refrigerator to take the room temperature.

2- Preparation of the reagents.

LAL reagent :( vial with silver cover)

The vial is dissolved in 6.5 ml LAL reagent water, then vortex for 1 min.

Chromogenic substrate (vial with golden cover)

The vial is dissolved in 6.5 LAL reagent water, then vortex for 1 min.

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The stop reagent (25% acetic acid solution)

In a graduated cylinder of a capacity 100cc put 25ml glacial acetic acid and fill the cylinder with 75ml distilled water.

i.e 25 ml acetic acid + 75 ml distilled water = (25%acetic acid).

The endotoxin standard (vial with red cover)

Dissolve the endotoxin standard with 1 ml LAL reagent water (blank) then vortex 15 mins (the concentration is 27EU/ml)

### Preparation of 4 concentrations (1,0.5,0.25&0.1EU/ml) of endotoxin standard (red cover):

1- Prepare solution of 1 EU/ml by diluting 0.1ml (100ul) of endotoxin standard (red cover) with 2.6 ml LAL reagent water (blank) in a test tube, then vortex for 1 min.

2- Prepare a solution containing 0.5 EU/ml by transferring 0.5 ml of the first dilution 1 EU/ml (first tube) into 0.5 ml LAL reagent water (blank) in another test tube, then vortex for 1min.

3- Prepare solution containing 0.25 EU/ml by transferring 0.5 ml of the 1EU/ml (first tube) into 1.5 ml of LAL reagent water in a test tube, then vortex in 1 min.

4- Preparation of 0.1 EU/ml by transferring 0.1 ml (100ul) of 1EU/ml tube into 0.9 ml of LAL reagent water (blank) in a test tube, then vortex in 1 min.

The above 4 concentrations are very critical for calculation of the standard curve as in the figure.

To prepare the unknown sample of the patient, remove the test tube containing the paper point taken from the root canal then leave it to gain the room temperature then add 1ml LAL reagent water to the paper point then shake well.

### The micro plate procedure:

1- Add 50 ul of LAL reagent water (Blank) in the first well. (A)

2- Then add 50 ul of the each of the 4 standards concentrations into their appropriate micro plate well (Corning Coaster, USA). (B,C,D,E)

3- Then add 50ul of patients' unknown samples to fill the hole wells.

4- Adding the 50ul of LAL reagent (silver cover) to all 96 wells.

5- Mix gently and incubate at 37°C for 10 mins.

6- Add 100ul the chromogenic substrate solution (golden cover) to all the 96 wells.

7- Mix gently and incubate at 37°C for 6 mins.

8- Add 50 ul of the stop reagent and mix immediately then read the absorbance at 405 nm in the special micro plate reader Synergy 2, USA.

9- Calculation of the endotoxin concentration by the graphic method.

- Until filling all the 96 wells completely then record their absorbances as in the upper figure showing the exact places of the samples in the 96 well plate.

- Adding the reagents to the samples in the 96 well plate must be done at the same time without delay.

### statistical Analysis:

Statistical analysis were done by SPSS program version 15 software, Kruskal–Wallis test and Wilcoxon tests were used as a non-parametric method for testing quantitative values. It is used for comparing two or more independent samples of equal or different sample sizes, while Chi square test was done in comparison between qualitative variables. Qualitative data was presented as frequency and percentages while quantitative data was expressed by means and standard deviation. The following items will be mentioned in the statistics: Mean, standard deviation, f- ration and p- variance.

### Results

In this study 45 patients were divided into 3 groups (n=15) while a patient from the control group was exempted (n=14), giving 2 homogenous group with heterogenicity if compared with the control group which is 14 patients.

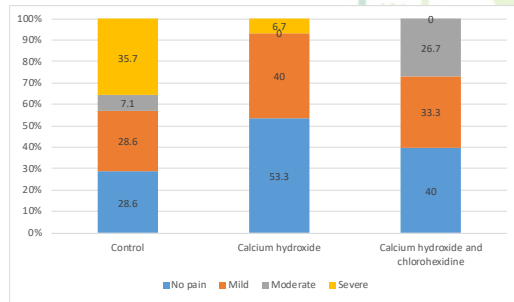
There was no significance between Ca(OH)<sub>2</sub> and Ca(OH)<sub>2</sub> & CHX groups (table 1). In (S3) there was a reduction in the mean of endotoxins at Ca(OH)<sub>2</sub> & CHX group than in Ca(OH)<sub>2</sub> group but that was statistically non-significant.

i.e Ca(OH)<sub>2</sub> paste mixed with 2% chlorhexidine gel was favorable than calcium hydroxide paste alone for endotoxins

reduction. Results bar chart 1,2,3,4 reveals the percent distribution of degree of pain among three groups in the different time intervals supported with bar charts.

