In Vivo Antimicrobial Effect Of 5.25% Sodium Hypochlorite In Root Canals Treatment In Duhok City, Iraq

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Abstract

Aim: This study aims to evaluate the bacterial reduction after conventional root canal treatment using 5.25% sodium hypochlorite.

Materials and Methods: The study criteria included 100 patients requiring root canal treatments due to pulpitis. The diagnosis was made based on objective clinical signs (examination, probing, percussion, palpation) and X-ray investigation. The clinical procedures were carried out under strict aseptic precautions. All patients were subjected to biomechanical preparation followed by irrigation using sodium hypochlorite at a concentration of 5.25% which is considered the highest concentration without causing any toxic effect on the patient. All microbiological samples were taken immediately after the access preparation baseline (S1) and after the completion of the root canal instrumentation and disinfection with 5.25% sodium hypochlorite (S2) and sent for microbiological analysis.

Results: One hundred patients with infected root canal were selected for this in vivo study. All S1 samples were positive for microbial isolates, 71 samples harbored cultivable bacterial isolates, 22 were fungus (Candida albican), and 7 samples showed no growth so in total adding up to 151 isolates identified from 100 patients. Bacterial isolates before chemomechanical treatment showed the highest prevalence belonging to Enterococcus faecalis followed by Streptococci, Staphylococci and Candida albican. After the chemomechanical treatment with 5.25% of sodium hypochlorite no cultivable bacteria were isolated from 38 patients root canals, and therefore the treatment managed to eradicate 54% of the 151 isolated bacterial species from the 71 infected root canals.

Conclusion: The antimicrobial effect of 5.25% sodium hypochlorite was effective in eradicating a high number of the bacterial isolates along with the fungus identified in infected root canals. This high concentration of sodium hypochlorite presented no toxic damage to the root canal or the area around it when handled with care.

INTRODUCTION

Root canal disinfection is vital since the successful eradication of the microbial flora is the only assurance for the ultimate success of the endodontic treatment, and to achieve this effective chemomechanical instrumentation is crucial (Mashalkar et al. 2014). Modern endodontic therapy comprises of mechanical debridement of dentine with chemicals for irrigation and cleansing, aiming to remove microbes off the root canal system. Although existing instruments can shape the walls of a root canal predictably, these can only contact part of the canal walls (Walsh and George 2017). Thus, the emphasis of the antibacterial effectiveness of instrumentation and irrigation is on the use of irrigating solutions with the strongest antibacterial activity that is necessary to accompany the mechanical preparation (Saleh 2010).

Even with the new technological advances in instrumenting root canals, about 35% of it surfaces remain un-instrumented, and therefore, cleaning of the canal regarding soft tissue removal and eradication of microorganisms mostly depend on the chemical action of the irrigating solutions due to the anatomic complexity of the pulp space (Peters et al. 2001). Otherwise, remaining pathogens may survive in sufficient numbers to jeopardize the outcome of the root canal treatment (Sjogren 1996, Sjogren et al. 1997, Sunqvist et al. 1998, Siqueira 2001). Therefore, it becomes evident that antibacterial irrigant must be used to maximize

bacterial elimination from the root canal. Irrigation is also essential to suspend and rinse away debris formed during instrumentation, acting as a lubricant for instruments and removing the smear layer that forms on instrumented dentine surfaces. These smear layers are inorganic and organic materials that may contain microorganisms.

Sodium hypochlorite is a commonly used irrigant since its introduction in endodontics treatment by Walker in 1936 (Walker 1936). Sodium hypochlorite was used due to its germicidal properties and its capability to dissolve soft tissues of the dental pulp (walker 1936, Grossman and Meiman 1982). Sodium hypochlorite is an excellent non-specific proteolytic and antimicrobial irrigation solution. Depending on the different concentrations of sodium hypochlorite, it is very efficient against most microorganisms typically found in the root canal system, including Enterococcus faecalis. The principle of this study is to evaluate the efficacy of 5.25% sodium hypochlorite in reducing or even eradication microbial isolates from root canals.

MATERIAL AND METHOD

Subject selection

The present in vivo study was conducted on 100 patients, who were clinically diagnosed with pulpitis with symptoms of continuous pain, swelling, tenderness to pressure. All patients were selected from the root canal treatment of Duhok Dental Polyclinic, Duhok City, Iraq. The study was approved by

the ethical committee of Duhok Directorate General of Health. The general health of all patients was in good shape, and the ages range was between 19 and 70. All included patients showed no presence of any significant systemic conditions and pregnant and nursing females were also excluded from the trial. Written informed consent was obtained from each patient before procedure.

