Antimicrobial Effect of Chlorhexidine and Sodium Hypochlorite on Some Microorganisms in the Root Canals of Non Vital Teeth - In Vivo Study

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Abstract: Aim: The aim of the study is to clinically evaluate and compare the antimicrobial effect of 2% chlorhexidine (CHX) and 1% sodium hypochlorite (NaOCl) irrigation solutions on some microorganisms in human root canals containing necrotic pulp tissue. **Methodology:** Forty five root canals of necrotic upper incisors and lateral incisors of 41 patients were included and divided into 3 groups according to irrigant to be used. After accessing the canal, the first root canal sample was collected using two sterile paper points. One paper point was placed in a tube containing Brain hurt infusion medium to transfer E.faecalis and Streptococcus species and the other paper point was placed in a tube containing thioglycolate broth for Gram positive anaerobes. The root canal was irrigated using 2%chlorhexidine solution or 1% sodium hypochlorite or normal saline. Immediately after irrigation at the end of the first visit the second sample was taken as before. A small sterile cotton pellet was placed at the root canal entrance and the cavity was sealed with zinc oxide-eugenol cement. After 48hrs third sample was obtained. All the samples were submitted to bacterial evaluation**. Results** showed that both 2% CHX and 1% NaOCl irrigation solutions showed statistically significant reduction in mean $\log_{10}$ CFU values of all target bacteria immediately and after 48 hrs. except for E faecalis after48 hrs. **Conclusions:** 2% CHX and 1% NaOCl were effective against all the types of tested bacteria at the end of first visit. E. Faecalis was the most resistant bacteria to both 2% CHX and 1% NaOCl irrigants after 48 hrs.

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1. Introduction

The success of endodontic treatment is directly influenced by elimination of microorganisms present in infected root canals ***(Cheung and Ho, 2001***). It is generally acknowledged that most treatment failures are caused by microorganisms persisting in the apical parts of root canals of obdurated teeth (***Zamany et al., 2003***).There is some diversity of species isolated from root filled teeth with persistent periapical disease, but there is a consensus among many studies that there is a high prevalence of enterococci and streptococci (***Siqueira and Ro^c¸as, 2004).***

Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are the most frequently widely used antimicrobial agents for treatment of root canal infection ***(Estrela et al., 2004***). These medicaments present chemical characteristics that are particularly responsible for their distinct results when compared. These variations occur probably due to differences in methodology, biological indicators, concentrations, exposure time, the potential for different anatomical and treatment differences between patients ***(Estrela et al., 2003 and Siqueira et al., 2002).***

Thus the aim of this study is to evaluate and compare the antimicrobial effect of 2% chlorhexidine and 1% sodium hypochlorite on Enterococcus Faecalis (E.faecalis), Streptococcus species (Spp) and Gram positive anaerobes.

Null hypothesis is there is no difference in the effectiveness of any of the tested irrigants on the target bacteria.

2. Material and Methods:

Forty five anterior teeth of forty one patient patients requiring root canal treatment attended the clinic of Pediatric Dentistry Department, Faculty of Oral and Dental Medicine, Cairo University were included in this study. Selected children fulfilled the following criteria:

1. Age range from 10 to14 years.
2. Free of systemic diseases.
3. Had not received antibiotic treatment during the previous 2 weeks.
4. All selected teeth were non vital, single rooted upper incisors which had not received previous root canal treatment, with no sinus tract, no immature apices or root resorption.

A signed informed consent was taken from the parents or guardian. The selected teeth were randomly divided into three groups, each of fifteen, according to the type of irrigation material to be used;

Group 1(control group): Included 15 root canals irrigated by normal saline.

Group 2: Included 15 root canals irrigated by 2% chlorhexidine gluconate.

Group 3: Included 15 root canals irrigated by 1% sodium hypochlorite.

The target bacteria were E.faecalis, Spp and Gram positive anaerobes.