**Table 1: Comparison between Ca(OH)<sub>2</sub> group and Ca(OH)<sub>2</sub> & CHX group concerning endotoxin quantification:**

Group	Sample number	Type of sample	mean	Std.dev.	F.ration	p-variance	t-value	Stat. significance
Ca(OH) <sub>2</sub>	15	S1	0.478	0.361	1.39	0.54	1.21	Statistically non significant
Ca(OH) <sub>2</sub> & CHX	15		0.628	0.306				
Ca(OH) <sub>2</sub>	15	S2	0.310	0.300	1.19	0.74	0.81	statistically non significant
Ca(OH) <sub>2</sub> & CHX	15		0.404	0.327				
Ca(OH) <sub>2</sub>	15	S3	0.437	0.358	1.11	0.841	0.16	Statistically non significant
Ca(OH) <sub>2</sub> & CHX	15		0.416	0.339				



Bar chart 1: percent distribution of degree of pain among three groups in 4 hour time interval.

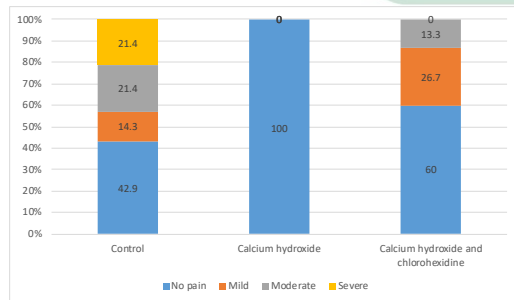
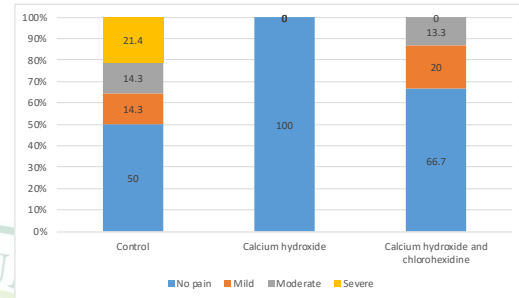
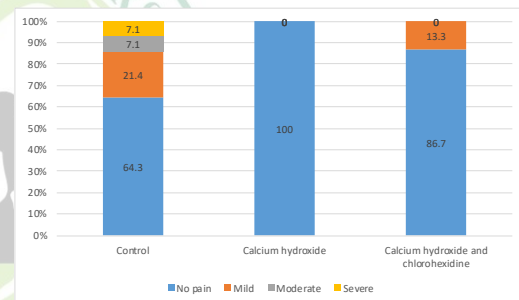


Chart 2: Percent distribution of degree of pain among three groups in 24 hour time interval.



Bar chart 3: Percent distribution of degree of pain among three groups in 48 hour time interval.



Bar chart 4: Percent distribution of degree of pain among three groups at time interval 2 weeks.

## Discussion

The basic principle of root canal treatment is to eliminate root canal irritants which are gram negative bacteria and their byproducts as endotoxins. Endotoxins are released during bacterial multiplication and at cell death in necrotic cases. Endotoxins are responsible for election of pain in endodontic infections, upon its egression through the apical foramen into the periapex can even perpetuate an apical periodontitis.<sup>1,2,10</sup>

In case of asymptomatic apical periodontitis there is a balance between infectious micro flora and defensive mechanisms of human immune system in periodontal tissues this is what is called "local adaptation syndrome. After mechanical preparation, extrusion of the debris from the apical foramen to periapical tissues leads to increase the inflammation due to imbalance between microorganisms and human immune

system leading to vasodilatation and release of inflammatory mediators.<sup>11</sup>

In this study a comparison between ready-made calcium hydroxide paste alone against combination of 2% chlorhexidine gel mixed with calcium hydroxide paste to assess their efficacy on reducing postoperative pain. After the first visit as the patient can be worried about pain severity and its duration, then to quantify endotoxin concentration after a period of 2 weeks of using intracanal medications. Calcium hydroxide paste is considered one of the most widely used intracanal medications due to its alkaline pH 10-12 which considered unsuitable condition for bacterial growth<sup>12</sup>, it affects microorganisms by 3 different mechanisms: the hygroscopic action due to absorption of the inflammatory exudates, formation of calcium proteinate bridges due to combination of  $\text{Ca}^{2+}$  ions with the proteins in the intercellular substances of endothelial cells thus prevents inflammatory exudates from spreading towards the blood vessels, finally phospholipase inhibition by calcium hydroxide decreases cellular lysis and consequently the liberation of PG which is one of inflammatory mediators.<sup>13</sup> Chlorhexidine gel is used in combination to calcium hydroxide to get synergistic effect.<sup>14</sup> The alkalinity of the calcium hydroxide remained unchanged when mixed with the chlorhexidine. Concerning endotoxins quantifications, the current study showed that calcium hydroxide mixed with 2% chlorhexidine gel was better than calcium hydroxide paste alone in reducing endotoxins in the root canal after 2 weeks of intracanal medication, that was in agreement with studies.<sup>15, 16, 17, 18, 14, 19</sup>

