ORIGINAL RESEARCH

Antibacterial Effect of Metronidazole vs Chlorhexidine Solutions in Treatment of Root Canals of Primary Anterior Teeth

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Abstract

The aim of this study was to evaluate and compare the antibacterial effect of 0.5% metronidazole, 2% chlorhexidine, and normal saline irrigant solutions against *Enterococcus faecalis* bacteria in the treatment of root canals of primary anterior teeth.

Materials and methods: This study was carried out on sixty nonvital primary anterior teeth of Egyptian children diagnosed with ECC. These teeth were classified equally into three groups according to irrigation materials. A fresh sample was collected from each root canal after access opening before and 3 days after irrigation with the help of a paper point. The number of organisms (*E. faecalis)* before and after irrigation was compared for each group.

Results: The bacterial count of *E. faecalis* was decreased in all groups however, these differences were statistically insignificant where ($p < 0.05$).

Conclusion: Both 0.5% metronidazole and 2.0% chlorhexidine appeared to be superior against *E. faecalis* bacteria as endodontic irrigants in pulpectomy anterior primary teeth with higher antibacterial efficacy compared to saline.

Clinical significance: The success of endodontic treatment depends on the removal of microbes, from the root canals and avoidance of reinfection thus; this study provides an insight on the effects of different irrigant solutions to further help dental practitioners in the endodontic management of primary dentition.

Keywords: Chlorhexidine, Dental caries, *E. faecalis*, Metronidazole, Pulpectomy.

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Introduction

When dental caries invades the pulp tissues, and it was necessary to treat the tooth endodontically, it is essential for endodontic success to eliminate or at least reduce the number of microorganisms and remove inflamed or necrotic pulpal tissue.[1] Irrigants should ideally have antimicrobial and tissue-dissolution actions as well as other advantageous properties, such as lubrication, demineralization, and the ability to remove debris and the smear layer.[2] The most common irrigants used during root canal preparation is sodium hypochlorite (NaOCl). It is an effective tissue solvent and has excellent antimicrobial property,[3] however, its tissue toxicity corrosive effect on endodontic instruments, and bad odor have to be of concern.[4] Also, chlorhexidine digluconate (CHX) has been suggested as an irrigant in endodontic treatment because of its antimicrobial activity however, its inability to dissolve organic matter is a drawback in its clinical use.[5] In the last years, a new concept has been developed, which employs the use of a combination of antibacterial drugs (metronidazole, ciprofloxacin, and minocycline) for disinfection of pulpal and periradicular tissues. It has been reported that this mixture can sterilize root dentin.[6] In instances where anaerobic bacteria are acting as sole or major pathogens, metronidazole acts specifically without distributing the commensal aerobic flora. The resistance to it develops very rarely.[7] Hence, it has been suggested for use as an irrigant solution. This study was conducted to evaluate and compare the antibacterial effect of 0.5% metronidazole, 2% chlorhexidine, and normal saline irrigant solutions against *E. faecalis* bacteria in the treatment of root canals of primary anterior teeth.

Materials and Methods

This study was carried out on sixty nonvital primary anterior teeth from 20 patients (three anterior teeth from each patient) of Egyptian children suffering from ECC and selected from the Pediatric Dental Outpatient Clinic, Faculty of Dental Medicine, Cairo, Al-Azhar University.

Inclusion Criteria

Children's age of 3–5 years irrespective of gender were selected. Decayed teeth with or without periapical pathosis, patients without systemic diseases, patients that did not report the use of antibiotics for at least 3 months prior to the treatment.

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Exclusion Criteria

Children younger than 3 years old and older than 5 years old, children with systemic diseases, patients that report the use of antibiotics 3 months prior to treatment. Teeth were divided into three equal groups according to the irrigant solutions used.

Group A

20 nonvital anterior primary teeth irrigated with 0.5% metronidazole after pulpectomy.

Group B

20 nonvital anterior primary teeth irrigated with 2% chlorhexidine solution after pulpectomy.

Group C (Control Group)

20 nonvital anterior primary teeth irrigated with normal saline after pulpectomy. After isolation with a rubber dam, access cavity was gained on the palatal surface of each tooth using sterile round bur No. 2 or No. 4. The first microbiological sample (baseline) was collected by introducing a sterile paper point with a diameter compatible with that of the canal. The paper points were kept in place for 60 seconds in the root canals and immediately transferred in a screw-capped test tube containing Amies transport media stored at room temperature. A sample was collected from each root canal before and 3 days after irrigation. All samples were transferred as early as possible to the bacteriological laboratory at the Faculty of Pharmacy, Al-Azhar University. The samples were streaked on M-Enterococci media (selective media for *E. faecalis*) by using cell spreaders. The plates were incubated in the anaerobic chamber for 7 days at 37°C. After 7 days, the total number of colonies on the incubated plates were counted with the help of a digital colony counter and expressed as the total colony-forming units per mL (CFU/mL). The organisms were identified on the basis of colony morphology, gram stain appearance, and standard biochemical reactions.

