Effect of Bio Mechanical Preparation on Endotoxin Levels in Patients with Chronic Apical Periodontitis

Nezar Boreak[1]*

1. Dept. Restorative Dentistry, College of Dental Sciences, Jazan University Jazan, Kingdom of Saudi Arabia.

Abstract

Background: Many studies have reported a strong connection between the level of endotoxins, periapical bone resorption, and progression of the symptoms of the disease contributing to the progression of endodontic ailments.

The current research was aimed to explore the effect of root canal treatment by using various chemical substances and the use of intracanal medicaments for a longer period of 30 days on endotoxin concentrations in teeth of patients with apical periodontitis.

188 patients with suffering from apical periodontitis were chosen for the current study. Participants were grouped into three different clusters (G1, G2, and G3) according to the irrigants used for chemo-mechanical preparation. The selected teeth of G1 participants were irrigated using 2.5% Sodium Hypo Chlorite (NaOCl), G2 with 2% Chlorhexidine (CHX), and G3 with Normal Saline solution. Endotoxin samples of the patients participating in the study were collected at baseline {sample 1 (S1)}, after use of auxiliary chemical substances {sample 2 (S2}, after use of EDTA washing {sample 3 (S3)}, and subsequently after 30 days of exposure to intracanal medicament {sample 4 (S4)}.

The present study revealed that participants of all the groups (G1, G2, and G3) exhibited a marked reduction in endotoxin concentrations from S1 to S4, irrespective of root canal procedure.

We concluded that instrumentation, followed by the use of auxiliary chemical disinfectants namely sodium hypochlorite and chlorhexidine and 30 days calcium hydroxide intracanal medicament significantly reduced the endotoxin echelons in chronic apical periodontitis patients.

Keywords: Chronic Apical periodontitis; Chlorhexidine; Endotoxins; Root canal; Sodium

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Introduction

Various Gram-negative bacteria species like Prevotella, Porphyromonas, and Fusobacterium have been reported in root canal infections.[1-7] The outer layer of gram negative bacteria's cell wall comprises a complex lipopolysaccharide (LPS) molecule.[8] This molecule is commonly known as endotoxin and is released by the bacteria during active cellular growth and after cell lysis.[8,9] Numerous studies reported of the existence of endotoxins in periapical infections.[3, 4, 8-10] The level of endotoxins in the lesion have been linked to pathological periapical bone resorption and progression of the symptoms contributing to the development of the endodontic disease. [5, 14-21] It has been reported that endotoxins released by the bacteria during root canal infection activate the formation of InterLeukins-1 and Tumor Necrotic Factor-alpha.[22, 23] These factors activate the macrophages which consequently up-regulates the Matrix Metalloproteinase to promote the periapical bone resorption.[22] Endotoxins can affect and disrupt the functioning of human humoral and cellular host mediation systems.[24]

Endotoxins are recognized by the human body as foreign material.25It has been found that even low concentrations of endotoxins can lead to strong immune responses through different

*Corresponding author:
Nezar Boreak
Department of Restorative Dental Science,
College of Dentistry, Jazan University, Saudi Arabia - 45142
E-mail: boreaknezar@gmail.com

mediators of inflammation like cytokines in pulp and periapical areas of teeth[11, 26] contributing to the advancement of apical periodontitis. Higher concentrations of endotoxins were reported to be extant in apical periodontitis. [2]6

These studies highlight the need for an effective clinical procedure that could be implemented to flush and remove these endotoxins from pulp spaces and periapical areas of teeth, leading to enhance the treatment efficacy during chronic clinical conditions.

Previous studies have reported that chemo-mechanical preparation together with the use of the auxiliary chemical substance may reduce the 50% endotoxin load in root canals.[2,15,20]

Bio-Mechanical Preparation using rotary instruments can reduce up to 90% of the endotoxin load.[19, 28, 29] Nevertheless, endotoxins were yet identified in all the samples collected from the root canals after instrumentation.

