ORIGINAL RESEARCH

Antifungal Efficacy of Sodium Hypochlorite and Four Intracanal Medicaments: An *in vitro* Evaluation

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ABSTRACT

Objective: To evaluate the effect of 2.5% sodium hypochlorite and four intracanal medications on *Candida albicans* harvested inside root canals.

Materials and methods: The contaminated canals were irrigated with sterile saline and then treated as follows: filled with (1) calcium hydroxide and saline, (2) calcium hydroxide and 2% chlorhexidine gluconate, (3) zinc oxide and 2% chlorhexidine gluconate, (4) amphotericin B powder and distilled water, (5) irrigation with 2.5% sodium hypochlorite with no medication and (6) no intracanal medication.

Canal access and apex were sealed with cavit and the roots were stored in an incubator at 37 ± 1°C for 14 days. The canals were reinstrumented and irrigated with saline. Sterile paper points were used to transfer the root canal contents to test tubes containing saline. Part of the suspension was harvested on Sabouraud dextrose agar with chloramphenicol and incubated at 37 ± 1°C for 48 hours.

Results: Group 5 was effective in 90% of the samples and least effective was group 1 (50% effective).

Conclusion: Within the limitations of this study, long-term intracanal medication was important to eliminate microorganisms especially *Candida albicans* present inside root canal.

Keywords: Amphotericin B, *Candida albicans*, Calcium hydroxide, Chlorhexidine gluconate, Sodium hypochlorite.

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INTRODUCTION

The control and elimination of microorganisms is very important during endodontic treatment because of their role in pulpal and periodontal diseases.[1]

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The chemomechanical cleaning of root canal is most important part of infection control process and can eliminate many of the microorganisms present. Although some may remain in the root canal system as they have the ability to penetrate deeper into dentinal tubules. Hence, an intracanal medicament is necessary to eliminate them.[2,3]

For selection of the proper intracanal medicament it is necessary to determine which microorganisms are present to destroy them and neutralize their byproducts. Calcium hydroxide alone or in combination with other medicament has been widely used in teeth with radiographic evidence of periapical lesions.[4] However, there are some cases where conventional therapy does not eliminate these microorganisms from the root canal system.[5]

Microbiological investigations of apical periodontitis have revealed that yeasts can be isolated from the root canal together with bacteria.[6-8] A total of 48 fungal species were isolated from root canals of teeth with apical periodontitis.[9] The most common isolated species is *Candida albicans* since it has the ability to colonize deeper into dentinal tubule.[10]

Hence, in the present study, we evaluated *ex vivo*, the effect of 2.5% sodium hypochlorite and four intracanal medications on *C. albicans* harvested inside root canals.

MATERIALS AND METHODS

Sixty human freshly extracted single rooted teeth (non-carious-closed apices) were used in this study. The crowns were sectioned and the average root size was ~16 mm. The root canals were instrumented 0.5 mm short of apex up to #25 file.

The roots were coated externally with two coats of nail polish, except the cervical access and apical foramen, and autoclaved at 123°C for 30 minutes. The remaining procedures were performed inside a laminar flux chamber using sterile materials and instruments (Fig. 1A).

Root apices were sealed with Cavit cement and root canals were contaminated with 0.1 ml of a suspension containing 10^8 *C. albicans* cells using sterile micropipette.

The access openings were sealed with a sterile cotton pellet and cavit cement and the roots were placed in a

sterile receptacle and maintained in an incubator at 37 ± 1°C for 48 hours following which the sealings were removed and the root canals were instrumented again up to #40 file using 5 ml sterile saline for irrigation.

The roots were divided into groups of 10 each according to medicaments used.

Groups are as follows:

- Group 1: Ca $(OH)_2$ powder and Saline
- Group 2: Ca $(OH)_2$ powder and 2% CHX
- Group 3: ZnO powder and 2% CHX
- Group 4: Amphotericin B powder and distilled water (Fig. 1B).
- Group 5: Irrigation with 2.5% NaOCl during the procedure with no intracanal medication.
- Group 6: Roots are sealed with cavit with no intracanal medication: (control group)

The roots are then incubated at 37 ± 1°C for 14 days in an incubator (Fig. 1C). After 14 days, canals are again instrumented with #25 file and irrigated with saline to remove medicaments.

For microbiological evaluation sterile paper points (#25) are placed in the root canal for 1 minute, and then placed in a test tube containing 1 ml sterile saline (Fig. 1D), shaken for 30 second and 0.1 ml aliquots were harvested on Sabouraud dextrose agar plates with chloramphenicol (1 mg/ml) and incubated at 37°C for 48 hours (Fig. 2). Pure culture, isolated from the canals, were identified and confirmed by Gram staining and germ tube testing.

