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The bactericidal efficiency of Chlorhexidine as an endodontic irrigant

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ABSTRACT

Aims: to determine the bactericidal efficiency of 0.75% Chlorhexidine in vitro . The time required for this irrigant to start its antimicrobial effect on the selected microorganisms isolated from the infected root canals and unidentified samples taken from root canals with necrotic pulps were evaluated. **Materials and Methods**: The substantive antimicrobial effect of 0.75% Chlorhexidine in vitro is also considered. Samples taken from 13 teeth with necrotic pulp from patients attended the Department of Conservative Dentistry, College of Dentistry at Mosul University. The turbidity method was applied to determine the antimicrobial effect of 0.75% Chlorhexidine and the combination of 0.5% Chlorhexidine and 0.5% sodium hypochlorite comparing with the antimicrobial effect of 2.5% sodium hypochlorite, the time required for these materials to start their antimicrobial effect on the selected microorganisms was determined using contact test. The antimicrobial effectiveness were evaluated at different time intervals, immediately, 5, 10, 15 minutes after the contact of the microorganisms with the irrigating solutions. **Results**: Both 0.75% Chlorhexidine and 2.5% sodium hypochlorite are effective on microorganisms collected from root canal. There is no significant difference between Chlorhexidine and sodium hypochlorite in their antimicrobial effect on the anaerobic microorganisms, but Chlorhexidine is more effective on the aerobic microorganisms. **Conclusion**: Chlorhexidine 0.75% and 2.5% sodium hypochlorite has an immediate effect on the selected microorganisms and unidentified samples from the teeth with necrotic pulps. The combination of Chlorhexidine 0.5% and 0.5% sodium hypochlorite has an effect started after 5 minutes.

Key Word: Chlorhexidine, Root canal, Irrigation solution

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INTRODUCTION

The infection of dental pulp can be developed in several ways, through penetration of oral bacteria to the dentinal tubules opened by caries, restorative procedures, dental trauma or tooth wear, or by infection of lateral canal from deep gingival pocket.(1) The result of the infection is unpredictable, depending on the virulence of the infecting microorganisms and the resistance of the host, the pulp may be partially or totally involved, and the infection may be acute or chronic and may lead to necrosis.(2)

The success of the endodontic treatment directly influenced by the elimination of microorganism in the infected root canal.(3) Failures in endodontic therapy may due to persistence of infection. Therefore, the endodontist should be acquainted with the pulpal and perapical pathosis and these inflammatory lesion, such information facilitate rational treatment toward microbial elimination.(4)

There are large number of irrigating solutions used in endodontic treatment, sodium hypochlorite is widely used as endodontic irrigant because it has a strong antimicrobial activity and has an important role in dissolving organic part of pulpal reminant and dentin, but sodium hypochlorite is caustic if accidentally expressed into the

periapical area. In addition, the active chlorine in the solutions may damage the patient's clothing through its strong bleaching effect, therefore, alternative irrigating solutions have been pursued that could replace sodium hypochlorite. Chlorhexidine has been in use for a long time in dentistry because of its antimicrobial activity and its low toxicity.[5]

Chlorhexidine is widely used in a concentration of 0.2% as a root canal irrigant.[6] But a viable microorganisms remained within the root canals irrigated with 0.2% Chlorhexidine. In other study, Sassone *et al*.,[7] found that 0.12% has no antimicrobial effect and 0.5% has antimicrobial effect that started after 5 minuet and 1% has an immediate effect on the selected microorganisms. Therefore in this study the bactericidal efficiency of 0.75% Chlorhexidine will be investigated.