Calcium hydroxide [Ca $(OH)_2$] has been studied as an intracanal medicament and is known to bring down the endotoxin concentrations during root canals infections.[2,28,31]

There is a notion that the percentage of removal of these endotoxins is related to the period of intracanal medication. Longer period intracranial medication has been found associated with more efficient removal of endotoxins during a root canal and acute apical abscesses. Xavier et al. have found that intracanal medication dressed for fourteen days has reduced 12% endotoxin levels.[21,29]

In addition, there are notions that bio-mechanical preparation along with the adjuvant use of auxiliary chemical affluences and the use of intra canal medicaments for an extended period may contribute to the total elimination of endotoxins from the infested root canals.[30]

Therefore, the current study was aimed to explore the effect of root canal treatment by using various auxiliary chemical substances and the use of the intracanal medicament for a longer period (30 days) on endotoxin concentrations in teeth of patients with apical periodontitis

Materials and methods

1. Study design and patient selection

The present study was planned, on the basis of repeated-measures design. Initially, 213 patients with apical periodontitis were screened at Elite Smile Private dental practice, Jazan, Saudi Arabia. Demographic data and clinical history of all the patients were determined before starting the experimental protocols. Patients in the age group of 18- 48 years with periapical infection in single rooted teeth such as incisors, canines and mandibular premolars were included in the study. Patients who took antibiotics in a span of 3 months were not considered for the study. 17 patients were eliminated from the study due to the use of different antibiotics in the last 3 months and 8 patients were having periodontal pockets larger than 4mm. Finally, 188 patients with apical periodontitis were selected for the current research. The average age of the samples were 35.17 years (range: 18-48 years). All the patients were well acquainted to the nature of the planned study. Approval and consent was taken before starting the study.

2. Baseline sampling

All the instruments that were used in the study were sterilized and the area of the operative field was disinfected according to standard protocols.32, 33The targeted teeth were isolated using rubber dam and sterilized with 30% Hydrogen peroxide and 2.5% Sodium hypochlorite (NaOCl), each having an exposure time of 30 seconds. After that, 5% sodium thiosulphate was used for inactivation. The same method was also implemented for the disinfection of the access cavity. Sterilized diamond bur were used for access cavity preparation. A sterile paper point was interleaved into the root canal to collect the endotoxin sample. The baseline samples (S1) were collected immediately mixed with 1mL Limulus Amebocyte Lysate (LAL) water for assessing the endotoxin levels.

3 Experimental Groups

After collecting the baseline samples, the canal length of all teeth included in the study were determined and confirmed by using an apex locator. Thereafter, participants were allocated into different groups:

I. Group 1 (G1) = 62 (2.5 % NaOCl)
II. Group 2 (G2) = 63 (2% Chlorhexidine)
III. Group 3 (G3) = 63 (Saline water)

The groups were divided according to irrigants used for chemo-mechanical preparation. The teeth of G1 participants were irrigated with 2.5% sodium hypochlorite, G2 participants with 2% Chlorhexidine (CHX), and

G3 participants with saline solution.

4 Preparation of root canals

The preparation of root canals was carried out by following the standard protocol [2, 5]. Step- back technique was used for canal preparation. The working length of the canal was estimated using pre- operative radiograph. A K- file of size 10 was inserted into the canal till the apex. The canal was prepared by using rotary files of the sizes 10/.04, 15/.05, 20/.06, 30/.05, 35/.04 and 40/.04 at the speed of 300 RPM along with specific irrigants. The working length was confirmed with an IOPA after interleaving the master file in the root canal upto the expected working length established using the apex locator. Step-back widening of the root canal was accomplished using files of larger diameter intermittently used in a filing motion. The last file that was used to design the apical stop was also used for recapitulation of the canal. Step back canal preparation was stopped when three files that were bigger than the file that designed the apical stop was used.