Media for Culturing of Clinical Specimens[8]

M-Enterococcus Agar is used for the selective isolation and enumeration of enterococci by membrane filtration in a laboratory setting.

The number of *E. faecalis* colonies before and after irrigation was compared for each group. The treated canals were sealed with temporary sealing. After 48 hours, the temporary sealing was removed, and the second sample was collected as done previously. Obturation was performed with zinc oxide and eugenol in the later appointments followed by composite resin as a final restoration (Table 1).

Table 1: Formula liter

Formula liter	
Enzymatic digest of casein	15 g
Enzymatic digest of soybean meal	5 g
Yeast extract	5 g
Dextrose	2 g
Dipotassium phosphate	4 g
Sodium azide	0.4 g
2,3,5-Triphenyl tetrazolium chloride	0.1 g
Agar	10 g

Final pH: 7.2 ± 0.2 at 25°C

Table 2: Mean and standard deviation values of bacterial count for *Enterococcus faecalis* after irrigation with 0.5% metronidazole

Group	*Time*	*Mean*	*SD*	*Effect*	*p value*
Metronidazole	Before	2120	147	91.4%	0.02*
	After	283	23		

*Significant ($p < 0.05$); ns, non-significant ($p > 0.05$)

Table 3: Mean and standard deviation values of bacterial count for *Enterococcus faecalis* after irrigation with 2% chlorhexidine

Group	*Time*	*Mean*	*SD*	*Effect*	*p value*
Chlorhexidine	Before	1650	117	96.1%	0.01*
	After	65	32		

*Significant ($p < 0.05$); ns, non-significant ($p > 0.05$)

Table 4: Mean and standard deviation values of bacterial count for *Enterococcus faecalis* after irrigation with normal saline

Group	*Time*	*Mean*	*SD*	*Effect*	*p value*
Normal saline	Before	1213	126	49.1%	0.07^{ns}
	After	513	16.6		

^{ns}Non-significant ($p > 0.05$)

Results

Metronidazole Group

The bacterial count ranged from 121 and 257 before irrigation, while it ranged from 19.02 and 31.23 after irrigation. The ratio of the decreased bacterial count of the metronidazole group was about 91.4%. There was a statistically significant difference ($p < 0.05$) between groups where the highest mean value of bacterial count for *E. faecalis* was (2236 ± 147) before the application of metronidazole while the least bacterial count was (163 ± 23) after application (Table 2).

Chlorhexidine Group

Microbiological study of samples collected before irrigation and 3 days after irrigation showed that the bacterial count ranged from 118 and 232 before irrigation while it was ranged from 11.02 and 24.23 after irrigation. The ratio of the decreased bacterial count of chlorhexidine group was about 96.1%. There was a statistically significant difference between groups where the highest mean value of bacterial count was (2141 ± 117) before the application of chlorhexidine while the least bacterial count was (138.06 ± 23) after application (Table 3).

Normal Saline Group (Control Group)

Microbiological study of samples collected before irrigation and 3 days after irrigation showed that the bacterial count ranged from 112 and 189 before irrigation while it was ranged from 54.06 and 63.02 after irrigation. The ratio of decreased bacterial numbers of the saline group was about 49.1%. There was a statistically significant difference between groups where the highest mean value of bacterial count was (2013 ± 126) before the application of normal saline while the least bacterial count was (1043 ± 23) after application (Table 4).

Comparison between the Three Irrigant Solutions

There was no significant difference between the action of 2.0% chlorhexidine and 0.5% metronidazole, against *E. faecalis* while there was a significant difference when compared with the action of normal saline (Fig. 1).

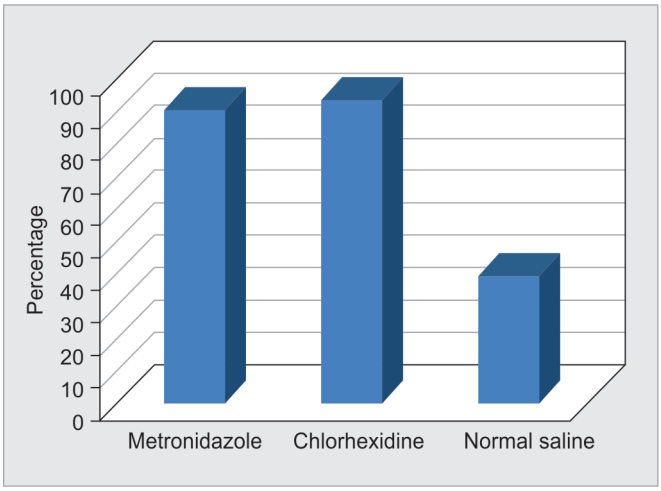

Fig. 1: Column chart of mean values of reduction in bacterial count percentage in *Enterococcus faecalis* after irrigation in all groups