Sample collection

Throughout the endodontic procedure, an aseptic setting was maintained. After local anesthetic administration, the teeth were cleaned and isolated under rubber dam and access opening was done with a sterile high-speed diamond round bur.

The first root canal sample (S1) before chemomechanical treatment was taken as follows. A sterile #15 K-file was introduced into the root estimating the working length approximately 1-mm short of the apex, this was confirmed using an electronic apex locator, and then using a small amount of normal saline in the root a gentle filing motion was then applied with the file. Sterile paper points were then placed in the canal to the same level to soak up the fluid from the canal and left for 1 minute. The paper points were then transferred aseptically into a vial containing thioglycollate broth medium for microbiological analysis.

As for the second root canal sample (S2) after the chemomechanical treatment with sodium hypochlorite (5.25%) the sample was taken as follows. In all cases the chemomechanical procedure was completed at the same appointment. Apical preparation was accomplished to the working length using hand nickel-titanium files in a back and forth alternating rotation motion. Depending on the root anatomy and initial diameter of the root canal, the apical files ranged from #50-70. A gauge needle was used to flush sodium hypochlorite (5.25%) into the open root canal and then dried off using sterile paper points. Using a paper point which was left in the canal for 1 minute then taken and transferred aseptically into a vial containing thioglycollate broth medium for microbiological analysis.

All the canals were then medicated with calcium hydroxide paste, after the chemomechanical treatment.

Microbiological Analysis

The vials were transferred to the Microbiology laboratory in the College of Health Sciences, Duhok University, and incubated at 37°C for 24 hours. All samples (S1 and S2) were immediately inoculated and streaked on (blood agar, chocolate agar, and MacConkey agar) incubated aerobically and anaerobically using anaerobic jars at 37°C for 24, 48 and 72h. The suspected Enterococcus faecalis were sub-cultured on bile esculine agar. The microorganisms were isolated and identified by means of cultural, morphological, physiology and biochemical characteristics according to methods described by (Cheesbrough 2006).

RESULT

One hundred patients with infected root canal were selected for this in vivo study, 51 were female, and 49 were male with an age range of 18-60. Using culture methods 71 root canal samples harbored cultivable bacterial isolates, 22 were fungus (Candida albican) and 7 root canal samples showed no growth. A total of 151 cultivable isolates belonging to 9 different bacterial species were identified from the 71 isolated root canal samples (Table 1). According to Gram staining characteristic of the 151 bacterial isolates identified, 113 (75%) were gram-positive, 16 (11%) were gram-negative species, and 22 (16%) were fungi (Candida albican) (Table 2).

Bacterial isolates before chemomechanical treatment (S1) showed the highest prevalence belonging to Enterococcus faecalis followed by Streptococci, Staphylococci and Candida albican as illustrated in table 1.

Table 1

Prevalence of microbial species in 100 isolated root canals before 5.25% sodium hypochlorite treatment.

Isolated Microorganisms	Number of isolates
Enterococcus faecalis	47
Streptococcus	31
Staphylococcus	23
Candid albican	22
Actinomyces	9
Escherichia coli	8
Bacteroides	4
Lactobacillus	3
klebsiella	2
Neisseria	1
Acinetobacter	1
Total isolates	151

Table 2

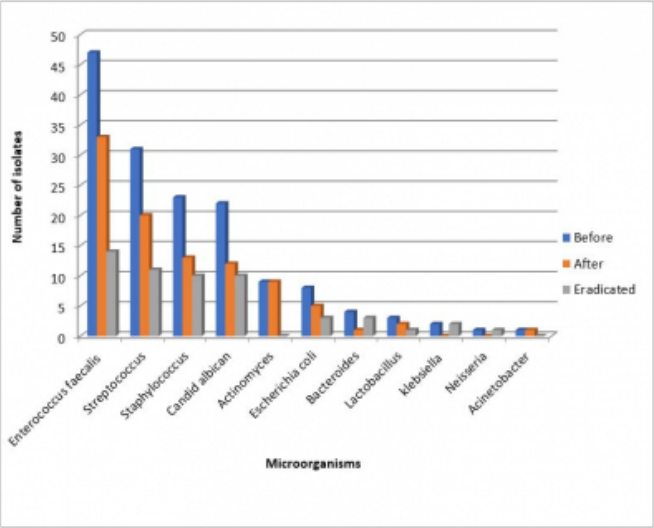
Prevalence of gram-positive, gram-negative and Fungus (Candida albican) in 100 root canals.