The tooth under treatment was isolated using rubber dam. The operative field disinfected with 1ml of 30% hydrogen peroxide for 30 seconds followed by 1ml of 2.5% NaOCl for 30 seconds. The antiseptic solution was inactivated with 1ml of 5% sodium thiosulfate in order to avoid interferences in the results ***(Mo¨ller, 1966 and Gomes et al., 2004).*** A swab sample was taken from the surface of the tooth and streaked on blood agar plates to check the sterility of the operative field ***(Dahle´n et al., 1993).*** The coronal access into the pulp chamber was prepared using sterile round burs. Sample 1 was taken immediately after opening the access cavity by introducing two subsequently sterile paper points into the full length of the root canal and kept in place for 60 seconds. One of the paper points was placed in 1ml thioglycolate broth transport medium for culture of Gram positive anaerobe and the other paper point was placed in1ml Brain hurt infusion (BHI) transport medium for Spp and E.faecalis bacteria.

Biomechanical preparation of the root canal was subsequently performed by step-back technique using K files (Dia Dent Group International Inc, Korea), with each instrument change accompanied by irrigation using 2 ml 2% CHX gluconate or 2ml 1% NaOCl or 2 ml normal saline by means of syringe with a sterile thin needle that was introduced to the middle portion of the root, also Irrigation was performed slowly with gentle movement of the needle to ensure that it is not binding in the canal.

After drying the root canals with sterile paper points Sample 2 was obtained as before and the root canals were left empty, a sterile cotton pellet was then placed at the canal entrance and temporarily sealed with zinc oxide-eugenol cement for 48hrs. After 48 hrs, under the same aseptic condition, the temporary restoration was removed using round burs in a high-speed handpiece and Sample 3 was taken as before. Mean difference of bacterial count was calculated immediately after irrigation at the end of first visit by subtracting sample 1 from sample 2 and after 48 hrs. by subtracting sample 1 from sample 3. Finally, all teeth were irrigated with normal saline as a final irrigation and filled using lateral compaction of gutta-percha cones (Dentsply-Herpo, Petro´polis, RJ, Brazil) with Endofill^{R} sealer (Dentsply-Herpo, Petro´polis, RJ, Brazil) and the access cavities were restored with composite restoration.

Bacteriological culturing and identification techniques:

Culturing of E. Faecalis bacteria:

10 µl from BHI of each sample were plated with L-shaped glass rods on a bile esculin agar plates, then incubated for 24 hrs at 37 C. Cultures purification was confirmed by colony morphology, Gram staining, and catalase production. Colony count was performed and expressed as CFU/ml.

Culturing of anaerobic microorganisms:

10 µl from thioglycolate broth of each sample were plated with L-shaped glass rods on Wilkins-Chalgren Anaerobic Agar plates, and then incubated at 37 C in anaerobic jars for 48 hrs. Colonies of different morphology were Gram stained and classified according to colony morphology, similar colonies were counted and the expressed as CFU/ml for each group.

Culturing *of Spp:*

10 µl from BHI of each sample were plated with L-shaped glass rods on mitis salivarius agar plates enriched with potassium toulirite, then incubated anaerobically in a candle jar at 37 C for 48 hrs. The purity of the cultures was confirmed by Gram staining, catalase production and using a biochemical identification kit (API Strep, bioMerieux: Marcy-I'Etoile, France).

Statistical analysis

Data were presented as mean and standard deviation ($\pm$SD) values. A logarithmic transformation ($\log_{10}$ transformation) of each CFU count was performed because of the high range of bacterial counts. However, bacterial data still showed non-parametric distribution; so Kruskal-Wallis test was used to compare between the three groups. This test is the non-parametric alternative to one-way ANOVA.

3. Results:

The results of this study revealed that both 2% CHX and 1% NaOCl irrigation solutions showed statistically significant reduction in mean log10 CFU values of all target bacteria immediately after irrigation at the end of first visit and after 48 hrs. except for E faecalis after 48 hrs. (Table 1, 2 and 3 & Fig. 1, 2 and 3).

Table (1): Mean difference and (±SD) standard deviation values of changes in log10 CFU of E. faecalis in each group of irrigants immediately after irrigation and after 48 hrs.