Saatchi *et al.* 2014<sup>20</sup> showed that calcium hydroxide mixed with chlorhexidine did not significantly increases the antimicrobial activity of calcium hydroxide, also Schafer and Bosmann 2005<sup>21</sup> showed that 2 % chlorhexidine was significantly

more effective than calcium hydroxide alone or mixture of calcium hydroxide and chlorhexidine, while, Sousa *et al.* 2014<sup>22</sup> showed that calcium hydroxide with chlorhexidine was very potent against endotoxins only after 30 days of application. There was no antagonistic effect between calcium hydroxide and chlorhexidine when they were mixed together according to the study of Signoretti *et al.* 2011<sup>23</sup> as the pH media inside the root canal is still alkaline, and ion release is not affected concerning calcium hydroxide. While in a study by Lindskog *et al.* 1998<sup>24</sup> positive ions of chlorhexidine attach to the negatively charged phosphate groups in the bacterial cell wall thus allows penetration of chlorhexidine to bacteria exerting its bactericidal effect.

The stomatognathic system is a very complex media showing fluctuation in temperature, masticatory forces, bacteria and saliva. The results from this study were limited to the patient's stomatognathic system and the oral hygiene motivation on contrast to the in vitro studies where the specimens are stored in a safe media (incubators) not subjected to the previous detrimental factors which can affect the results at the end. The difference between the working lengths of the root canals and the width of root canals vary from patient to another and this is an important factor determining the amount (volume) of the intracanal medication applied also the amounts of endotoxins at sample 1, thus leading to differences between in vivo results and in vitro results where a lot of factors are constant and no variables for example same working length, same tooth type, no masticatory forces were applied, and sterile safe media with fixed temperature as in case of in vitro studies which can affect the final obtained results.

## Conclusions

- 1- Cleaning and shaping step in necrotic pulp cases is mandatory but that was not enough.
- 2- For better results the use of any of intracanal medications like calcium hydroxide or mixture of chlorhexidine and calcium hydroxide paste is a must to reduce both bacterial endotoxins and post-operative pain and getting better results otherwise the time of the treatment and number of visits will increase with sever drawbacks.
- 3- There is synergistic effect between chlorhexidine gel and calcium hydroxide and no interaction between them.
- 4- Calcium hydroxide was efficient in reducing postoperative pain while combination of both calcium hydroxide and 2% chlorhexidine gel was better in reducing endotoxin levels without statistically significant differences between the groups.