5 Irrigation of root canals and sampling

During the process of canal irrigation, the canals of selected teeth in G1 participants was irrigated with 5 mL of 2.5% Sodium hypochlorite. After some time Sodium hypochlorite solution was removed by suction and was disabled using sterile 5 ml 5% sodium thiosulphate solution. Finally, washing was carried out using 5mL sterile NaCl solution and second-time samples (S2) were collected for the determination of endotoxin level. Likewise, root canals in selected teeth in G2 participants were irrigated with 1 mL of 2% CHX. After that first washing was carried out with 5 mL sterile NaCl solution and then chlorhexidine was washed out by 5% Tween 80 as well as 0.07% (v/v) lecithin. Finally, root canal cleansing was done using a 5 mL antiseptic NaCl solution, and second-time samples (S2) were collected. The targeted area G3 participants were first moistened with 5 mL of the NaCl solution followed by suction and collection of second-time samples (S2). Irrigation in all the groups was carried out using 27-gauge needles.2, 5 Next 17% EDTA solution was used to clear the smear layer. First 3 mL of the solution was applied for 3 minutes, thereafter the area was washed with 5 mL of sterile NaCl solution and third-set of samples (S3) were taken. Consequently, a paste of Calcium hydroxide in sterile salt solution in 1:1 ratio was prepared. A sterile Lentulo spiral was used to seal the root canals with Calcium hydroxide paste and light-cured resin composites were used to seal the access cavities for 30 days. After the completion of 30 days, the selected teeth were aseptically accessed. The selected teeth were secluded using a rubber dam. The intracanal medicament was completely detached with a file size 45 with 0.02 taper. Finally, the root canals were flushed with 10mL of sterile saline solution and fourth time samples (S4) were collected for the determination of endotoxin level.

6 Assessment of Endotoxins using the Kinetic Chromogenic Limulus Amebocyte Lysate Assay

Endotoxin concentrations were measured by using Lonza's Kinetic Turbidimetric LAL Assay kit. LAL method is a quantitative method for the determination of endotoxin levels that is commonly used.11, 13, 14According to this method, to assess the type and quantity of endotoxins, in unfamiliar samples, each turbidimetric LAL assay must be first compared to a valid standard curve. LAL Assay kit is supplied with known endotoxin concentration (100 EU/mL). From this known endotoxin concentration, dilutions of 0.01, 0.1, 1, and 100 EU/mL were prepared and subjected to standard curve plotting. Initially, all the test samples collected were suspended in 1 mL LAL reagent. Thereafter, 100 µl of the LAL reagent, endotoxin standards, endotoxin samples, and positive controls were carefully dispensed into 96-well microplates in triplicates. All the wells were carefully monitored to remove the bubbles if any. Microplates were incubated at heat block 37°C for 15 minutes then transferred to a microplate reader to record the absorbance at 340 nm.

7 Statistical analyses

The data was collected in MS Excel datasheet. The recorded data was subjected to descriptive statistical analysis.34, 35 Average values of endotoxin concentrations were examined by the Friedman test and one way-ANOVA test.36 Differences among the various blends of associated measurement levels was determined by Wilcoxon test. Mean difference value was set at 0.012 by Bonferroni adjustment i.e. the conventionally used significance level which means 0.05 was

divided by the number of measurement levels i.e. 4, to get new level of significance i.e. 0.012 (0.05/4). The mean scores of endotoxin levels with p<0.012 connoted the statistically significant difference among the related measurement levels. Data was scrutinized using the statistical package SPSS (Version 21.0).

Results

Average values of endotoxin levels of samples collected at different time intervals in participants of different groups are displayed in Table 1. Results obtained from the study showed that the endotoxin level were higher in baseline samples when compared to samples collected at other time intervals of different root canal procedures. These results reflect that the instrumentation procedure followed by intracanal medication for 30 days is an effective strategy to decrease the endotoxin level in dental patients.

	Sample Mean ± SE			
Group	S1	S2	S3	S4
NaOCl (G1)	32.51±2.13^{a}	0.43±0.01^{b}	0.41±0.01^{bc}	0.01±0.00^{d}
CHX (G2)	21.75±1.50^{a}	0.72±0.01^{b}	0.67±0.01^{bc}	0.01±0.00^{d}
SS (G3)	16.40±1.23^{a}	0.89±0.01^{b}	0.69±0.01^{c}	0.03±0.00^{d}

Table 1. Mean ± SE of endotoxin levels of samples collected at different time intervals in participants ofdifferent groups.
Mean values bearing matching letters are not ominously different from each other at *p< 0.05.*
NaOCl- Sodium hypochlorite; CHX- Chlorhexidine; SS-Saline solution; G1- Group 1; G2-Group 2; G3-Group 3.