MATERIALS AND METHODS

Samples taken from thirteen patient with asymptomatic uniradicular teeth with necrotic pulp which was determined by radiographic presence of apical rarefaction and lack of response to pulp vitality test by using electric pulp tester (Dentotest TB 09, Germany), the patients who were included in this study had not been treated with antibiotic in preceding three months.[6,8] The samples was taken directly to the laboratory at the same day for the microbiological work and placed in an incubator for 18 hours at 37°C. Four irrigating solutions were used 0.75% Chlorhexidine, 2.5% sodium hypochlorite, combination of 0.5% Chlorhexidine and 0.5% sodium hypochlorite, and normal saline. Chlorhxidine (ENECA limited, UK) was diluted to 0.75% Chlorhexidine with a sterile distilled water[9], at the same day of use. Sodium hypochlorite (Household bleach Clorox 6%, Turkey) was diluted in a similar manner, combination of Chlorhexidine and sodium hypochlorite prepared by mixing the equal amount of 0.5% sodium hypochlorite and 0.5% Chlorhexidine, which are diluted according to previous method. Normal Saline used as a control group (0.9 w/v sodium chloride, Mosul I.V. Plant, Iraq).Turbidity method was used to determine the antimicrobial effect of the Chlorhexidine, the measurement of light absorption was done by the use of Spectrophotometer (CEIL CE 1021/ England) at 590 nanometer (10,11).This method was achieved by using a series of test tubes containing an equal amount of sterilized brain–heart infusion broth (4ml) for aerobic microorganisms and 4ml of Thioglycolate broth for anaerobic microorganisms. The contact test was used to determine the time required for each of the four irrigating solutions to start its effect.[7] The following strains of bacteria were used *Escherichia.coli, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella spp., Candida albicans* and *actinobacillus actinomycetecomitans.* All these microorganisms were isolated and identified from clinical samples at the Microbiology Laboratory of college of Dentistry, University of Mosul. The residual effect after the canal irrigation with Chlorhexidine was studied using White *et al*., method.[12] Twenty–six freshly extracted, single–rooted teeth were obtained from dental clinics. The teeth were stored in tap water at 4°C until used. These teeth divided into two equal groups, in the first group, Chlorhexidine used as a root canal irrigant (experimental group); in the second group normal saline used as root canal irrigant (control group).

The following statistical methods were used to analyze the results. Calculation of statistical mean and standard deviation, One way analysis of variance at level of significance 0.05 was used by the aid of computer program (SAS) to analyze the data, And Duncan Multiple Range Test to compare between the studied solutions.

RESULTS

Both 0.75% Chlorhexidine and 2.5% sodium hypochlorite are effective on microorganisms collected from root canal. There is no significant difference between Chlorhexidine and sodium hypochlorite in their antimicrobial effect on the anaerobic microorganisms, but Chlorhexidine is more effective on the aerobic microorganismms. The combination (0.5% Chlorhexidine and 0.5% sodium hypochlorite) and normal saline have lesser antimicrobial activity, and there are no significant difference in their antimicrobial activity between the combination and normal saline on the anaerobic but the normal saline is ineffective on

the aerobic when compared to the control group (normal growth).The results are shown in Tables (1–4)

Both 0.75% Chlorhexidine and 2.5% sodium hypochlorite eliminated all the microorganisms that were used in this experiment at all time period. The combination of 0.5% Chlorhexidine and 0.5% sodium hypochlorite has an effect started after 5 minutes on all tested microrganisms, normal saline was not showed any antimicrobial effect.

Table 1: ANOVA for the antimicrobial effect of the irrigating solution on the aerobic microorganisms isolated from infected root canals.

Aerobic	Degree of freedom	Sum of Squarees	Mean square	f –value	*p*–value
Between groups	4	7.28	1.82	106.07	0.000
Within groups	60	1.02	1071		
Total	64	8.30			

Table (2): Duncan's Multiple range tests for the antimicrobial effect of the irrigating solution on the aerobic microorganisms isolated from infected root canals

Treatment	Absorbance Mean(nm) ±SD	Duncan grouping
Normal Growth	1.03 ± 0.19	A
Normal saline	0.93 ±0.13	A
Combination	0.74 ±0.11	B
Sodium hypochlorite (2.5%)	0.31 ±0.11	C
Chlorhexidine (0.75%)	0.19 ±4.16	D

Different letters mean significant differences; SD: standard deviation

Table (3): ANOVA for the antimicrobial effect of the irrigating solution on the anaerobic microorganisms isolated from infected root canals.

Anaerobic	Degree of freedom	Sum of Squarees	Mean square	f – value	*P*–value
between groups	4	6.57	1.64	183.18	0.000
Within groups	60	0.53	8.96		
Total	64	7.1			

Table (4): Duncan's Multiple Range Tests for the antimicrobial effect of the irrigating solution on the anaerobic microorganisms isolated from infected root canals.

Treatment	Absorbance Mean(nm) ± SD	Duncan grouping
Normal Growth	0.97 ±0.14	**A**
Normal saline	0.87 ±9.6	**B**
Combination	0.80 ±0.10	**B**
Sodium hypochlorite (2.5%)	0.24 ±0.10	**C**
Chlorhexidine (%٠.٧٥)	0.24 ±4.5	**C**

Different letters mean significant differences; SD: standard deviation

Tables (5–11) showed the antimic-robial effect of the irrigating solutions us-ed in the study on *Streptococcus pyogenes, Staphylococcus aureus, Escherichia Coli, Klebsiella spp., Candida albicans, Actino-bacillus actinomycetecomitans* and unide-ntified samples from infected root canals respectively.