The Fisher test was used for statistical analysis and p-value <0.05 was considered to indicate significant statistical difference and software used for analysis and statistical package for social science (SPSS).

RESULTS

The results are reported in Graph 1.

2.5% NaOCl was effective in 90% of the samples followed in decreasing order of effectiveness by amphotericin B powder and distilled water (80% effectiveness), ZnO powder and 2% CHX (70% effectiveness), $Ca(OH)_2$ powder and 2% CHX (60% effectiveness), $Ca(OH)_2$ powder and saline (50% effectiveness) and saline + no intracanal medication.

Figs 1A to D: (A) Laminar flux chamber, (B) amphotericin B ampule, (C) roots are incubated at 37 ± 10ºC for 14 days in an incubator and (D) sterile paper points placed in a test tube containing 1 ml sterile saline

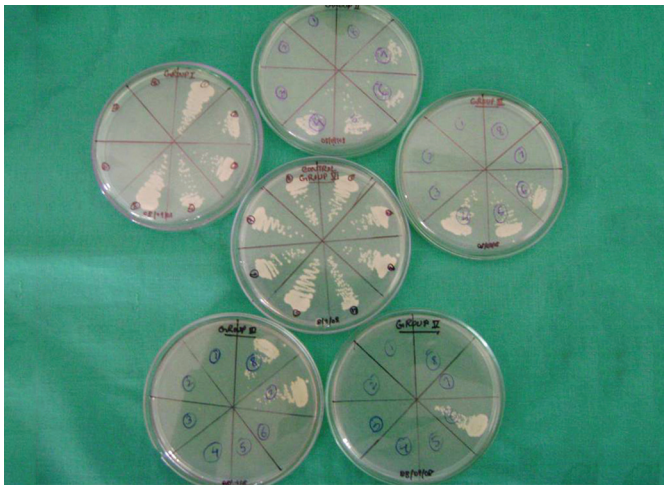
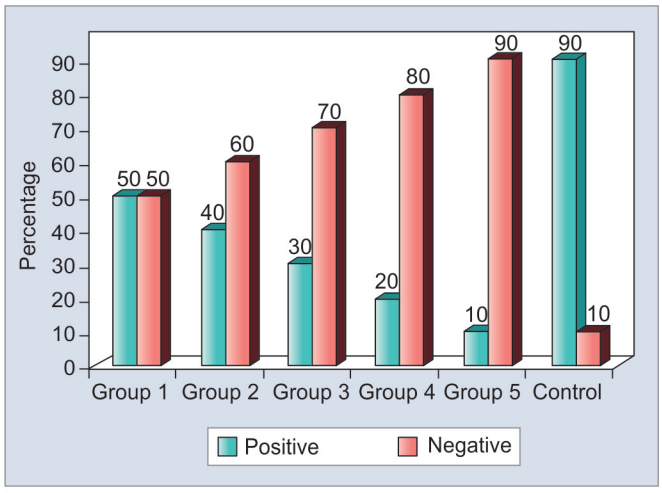


Fig. 2: Fungi growth on Sabouraud dextrose agar plates with chloramphenicol (1 mg ml) after 48 hours



	Group 1	Group 2	Group 3	Group 4	Group 5	Control
Positive	5 (50%)	4 (40%)	3 (30%)	2 (20%)	1 (10%)	9 (90%)
Negative	5 (50%)	6 (60%)	7 (70%)	8 (80%)	9 (90%)	1 (10%)

Graph 1: Positive and negative growth of *C. albicans* in six groups

DISCUSSION

The objective of endodontic therapy is to achieve a root canal free of microorganisms. This can be achieved by shaping and cleaning of root canal with the use of antimicrobial irrigants and intracanal dressings.[12]

Sodium hypochlorite[13] has been widely used for irrigation. However, some microorganisms may be resistant to conventional endodontic treatment.

2.5% NaOCl used as an irrigant during instrumentation-showed best antifungal efficacy. 2.5% NaOCl was used since it is known to posses similar antifungal effects but low toxicity compared to 5.25%.[14] Ninety percent of samples presented negative *C. albicans* culture after use of 2.5% NaOCl solution. The growth of yeast in 10% of the samples may be due to the difficulty of penetration of this irrigant into root canal irregularities.[15]

The root canals irrigated with 2.5% NaOCl were not treated with an ICM and also since EDTA was not used during instrumentation deeper smear layer may not have been removed, which otherwise might have enhanced action of chemomechanical preparation thus eliminating all microorganisms.

The control group, irrigated only with sterile saline showed *C. albicans* growth in 90% of the samples. The elimination of *C. albicans* in one control sample may be due to the physical effect of irrigation.[16]

Second best result was shown by amphotericin B – (commonly used antifungal agent).[17] It is an antifungal polyene antibiotic derived from the species *Streptomyces nodosus*. Amphotericin B is fungistatic as well as fungicidal depending on concentration used. Drug acts by binding to the steroids in the cell membrane of fungi, with resultant changes in membrane permeability allowing leakage of intracellular components.