	Friedman test (*p<0.05*)			Wilcoxon test (*p<0.012*)				
Group	X^2	df	*p-value*		S1-S2	S2-S3	S3-S4	S1-S4
NaOCl (G1)	36.789	3	<0.001	*Z*	-4.392	-1.652	-4.210	-4.142
				P	<0.001	0.152	<0.001	<0.001
CHX (G2)	27.231	3	<0.001	*Z*	-3.958	-1.531	-3.88	-4.444
				P	<0.001	0.043	<0.001	<0.001
SS (G3)	33.542	3	<0.001	*Z*	-4.010	-3.874	-4.921	-4.000
				P	<0.001	<0.01	<0.001	<0.001

Table 2. Friedman test and Wilcoxon test results of endotoxin levels of samples collected at different time intervals in participants of different groups.
NaOCl- Sodium hypochlorite; CHX- Chlorhexidine; SS-Saline solution; G1- Group 1; G2-Group 2; G3- Group 3. For the Friedman test, the statistical significance level was set at *p<0.05* and for the Wilcoxon test p- value was set at *p<0.012* (Bonferroni adjustment).

Results of Friedman test and Wilcoxon test are presented in Table 2. Friedman test results specified that the "root canal procedure" had a statistically significant effect (*p<0.001*) on the endotoxin levels, irrespective of the group (Table 2). Outcomes of the Wilcoxon test displayed that average values of the endotoxin concentration significantly decreased (*p<0.001*) from S1 to S2 and S3 to S4 and S1 to S4 in all the groups. Moreover, a significant reduction was also observed from S2 to S3 in the case of G3 participants (Table 2 and Figure 1, 2, and 3).

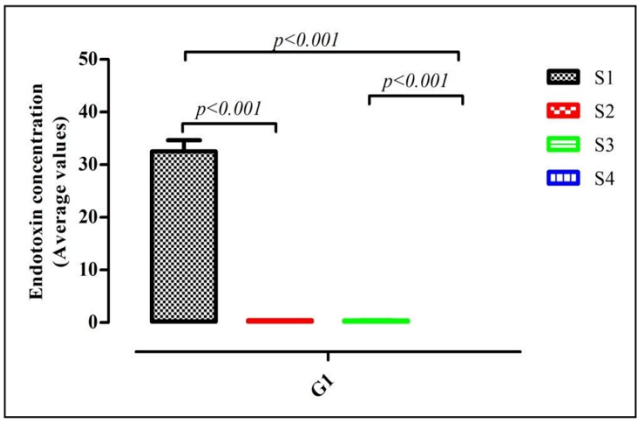

Figure 1. Mean±SE of endotoxin levels of samples collected at different time intervals in participants of G1. The p – value was set at *p<0.012* (Bonferroni adjustment).

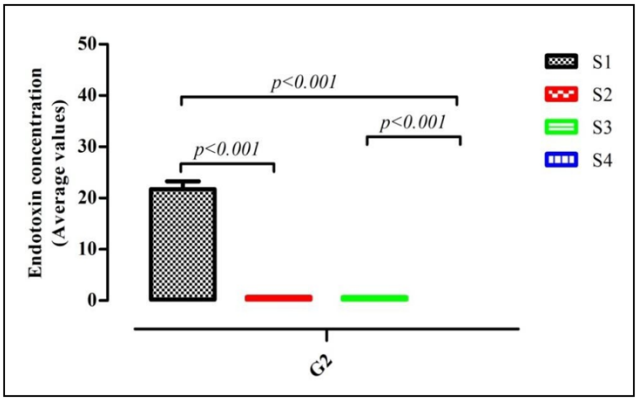

Figure 2. Mean ± SE of endotoxin levels of samples collected at different time intervals in participants of G2. The p- value was set at p<0.012 (Bonferroni adjustment).