Discussion

The main goal of endodontic intervention is to recover the functional aspects of the affected tooth by healing or preserving the integrity of the periapical tissue while eliminating the microorganisms from root canals to prevent subsequent reinfection.[9] During pulpectomy, irrigation is necessary as it renders the canal system free of necrotic pulp tissues, biofilms, bacteria, and bacterial products and also serves as a physical flush to remove dentinal debris; this creates an environment favorable to successful obturation, and ultimately to clinical success.[10] Some researches indicate 2.0% CHX may be a good choice for maximized antibacterial effect at the end of the chemomechanical preparation,[11,12] while previous studies have shown that treatment with local metronidazole resulted in significant improvements in clinical parameters and reduction in the number of anaerobic periodontal-pathogens.[13] However, others indicate the resistance to metronidazole develops very rarely.[7] Both irrigants were selected for this study to compare their effect against *E. faecalis* using the normal saline as a control group in this study. *Enterococcus faecalis* was chosen as the test organism because its prevalence has been a conspicuous finding in a high percentage of root canal failures in pulpectomy.[14] The 2% CHX produced complete inhibition of *E. faecalis* in 5 minutes contact time, which is evidenced by the study conducted by Önçağ et al.[13] in this research, the irrigation time was 5 minutes for 5% metronidazole and normal saline to simulate that for 2% chlorhexidine.[15] Furthermore, the reduction of the number of *E. faecalis* bacterial colonies before and after irrigation for 0.5% metronidazole solution was 91.4%, and 96.1% for 2.0% chlorohexidine solution and lastly for normal saline it was 49.8%. Comparing the results at different appointments among the three groups, it was found that the mean bacterial count of *E. faecalis* before irrigation was not significantly different ($p > 0.05$) for all groups. This indicated that bacterial counts for all groups were comparable before treatment. Metronidazole acts preferentially on anaerobic germs; it prevents hydrogen production, exercising its toxic action by depriving anaerobic microorganisms of reducing equivalents essential for certain anabolic processes.[16] In addition, the metabolite resulting from the reduction of the nitro group of metronidazole molecule damages the DNA chain, this results in DNA damage in the form of loss of helical structure, probably acting as a nuclease, thus leading to bacterial cell death.[17]

The antimicrobial effect of chlorhexidine is due to the attraction and adsorption of the chlorhexidine cationic molecules on the surface of the microorganism's cells.[18,19] This interaction promotes the alteration of the cell membrane permeability, resulting in the loss of intracellular components and the osmotic imbalance of the cell.[20] Normal saline has no antimicrobial action and will not decrease bacterial load considerably.[21] Therefore, in this study, it was used as a control group to compare the effectiveness with that of the experimental irrigants used. The effect of 0.5% metronidazole against *E. faecalis* (91.4%) does not significantly differ from it for 2.0% chlorhexidine (96.1%) while both of them are more significantly different from normal saline (49.8%). These results may be due to superior antimicrobial action, and dentinal tubules penetration of chlorhexidine followed by metronidazole, while there was no antimicrobial action for normal saline. These results are in accordance with previous studies by Sassone et al.[17] and Zamany et al.[22] evaluated the efficacy of CHX against *E. faecalis*. They indicated that CHX showed highly significant antibacterial activity. Also, Menezes et al.[19] conducted an *in vitro* study to assess the efficacy of sodium hypochlorite and 2% chlorhexidine used as irrigation solution on teeth that had been contaminated by *E. faecalis*. Siqueira et al.[20] demonstrated that chemomechanical preparation with 0.12% CHX solution significantly reduced the number of intracanal bacteria in teeth from patients with primary intraradicular infections and chronic apical periodontitis.[23] However, according to Shen et al.,[21] bacteria in mature biofilms and nutrient-limited biofilms are more resistant to CHX killing than in young biofilms. The results emphasize the importance of standardization of factors such as biofilm age when studying the comparative effectiveness of disinfecting agents against biofilm bacteria.[21] Furthermore, according to Hoshino et al.,[24] metronidazole can penetrate the deep layers of carious lesions, disinfect the lesions *in vivo* and diffuse throughout the dentine. While Sato et al.[25] indicated that metronidazole cannot kill all bacteria and that other drugs may be necessary to sterilize infected root dentine. Thus, ciprofloxacin and minocycline, in addition to metronidazole, were required to sterilize infected root dentine. A limitation of this study which can be considered in future studies was that we only tested the irrigants against *E. faecalis* bacteria, however, other isolated species—that might contribute to the success and failure of irrigants—found in infected root canals were *A. odontolyticus*, *L. aerophilus*, *S. salivarius*, *S. sanguis*, *P. corporis*, *P. gingivalis*, and *P. odontoma*.[23]

Conclusion

Both 0.5% metronidazole and 2.0% chlorhexidine appeared to be superior against *E. faecalis* bacteria as endodontic irrigants in pulpectomy anterior primary teeth with higher antibacterial efficacy compared to saline.

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