Gram stain	Before treatment (S1)	After treatment (S2)
Gram-positive	113 (75%)	77 (51%)
Gram-negative	16 (11%)	7 (5%)
Candida albican	22 (15%)	10 (6%)

After the chemomechanical treatment with 5.25% of sodium hypochlorite (S2) no cultivable bacteria were isolated from 38 patients root canals, and therefore the treatment managed to eradicate 54% of the 151 isolated bacterial species from infected root canals (Figure 1).

Figure 1

Prevalence of microbial isolates before and after chemomechanical treatment with 5.25% sodium hypochlorite.



DISCUSSION

A protocol routinely carried out by the Duhok Dental Polyclinic is the use of 5.25% of sodium hypochlorite. This in vivo study is designed to evaluate the effectiveness of the 5.25% sodium hypochlorite used in removing bacteria present in the infected root canal as final irrigation on patients in the clinic. The nature of this in vivo study makes the steps in root canal therapy more clinically appropriate than in vitro studies since in vitro studies can control working lengths correctly by seeing files at the apical foramen, and instrumenting teeth without any concern to access or time restrictions. Consequently, this in vivo study may be more clinically significant. The culture-dependent method was used in the identification of root canal flora of this study. The method is reliable in identifying viable bacteria, especially if samples are taken directly after antimicrobial treatment where most of the culture-independent methods may not ascertain viability (Anderson et al. 2012). Nevertheless, it is well known that around 40-55% of endodontic bacterial isolates are un-cultivated by the standard culture method (Anderson et al. 2012).

The main principle of endodontic treatment or therapy is to eradicate microorganisms from the root canal system to prevent reinfection. This can be accomplished by the use of mechanical instrumentation along with chemical irrigation.

This study confirmed the strong relationship between bacteria and apical pathology since all the studied root canals harbored bacteria before the chemomechanical

treatment (S1). The most prevalent bacterial species isolated in S1 as presented in table 1, were Enterococcus faecalis (66%) followed by Streptococcus (43%), Staphylococcus (32%) and Candida albican (30%), along with a small numbers of other bacterial isolates (Actinomyces, Escherichia coli, Klebsiella, Bacteroides, Lactobacillus, Neisseria and Acinetobacter) accounting for 39%. The microbiological results before the chemomechanical treatment of this study are very much in agreement with other in vivo and in vitro studies by Savitri Et al. 2018, EL-Sherbiny 2015, Siqueira Et al. 2007, Rôças and Siqueira 2011, and Mashalkar Et al 2014.

There was a substantial reduction in bacterial isolates after the chemomechanical (5.25% sodium hypochlorite) treatment (S2). This illustrated the vital role of instrumentation and antimicrobial substance of the irrigation in reducing bacterial isolates of the infected root canals (Savitri et al. 2018). There were no cultivable bacteria in 38 (54%) of the 71 isolated root canal after the treatment. The other 33 isolated root canals showed a massive reduction in the number of bacterial isolates. The most persistent bacteria isolated after the chemomechanical (5.25% sodium hypochlorite) treatment was Enterococcus faecalis, which was eradicated by 20% followed by Streptococcus (16%), Staphylococcus (14%) and Candida albican (14%) in Duhok city. These results displayed the effectiveness of 5.25% sodium hypochlorite against the growth of many if not all the microorganisms of infected root canals as documented by many other studies (El-Sherbiny 2015, Siqueira et al. 2007).

Numerous in vitro and in vivo studies support the strong antibacterial activity of sodium hypochlorite such as Siqueira et al. in vitro study who reported that 4% of sodium hypochlorite was significantly more effective than saline in disinfecting the root canal (Siqueira et al. 2007). Berber et al. evaluated the efficacy of different concentrations of sodium hypochlorite with hand rotary instrumentation technique against Enterococcus faecalis (Berber et at. 2006). This in vitro study concluded that sodium hypochlorite at a 5.25% concentration was most effective. As for in vivo studies, both Bystrom and Sundqvist showed that even a low concentration of sodium hypochlorite such as 0.5% was much more effective in eradicating bacteria from the root canal than saline (Bystrom and Sundqvist 1983). Similarly, other in vivo studies that supported this, were by Shuping et al. and Vianna et al and others (Bellingham 2011).

CONCLUSION

This in vivo study, the number of microorganisms were significantly reduced after the chemomechanical treatment with sodium hypochlorite 5.25%.

The 5.25% of sodium hypochlorite did not show a toxic effect on the teeth or the surrounding and therefore provide to be more acceptable than smaller concentrations.

Until this day no procedure will completely sterilize an infected root canal. Therefore, it is important to study new techniques and treatment protocols that could carry out a complete bacterial eradication.

ETHICAL DISCLOSURES

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

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