Combination of ZnO and 2% CHX was better compared to $Ca(OH)_2$ and 2% CHX.[18] $Ca(OH)_2$ mixed with saline was least effective in eliminating *C. albicans*.[19] *Candida albicans* has been demonstrated to be highly resistant to $Ca(OH)_2$ if used along with inert material, such as glycerine, saline.[17]

Recent studies have suggested that CHX combined with $Ca(OH)_2$ is effective against $Ca(OH)_2$ resistant microbes.[20]

Chlorhexidine is more effective than $Ca(OH)_2$ in eliminating *C. albicans*.[21] Since, CHX is known to have substantivity showed slightly better result when mixed with $Ca(OH)_2$ compared to saline.

CONCLUSION

This study reinforces the importance of endodontic treatment in two sessions with the use of long-term intracanal medication to eliminate microorganisms present inside root canal. *Candida albicans* is an opportunistic microorganism and a poorly functioning immune system might increase the risk of fungal infection in the root canal space. This study also highlights the presence of *C. albicans*, which may persist even after endodontic treatment.

Further evaluation has to be done to find an irrigant along with a medicament to eliminate all microorganisms under clinical situation.

REFERENCES

1. Sundqvist G, Johansson E, Sjogren U. Prevalence of black-pigmented bacteroides species in root canal infections. J Endod 1989;15(1):9-13.
2. Stevens RH, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament. J Endod 1983;9(9):372-374.
3. Gomes BPFA, Lilley JD, Drucker DB. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. Int Endod J 1996;29(4):235-241.

4. Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985;1(5):170-175.
5. Sundqvist G, Reuterving CD. Isolation of Actinomyces Israelii from periapical lesion. J Endod 1980;6(6):602-606.
6. Nair PNR, Sjogren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy resistant periapical lesion: a long term light and electron microscopic follow-up study. J Endod 1990;16(12):580-588.
7. Najzar-Fleger D, Filipovic D, Prpic G, Kobler D, Candida in root canal in accordance with oral ecology (abstract). Int Endod J 1992;25:40.
8. Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. Endod Dent Traumatol 1995;11(1):6-9.
9. Waltimo TMT, Siren EK, Torkko HLK, Olsen I, Haapasalo MPP. Fungi in therapy-resistant apical periodontics. Int Endod J 1997;30(2):96-101.
10. Siqueira JF, Rocas IN, Lopes HP, Elias CN, Uzeda MD. Fungal infection of the radicular dentin. J Endod 2002;28(11):770-773.
11. Waltimo TMT, Haapasalo M, Zehnder M, Meyer J. Clinical aspects related to endodontic yeast infections. Endod Topics 2004;9(1):66-78.
12. Gomes BPFA, Lilley JD, Drucker DB. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. Int Endod J 1996;29(4):235-241.
13. Siqueira JF, Machado AG, Silveira RM, Lopes HP, Uzeda M. Evaluation of the effectiveness of sodium hypochlorire used with three irrigation methods in the elimination of Enterococcus faecalis from root canal, in vitro. Int Endod J 1997;30(4):279-282.
14. Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, Drucker DB. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. Int Endod J 2004;37(7):438-446.
15. Siqueira JF, Uzeda M, Fonseca MAF. A scanning electron microscopic evaluation of in vitro dentinal tubules pene-tration by selected anaerobic bacteria. J Endod 1996;22(6):308-310.
16. Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5% sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol 1983;55(3):307-312.
17. Ferguson JW, Hatton JF, Gillespie MJ. Effectiveness of intracanal irrigants and medications against the Yeast Candida albicans. J Endod 2002;28(2):68-71.
18. Siqueira JF, Rocas IN, Lopes HP, Magalhaes FAC, Uzeda MD. Elimination of Candida albicans infection of the radicular dentin by intracanal medications. J Endod 2003;29(8):501-504.
19. Siqueira JF, Uzeda M. Intracanal medicaments evaluation of the antibacterial effects of chlorhexidine, metronidazole, and calcium hydroxide associated with three vehicles. J Endod 1997;23(3):167-169.
20. Basrani B, Santos JM, Tjaderhane L, Grad H, Gorduysus O, Huang J, Lawrence HP, Friedman S. Substantive antimicrobial activity in chlorhexidine-treated human root dentin. Oral Surg Oral Med Oral Pathol 2002;94(2):240-245.
21. Waltimo TMT, Orstavik D, Siren EK, Haapasalo MPP. In vitro susceptibility of Candida albicans to four disinfectants and their combinations. Int Endod J 1999;32(6):421-429.