Group	Sampling time	Mean difference	±SD	*P*-value
Saline	Immediately	1.4	±1.4	0.053
	After 48 hrs.	0.3	±0.3	0.439
CHX	Immediately	2.1	±1.4	0.027*
	After 48 hrs.	1.4	±1.9	0.115
NaOCl	Immediately	1.7	±1.3	0.026*
	After 48 hrs.	0.2	±0.5	0.400

**: Significant at $P \leq 0.05$*

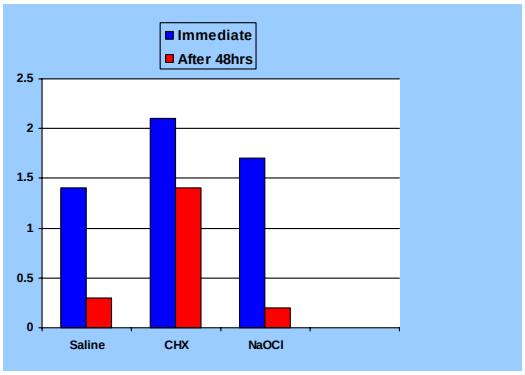

Figure (1): Changes in mean E. faecalis counts in the three groups immediately after irrigation and after 48 hrs.

Table (2): Mean difference and (±SD) values of changes in $\log_{10}$ CFU of Spp in each group of irrigants immediately after irrigation and after 48 hrs.

Group	Sampling time	Mean difference	±SD	*P*-value
Saline	Immediately	0.73	± 0.50	0.018*
	After 48hrs.	0.26	± 0.56	0.397
CHX	Immediately	1.32	± 0.63	0.012*
	After 48hrs.	1.19	± 0.38	0.012*
N**aOCl**	Immediately	0.92	± 0.62	0.018*
	After 48hrs.	0.43	± 0.40	0.043*

**: Significant at $P \leq 0.05$*

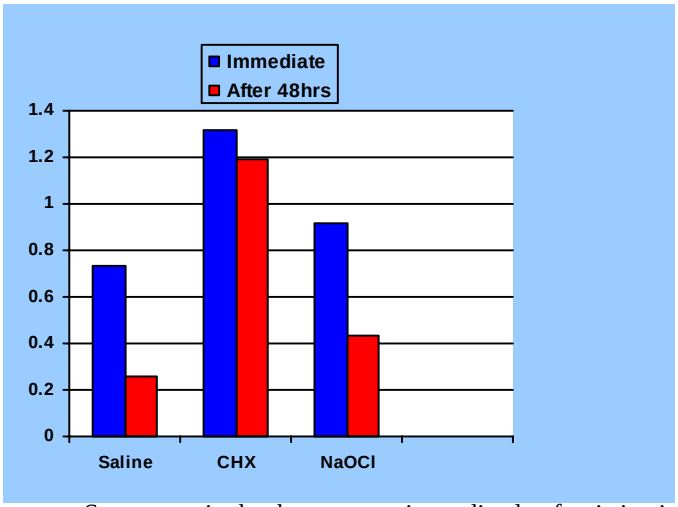

Figure (2): Changes in mean Spp counts in the three groups immediately after irrigation and after 48 hrs.

Table (3): Mean difference and (±SD) values of changes in log10 CFU of Gram positive anaerobes in each group of

irrigants immediately after irrigation and after 48 hrs.

Group	Sampling time	Mean difference	±SD	*P*-value
Saline	Immediately	0.59	± 0.68	0.017*
	After 48hrs.	0.10	± 0.23	0.092
CHX	Immediately	0.56	± 0.33	0.012*
	After 48hrs.	0.49	± 0.37	0.012*
NaOCl	Immediately	1.13	± 0.42	0.012*
	After 48hrs.	0.86	0.62	0.017*

**: Significant at $P \leq 0.05$*

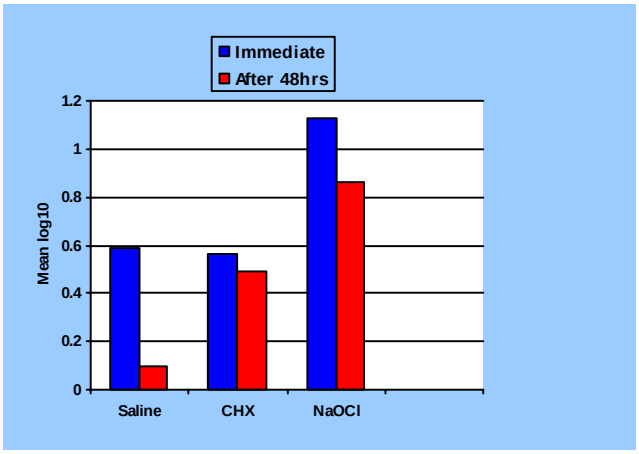

Figure (3): Changes in mean Gram positive anaerobes counts in the three groups immediately after irrigation and after 48 hrs.