Table 5:Contact test result of the irrigating solutions on *Streptococcus pyogenes.*

Time after contact	0.75% CHX	2.5% NaOCl	Combination	Normal saline
Immediate	+	+	–	–
5 min	+	+	+	–
10 min	+	+	+	–
15 min	+	+	+	–

CHX: Chlorhexidine; NaOCl: Sodium hypochlorite; Combination= (0.5% Chlorhexidine + .5%sodium hypochlorite)

Table 6*:* Contact test result of the irrigating solutions on *Staphylococcus aureus.*

Time after contact	0.75% CHX	2.5% NaOCl	Combination	Normal saline
Immediate	+	+	–	–
5 min	+	+	+	–
10 min	+	+	+	–
15 min	+	+	+	–

CHX: Chlorhexidine; NaOCl: Sodium hypochlorite; Combination= (0.5% Chlorhexidine + .5%sodium hypochlorite)

Table 7: Contact test result of the irrigating solutions on *Escherichia. Coli.*

Time after contact	0.75% CHX	2.5% NaOCl	Combination	Normal saline
Immediate	+	+	–	–
5 min	+	+	+	–
10 min	+	+	+	–
15 min	+	+	+	–

CHX: Chlorhexidine; NaOCl: Sodium hypochlorite; Combination= (0.5% Chlorhexidine + .5%sodium hypochlorite)

Table 8: Contact test result of the irrigating solutions on *Klebsiella spp*

Time after contact	0.75% CHX	2.5% NaOCl	Combination	Normal saline
Immediate	+	+	–	–
5 min	+	+	+	–
10 min	+	+	+	–
15 min	+	+	+	–

CHX: Chlorhexidine; NaOCl: Sodium hypochlorite; Combination= (0.5% Chlorhexidine + .5%sodium hypochlorite)

Table 9: Contact test result of the irrigating solutions on *Candida albicans*.

Time after contact	0.75% CHX	2.5% NaOCl	Combination	Normal saline
Immediate	+	+	–	–
5 min	+	+	+	–
10 min	+	+	+	–
15 min	+	+	+	–

CHX: Chlorhexidine; NaOCl: Sodium hypochlorite; Combination= (0.5% Chlorhexidine + .5%sodium hypochlorite)

Table 10: Contact test result of the irrigating solutions on *Actinobacillus actinomycetecomitans*

Time after contact	0.75% CHX	2.5% NaOCl	Combination	Normal saline
Immediate	+	+	–	–
5 min	+	+	+	–
10 min	+	+	+	–
15 min	+	+	+	–

CHX: Chlorhexidine; NaOCl: Sodium hypochlorite; Combination= (0.5% Chlorhexidine + .5%sodium hypochlorite)

Table 11: Contact test result of the irrigating solutions on unidentified samples from infected root canals

Time after contact	0.75% CHX	2.5% NaOCl	Combination	Normal saline
Immediate	+	+	–	–
5 min	+	+	+	–
10 min	+	+	+	–
15 min	+	+	+	–

CHX: Chlorhexidine; NaOCl: Sodium hypochlorite; Combination= (0.5% Chlorhexidine + 5%sodium hypochlorite)

DISCUSSION

Root canal infection possesses some peculiarities that differentiated them from infection in other human sites. Once established, a root canal infection cannot be eliminated by host defense mechanisms nor by systemic antibiotic therapy. This is due to the absence of the blood supply in a necrotic pulp impedes the transport of defense cell and systemically administrated antibiotic to the infected site. Due to microbial localization of endodontic infection, it can be treated by professional intervention using both chemical and mechanical procedure.[13]

The mechanical instrumentation of root canal not totally eliminate the microrganisms from the root canal.[14] Therefore, the use of the irrigating solutions during the endodontic procedure is very important, because it enhance a bacterial elimination and facilitates the removal of necrotic

tissue and dentin chips from the root canal; the irrigant also prevent packing of the infected tissue apically in the root canal and into the periapical area, Chlorhexidine is used for long time in dentistry because of its antimiocrobial properties and low toxicity.[5]