## References

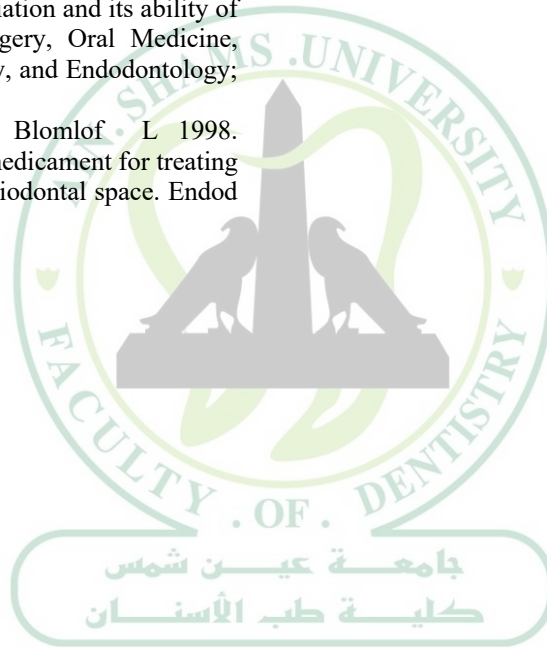
- 1-Mohammadi Z and Shalavi S 2012. Is Chlorhexidine an Ideal Vehicle for Calcium Hydroxide? A Microbiologic Review. Iranian Endodontic Journal; 7(3): 115–122.
- 2-Martinho FC & Gomes B PFA 2008. Quantification of endotoxins and cultivable bacteria in root canal infection before and after chemomechanical preparation with 2.5% sodium hypochlorite. Journal of Endodontics; 34(3): 268–272.
- 3- Weine FS 2004. Endodontic therapy; 6<sup>th</sup>edn. Mosby: 226-228
- 4- 71. Siqueira JF 2001. Strategies to treat infected root canals. Journal of California Dental association; ;29(12):825-837.
- 5- 70. Siqueira JF & Lopes HP 1999. Mechanisms of antimicrobial activity of calcium hydroxide: A critical review, International Endodontic Journal; 32(5):361-369.
- 6-Macdonnel G & Russell D 1999. Antiseptics and disinfectants: activity, action and resisittance . Clin Microbiol Rev; (12):147-179.
- 7-Portenier I, Waltimo T, Ostavik D, Haapasalo M 2006. Killing of Enterococcus faecalis by MTAD and Chlorohexidene Digluconate with or without Cetrimide in the presence or absence of dentin powder. Journal of Endontics;32 (2):138-141.
- 8-Raddiffe CE, Potouridou L and Quresh R 2004. Antimicrobial activity of varing concentrations of sodium hypochlorite on the endodontic micro-organisms Actinomyces israelii. International Endodontic Journal;37(7):438-46.
- 9- 49. Nandini S, Velmurugan N and Kadaswany D 2006. Removal efficiency of calcium hydroxide intracanal medicament with two calcium hydroxide chelators: volumetric anlysis using spiral CT, an in vitro study. Journal of Endodontics; 32(11): 1097-1101.
- 10- Seltzer S and Faber PA 1994. Microbiologic Factors in Endodontology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 78 (5) :634-645.
- 11- Seltzer S & Naidorf IJ 2004. Flare-ups in Endodontics: I. Etiologic factors. Journal of Endodontics; 30(11):476-481.
- 12- Safavi KE & Nicols FC 1993. Effect of Calcium Hydroxide on Bacterial Lipopolysaccharide. Journal of Endodontics; 19(2):76-78.
- 13-Souza V, Bernabe PFE and Holand R 1989. Tratamento nao cirurgico de dentes comlesos periapiciais.Revista Brasileira de Odontologia; 46:39-46.
- 14-Haenni S, Schmidlin PR, Mueller B, Sener B, Zehnder M 2003. Chemical and antimicrobial propertites of calcium hydroxide mixed with irrigating solutions. International Endodontic Journal;36(2):100-105.
- 15-Wang CS, Arnold RR, Trope M and Teixeira FB 2007. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. Journal of Endodontics; 33(11): 1283–1289.
- 16- Athanassiadis B, Abbott PV and Walsh LJ 2007. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics, Australian Dental Journal, 52(suppl), 64-82.
- 17- Salthorn C, Parashos P and Messer H 2007. Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis. International Endodontic Journal; 40(1): 2–10.
- 18- Yeung SY, Huang CS, Chan CP, Lin CP, Lin HN and Lee PH 2007. Antioxidant and pro-oxidant properties of chlorhexidine and its interaction with calcium hydroxide solutions. International Endodontic Journal; 40(11):837-844.
- 19- Sinha N, Patil S, Dodwad PK, Patil AC and Singh B 2013. Evaluation of antimicrobial efficacy of calcium hydroxide paste, chlorhexidine gel, and a combination of both as intracanal medicament: An in vivo comparative study. Journal of Conservative Dentistry; 16(1): 65–70.
- 20-Saatchi M, Ali S, Hooman N, Mohammad RM and Hasan S 2014. Antibacterial effect of calcium hydroxide combined with chlorhexidine on Enterococcus faecalis : a systematic review and meta-analysis. Journal of applied Oral Science; 22(5): 356–365.

21-Schafer E & Bossmann K 2005. Antimicrobial Efficacy of Chlorhexidene and two Calcium Hydroxide Formulations against E. Fecalis. Journal of Endodontics; 31(1):53-56.

22-Sousa ELR, Martinho FC, Nascimento G, Leite RM, Gomes, PFA 2014. Quantification of endotoxins in infected root canals and acute apical abscess exudates: monitoring the effectiveness of root canal procedures in the reduction of endotoxins. Journal of Endodontics; 40(2): 177–181.

23-Signoretti C, Fernanda G, Brenda P, Figueiredo DAG, Francisco M, Fernanda BT, Rogério CJP and Porto A 2011. Influence of 2% chlorhexidine gel on calcium hydroxide ionic dissociation and its ability of reducing endotoxin. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology; 111(5): 653–658.

24-Lindskog S, Pierce AM, Blomlof L 1998. Chlorhexidine as a root canal medicament for treating inflammatory lesions in the periodontal space. Endod Dent Traumatol; (14):186-190.



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