Curbing the growth of candida-A comparative in-vitro study

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Abstract:
Endodontics is the least forgiving branch of dentistry in terms of success or failure. Therefore, re-treatment has become a significant part of today’s endodontic practice. There is a growing evidence showing the presence of fungi especially Candida albicans in root canals of obturated teeth in which endodontic treatment has failed. It has been demonstrated that Candida species are resistant to some medications used in endodontics. The aim of this study was to examine the in-vitro susceptibility of Candida albicans to materials used in endodontics such as mineral trioxide aggregate (MTA), Calcium hydroxide Ca(OH)\textsubscript{2}/camphorated paramonochlorophenol (CMCP)/glycerine and chlorhexidine (CHX).

MTA and CHX are effective anti-fungal agents each with 67% anti fungal activity while Ca(OH)\textsubscript{2}/CMCP showed 33% anti-fungal activity.

Key Words: Candida albicans; MTA; Calcium hydroxide / CMCP / glycerin; chlorhexidine; tubedilution test.

Introduction:
The aim of endodontic treatment is to eliminate infections from the root canal and prevention of reinfection (Nair et al, 1990). Micro-organisms isolated from primary endodontic infections are predominately strict anaerobes. On the other hand, facultative anaerobic bacteria and yeasts prevail in therapy-resistant cases and chronic apical periodontitis as they are more resistant to antimicrobial agents usually used in endodontics (Ferrari et al, 2005; Siqueira (Jr), 2001).

The incidence of yeasts, specially Candida albicans (C. albicans) ranges from 7 to 55% in therapy resistant cases and can be greater in individuals with local or systemic factors predisposing to mycoses (Siqueira (Jr) & Sen, 2004). In all such situations, it becomes mandatory to use anti-fungal agents in one form or the other (Siqueira et al, 2002; Waltimo et al, 1997; Sequeira & Rocasl, 2004; Fergusian et al, 2002).

Therefore, the purpose of this study is to examine the in-vitro susceptibility of C. albicans to agents used in endodontics such as MTA, CHX and Ca(OH)\textsubscript{2} / CMCP using the tube dilution method.

Material & Method:
Pure culture of Candida albicans (MTCC 227, batch – Nov 2003, Chandigarh) was added to 2 test tubes each containing 10 ml Sabouraud’s broth and incubated at 37°C for 7 days. The cell viability of C. albicans was checked by inoculation in Sabouraud’s dextrose broth and plating onto Sabouraud’s dextrose agar plates. The cells were also observed under microscope to check their viability.

One ml of Sabouraud’s dextrose broth was taken in 24 test tubes and to each, 1 ml suspension of viable C. albicans suspension was added. The tubes were divided into four equal groups. Three hundred mg of MTA, Ca(OH)\textsubscript{2} /CMCP/glycerin and CHX were added to all the six tubes of group 1, 2 and 3 respectively. In group 4, no medicament was added and it served as a negative control.

All the test tubes were incubated at 37°C for 5 days after which 0.1 ml sample from each was transferred to test tubes containing 1 ml Sabouraud’s dextrose broth, vortexed and incubated at 37°C for 7 days. After 7 days, samples from each test tube were streaked on Sabouraud’s dextrose agar plates. Growth of C. albicans was observed.

Statistical analysis was done by using the Kruskal Wallis method.

Observations:
The growth of Candida albicans was significantly inhibited in group 1 with MTA as compared to the control. Four samples showed complete inhibition where as 2 samples showed 1-2 colonies of C. albicans.

Group 2 containing Ca(OH)\textsubscript{2} - CMCP- glycerin showed less inhibitory effect on the growth of C. albicans; 2 samples showed complete inhibition while 4 samples showed 3-4 colonies of C. albicans.

Group 3 with CHX showed significant inhibition of C. albicans with 4 samples showing no growth and 2 samples showing 1-2 colonies. All the tubes of group 4 were positive for C. albicans growth.
Statistical analysis showed significant anti-fungal action by MTA and CHX (p < 0.05). Where as no significant difference was observed between group 2 and group 4 (p > 0.05).

Discussion:
Yeasts have been demonstrated in the Plaque, Caries, Dentinal tubules, Sub-gingival flora, Root canals and Peri radicular tissues (Siqueira (Jr), 2003).

C. albicans was chosen as a test organism in this study because it has been found in infected root canals, peri-radicular tissue, persistent infections and in apical periodontitis. It is a dentinophillic organism and lodges in the dentinal tubules using the calcium in the dentin for its nourishment (Waltimo et al, 1997).

The method used in this study is the tube dilution susceptibility test which is an effective method to evaluate the antifungal and antibacterial properties of any filling material/solution. It allows direct contact between fungal cells and the test agents (Al-Nazhan & Al-Judai, 1997).

Mechanisms of pathogenicity of C. albicans is due to:
(i) its power of adaptability to a variety of environmental conditions attributable to the switching of gene expression dictated by environmental changes, (ii) it adheres to a variety of surfaces by surface molecules that mediate adherence to host tissues, (iii) it produces hydrolytic enzymes which result in the degradation of extra-cellular matrix proteins, (iv) C. albicans has the ability to form biofilms on different surfaces, (v) evasion and immunomodulation of the host defenses by different mechanisms and (vi) dentinophillic nature of candida (Sen et al, 1997). Mineral trioxide aggregate had been developed in 1994 to seal pathways of communication between the root canal system and the external surface of the tooth. It elicits minimum inflammatory response, exhibits low toxicity, good sealing ability, bacteriostatic and fungicidal effects. Its anti-fungal property is said to be due to its high pH (Al-Nazhan & Al-Judai, 2003; Schwartz et al, 1999). Chlorhexidine is a dicationic molecule effective against bacteria and fungi. Its mode of action is membrane disruption and it is known for its substantivity (Newman et al, 2003).

Calcium hydroxide / CMCP has the property to hydrolyze the lipid moiety of bacterial lipopolysaccharides and making them incapable of producing toxicity and pathogenicity (Foreman & Barnes, 1990; Zamany et al, 2003). Chlorhexidine is a dicationic molecule effective against bacteria and fungi. Its mode of action is membrane disruption and it is known for its substantivity (Newman et al, 2003).

MTA and CHX are effective anti-fungal agents each with 67% anti fungal activity. Ca(OH)\(_2\)/CMCP showed less effective anti-fungal activity at 33%. As
MTA is cost prohibitive, CHX could be the material of choice when the root canal culture shows the presence of C.albicans. However, further \textit{in-vivo} studies are required before we can definitely say what the action of these agents would be in the root-canal system.

\textbf{Bibliography:}