4. Discussion:

The pathogenicity of endodontic microorganisms responsible for stimulating apical periodontitis creates the need for finding effective antimicrobial medicaments *(Nair et al., 2005).* NaOCl and CHX are the most frequently widely used antimicrobial agents for treatment of root canal infection *(Estrela et al., 2004)*. These medicaments present chemical characteristics that are particularly responsible for their distinct results when compared *(Estrela et al., 2003 and Siqueira et al., 2002).*

We used 1% NaOCl as an irrigant in this study as it has been reported that toxicity of NaOCl is directly proportional to its concentration *(Bondestam et al., 1996).* The increase in concentration is directly proportional to the antimicrobial effect and tissue dissolution capacity and inversely proportional to biologic compatibility. Thus, considering the high surface tension of NaOCl and that antimicrobial action that can be achieved with the less concentrated solution, the best option is 1% NaOCl *(Estrela et al., 2002).*

2% CHX used in this study, as it is known to have a substantive broad antimicrobial spectrum, it has the ability to be adsorbed and release gradually from the hydroxyapatite surfaces. It is also effective against Gram +ve and Gram -ve bacteria as well as yeasts, but more effective against Gram +ve bacteria *(Athanassiadis, 2007).* 2% CHX was used in this study as it produces bactericidal action as precipitation of the cytoplasmic contents, which results in cell death *(Gomes et al., 2003).* However, low concentration of CHX (0.12%) did not eliminate E. faecalis in any time interval *(Sassone L M et al., 2003).*

In this study we evaluate the effect of 2% CHX and 1% NaOCl on three types of bacteria. First *E.* Faecalis was tested because it was reported as therapy-resistant bacteria in the root canals. It has been frequently found in root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases *(Molander et al., 1998).* E. Faecalis can be present in primary endodontic infections and its persistence can lead to post-treatment disease. These bacteria over long periods of time may induce or maintain a periapical lesion *(Dahle´n et al., 2000).* Second Streptococci as it comprise a relatively high proportion, approximately 20% (range 16–50%) of the microorganisms recovered from the canals of teeth with post-treatment disease *(Peciuliene et al., 2001and Cheung et al., 2001)*.Third Gram +ve anaerobes as they may cause persistent endodontic infections, the investigation of their prevalence in

primary infections assumes special importance *(Siqueira et al., 2002).*

The precautions taken in this study, such as getting a sterile environment for opening the pulp cavity, minimization of the time taken to culture the samples reduced the risk of contamination to a minimum.

The root canals were left empty for 48hrs before the third sample to allow surviving bacteria in the root canal to multiply to a level that would be detectable at a subsequent appointment *(Gomes et al., 1996, Sundqvist et al., 1998 and Ferrari et al., 2005).*

Under the condition of our study the results showed that there was similar effect of both 2% CHX and 1% NaOCl on tested bacteria in all times of samples collection, this was in accordance with *Gomes et al., 2001.*

As for E. Faecalis both 2% CHX and 1% NaOCl showed statistically significant reduction in mean log10 CFU values at the end of the first visit. However, there was no statistical significant reduction in mean log10 CFU values in both CHX and NaOCl groups after 48hrs, this may be due to environmental change due the biomechanical preparation, which eliminated the most sensitive microorganisms, provided better growth conditions for E.Faecalis, in addition to the fact that E.Faecalis have the ability to penetrate dentinal tubules *(Sydney 1996 and Ferrari et al., 2005).*

Similar results were found by *Siren et al., 1997, Kalfas et al., 2001 and Cha´vez de Paz et al., 2003* who Attributed this to the in-adequate seal which may allow entrance of bacteria as well as fluid leakage from oral environment into the root canal.

Contradicting to our results concerning the limited effect of CHX on E. Faecalis *Leonardo et al., 1999* reported that 2% CHX reduce microbial activity in the root canal for up to 48 hrs. After application. This may be due to difference in the time of sample collection as they keep the paper points for very short time only 30 seconds in the root canal not 60 second as in our study. Moreover they used different microbiological processing technique culture technique and measurement of inhibition zone were used .

Conclusion:

1. 2% CHX and 1% NaOCl were effective against all the types of tested bacteria immediately after irrigation at the end of first visit.
2. E. Faecalis was the most resistance bacteria to both 2% CHX and 1% NaOCl irrigants after 48 hrs.

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