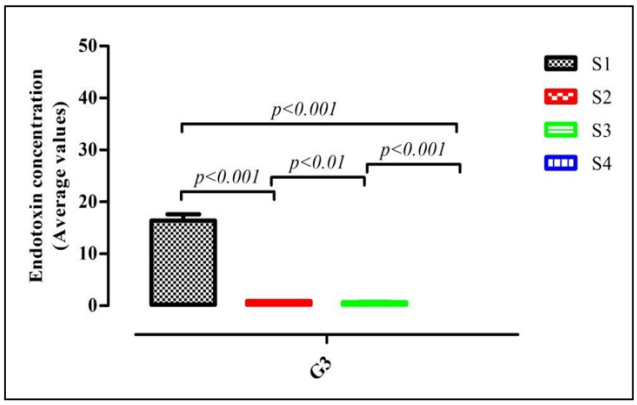

Figure 3. Mean ± SE of endotoxin levels of samples collected at different time intervals in participants of G3. The statistical significance level was set at *p<0.012* (Bonferroni adjustment).

Discussion

The current clinical research was planned to assess the endotoxin concentrations in patients diagnosed with apical periodontitis and underwent root canal treatment using different chemical substances as intracanal medicaments for a period of 30 days. Results of our study revealed that participants of all the groups (G1, G2, and G3) exhibited a marked reduction in endotoxin concentrations from S1 to S4, irrespective of root canalprocedure.

Baseline assessment showed the presence of endotoxins in all apical periodontitis patients. Our finding corroborates with several other clinical experiments which have reported the manifestation of endotoxins during root canal infections.[2-4,10-13] Further many studies have revealed that the presence of a higher concentration of these endotoxins causes periapical bone destruction leading to the progression of clinical symptoms contributing to the development of endodontic disease. [10, 14-16, 18, 19, 21, 29, 33]

Based on the baseline finding, patients were distributed in 3 different clusters i.e. G1, G2, and G3, based on the irrigation they received during chemo- mechanical preparation. The selected teeth in G1 participants were irrigated with 2.5% Sodium hypochlorite, G2 participants with 2% chlorhexidine (CHX), while G3 participants with saline solution. Previous studies have reported that chemo - mechanical preparation using rotary instruments together with the use of the auxiliary chemical substance may reduce endotoxin load in root canals.[2, 15,17,20,28,29] Endotoxin assessment after irrigation with these auxiliary chemical substances revealed a statistically significant reduction in all three groups. However, the average values of these endotoxins were lower in G1 (irrigated with 2.5% NaOCl) as compared to G2 (Irrigated with CHX) and G3 (Irrigated with saline solution). However, endotoxins have still been detected in all root canal samples even after instrumentation.[2,15,29] Our results revealed that 17% EDTA significantly lowered down the endotoxin level in G3 but not in G1 and G2. Our observation is in agreement with findings of other studies that could not find any significant effect of EDTA on the growth of some of the microbes and on their endotoxin concentrations in Ex Vivo human root canals.[37, 38]

Next, we explored the effect of 30 days sealing of intracanal medicaments ($Ca(OH)_2$) on the endotoxin level in apical periodontitis patients. Some reports provide a notion that exposure to intracanal medicaments for a long duration may reduce the endotoxin level.[21, 29]Results of our study revealed the significant effect of 30 days of sealing of Ca (OH) 2 intracanal medicaments on the endotoxin levels, irrespective of the group. Endotoxin levels were significantly reduced from S3 to S4. Our findings are supported by few other studies. Xavier et al. found that 14 days exposure to $Ca(OH)_2$ intracanal medicaments enhanced the reduction endotoxins by 12%.[21] While as Sousa, et al have reported even more endotoxin reduction on increasing the exposure time by more than 14 days.[29]A review on the efficacy of single- sessions disinfection protocols and multiple- sessions disinfection protocols against endotoxins in root canal infections also reported that when compared to single-session disinfection procedures with the application of $Ca(OH)_2$ medications, multiple-session disinfection protocols with the placement of $Ca(OH)_2$ medications are more effective in reducing the levels of endotoxin from root canal infections when used for 14 and 30 days.[39]

It has been reported that Calcium hydroxide powder when mixed with alkaline water renders high pH that is necessary for better disinfection and prevent postoperative flare-up.[40]

Conclusions

Based on our findings we conclude that an extended exposure to Ca (OH)2 intracanal medicine contributes to eradication of endotoxins present in infected root canals. Instrumentation, followed by the use of auxiliary root canal irrigants namely sodium hypochlorite and chlorhexidine and 30 days C intracanal medicaments significantly reduced the endotoxin levels in apical periodontitis patients.

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Declaration of Interest

The authors reported no declarations of interest.

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