The results of the in vitro study of the antimicrobial effect of 0.75% Chlorhexidine, showed that there are no significant differences between Chlorhexidine and sodium hypochlorite on the anaerobic growth of microorganisms isolated from the infected root canal both combinations (0.5% Chlorhexidine and 0.5% sodium hypochlorite) and normal saline show less effect on anaerobic microorganisms than Chlorhexidine (0.75%) and sodium hypochlorite (2.5%) but it is found that Chlorhexidine is more effective on the aerobic microorganisms isolated from infected root canal than sodium hypochlorite; normal saline has no effect on the aerobic microorganisms when compared to the control groups (normal growth). Chlorhexidine and sodium hypochlorite equally effective antimicrobial agents at similar concentrations against Enterococcus faecalis.[15,16]

In another study Chlorhexidine (2%) has a better antimicrobial activity than sodium hypochlorite (2.25%) [17] and the number of the post–irrigant positive cultures and the number of colony forming unit positive culture obtained from Chlorhexidine treated teeth were lower than the number obtained from sodium hypochlorite treated teeth ; this is coincide with our results. From the result it appears that the use of the combination (Chlorhexidine and sodium hypochlorite) will not increase the effect of the individual irrigating solution and it may decrease the effect of the irrigating solutions when compared to the effect of the irrigant when used separately. The combination of 2% Chlorhexidine and 2% sodium hypochlorite had antimicrobial activity similar to Chlorhexidine and greater than sodium hypochlorite alone. The combination of 0.05% Chlorhexidine and 0.5% sodium hypochlorite was more effective than Chlorhexidine alone but less effective than sodium hypochlorite alone.[18] While, the result of this study showed that both 0.75% Chlorhexidine and 2.5% sodium hypochlorite have an antimicrobial effect greater than that obtained by combination of the 0.5% Chlorhexidine and 0.5% sodium hypochlorite.In contact test, the time required for each irrigant used to start its antimicrobial effect was determined, the results show that both 2.5% sodium hypochlorite and 0.75% Chlorhexidine have an immediate effect on *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Klebsiella spp., Candida albicans, Actinobacillus actinomycetecomitans* and unidentified samples from infected root canals. The antimicrobial effect of combination of 0.5% Chlorhexidine and 0.5% sodium hypochlorite) started after 5 minutes this means that mixing both irrigants will decrease their individual effect. Also this test shows no antimicrobial effect for normal saline at all intervals. Chlorhexidine 0.5% and 0.12 have no immediate effect on the selected microorganisms but 1% Chlorhexidine has an immediate effect on the tested microorganisms.[7] A superior antimicrobial activity of 5.25% sodium hypochlorite has been shown compared to 2% Chlorhexidine on the *Staphylococcus aureus, Enterococcus faecalis, Streptococus salivarius, Sterptococcus pyogenes, Escherichia coli* and *Candida albicans*. [19] Another study for the effect of different concentrations of sodium hypochlorite (0.5, 1, 2.5 and 5.25 %) on different microrganisms (*Actinomyces naeslundii, Candiada albicans* and *Enterococcus faecalis)* was done and found that all solutions started their effect after 10 seconds in case of *A. naesluni* and *C. albicans.* but *E. faecalis* resist the effect of 0.5%, and 1%o sodium hypochlorite and affected by 2.5% sodium hypochlorite after 5 minutes.[20] Sodium hypochlorite (4% and 5.25%) have antimicrobial effect greater than than 2% Chlorhexidine when studied on black pigmented gram–negative anaerobes and facultative anaerobic bacteria.[13] One percent and 2% Chlorhexidine solution, and 5.25% sodium hypochlorite require 15 seconds to eliminate *Prophyromonas endodontalis, Porphyromonas gingivalis* and *Perovtella intermedia*.[21]

The various methodologies can be used to assess the antimicrobial activity of endodontic irrigants. Indeed, the methodology can be possible explanation for the difference found in the results between st-

udies. In some methodologies allow direct contact of the substances with the microrganisms. With others microorganisms located inside the dentinal tubules did not necessary have direct contact with the antimicrobial substance.(22) The differences in the finding of the studies in which same method were used is explained by the fact that the result depend on the microbial strains, contact conditions, volume of microbial inoculum, type and concentration of irrigating solution, temperature, contact period, pH and electrolytes.(22) he irrigant of choice should be one that exerts its antimicrobial activity quickly against the majority of microorganisms found in the root canal and dentinal tubules.

CONCLUSIONS

Chlorhexidine gluconate (0.75%) soluteon is an effective antimicrobial agent when utilized as an irrigant in the endodontic treatment of the teeth with necrotic pulps. There is no significant difference between the antimicrobial effect of 0.75% Chlorhexidine and 2.5% sodium hypochlorite. he combination of 0.5% Chlorhexidine and 0.5% sodium hypochlorite reduce their individual antimicrobial activity.

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