Comparative evaluation of effectiveness of 3% Sodium hypochlorite, 17% Ethylene diamine tetra-acetic acid (EDTA) and Fluconazole on Candida Albicans – An in vitro study

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ABSTRACT:
Objective: Candida albicans, is one of the most resistant pathogens found in root canals with necrotic pulp and in recurrent root canal failure cases. Removal of this resistant microorganisms from the root canal poses a special challenge by routine instruments and conventional irrigants. The objective of the study is to compare the anti-fungal efficacy of Fluconazole with two most commonly used root canal irrigating solution (3% NaOCl) Sodium hypochlorite and 17% EDTA through specific methodology that can project Fluconazole as an antifungal irrigant of choice.

Method: Twenty-five extracted human maxillary central incisors were used in this study with biomechanical preparation done to Protaper F3. Teeth were randomly divided into three test groups (n=5) and a positive control group. After cleaning and shaping, root canals were contaminated with C. albicans and incubated for 72 hours. Then the root canals were irrigated with the antifungal solutions and enlarged to Protaper F4. Generated aliquots were cultured on Sabouraud’s dextrose agar plate and numbers of colony-forming units (CFU) were counted.

Result: One way ANOVA showed that there was significant difference in mean number of Candida albicans colonies in S.D. agar plates for different intracanal irrigants (F3,16 = 347529.69; p<0.0001) As per Critical Difference (CD) the mean number of colonies for normal saline were significantly higher those that of others followed by 17% EDTA and 3% Sodium hypochlorite (p<0.001). No growth was found for Fluconazole.

Conclusion: Fluconazole showed greatest antifungal efficacy out of the three agents tested. 3% Sodium hypochlorite exhibited lower level efficacy followed by 17% EDTA.

KEYWORDS: Irrigation, EDTA, Sodium Hypochlorite, Fluconazole, Candida Albicans.

I. INTRODUCTION
Candida albicans is one of the most resistant pathogens found in root canals of treatment failure cases. In last decade, incidence of Candida albicans in endodontic infection has received attention and fungi were observed in primary and refractory endodontic infections. Candida is versatile and can adapt to a range of pH, change gene expression in response to environmental conditions, adhere to a variety of surfaces, produce degradative enzymes, and change morphologic forms to evade the immune system. Clinically important candida species grow well in vitro over a pH range of 3.0-8.0.
(1) So, this is a challenge for endodontists to eliminate candida albicans from root canal system to avoid recurrent root canal failure. Residual pulpal tissue, bacteria, fungi and dentine debris may persist in the irregularities of root canal systems, even after meticulous mechanical preparation. Therefore, irrigant solutions should be used in combination with canal preparation. (2)

Numerous root canal irrigants have been recommended out of which Sodium hypochlorite is the irrigant of choice universally. But undesired extrusion of sodium hypochlorite beyond the root apex can cause mild to severe degree of tissue reactions.

Ethylendiaminetetraacetic acid (EDTA), a chelating agent that dissolves inorganic dentin components but not the organic components, and which is used mainly to remove the smear layer, may also act as an antifungal irrigant; however, dentin erosion has been reported with prolonged exposure.

Fluconazole is a potent anti-fungal agent. It is a Triazole anti-fungal drug used in different topical, invasive fungal diseases including Candidiasis.

II. MATERIAL & METHOD

A total of 20 extracted maxillary central incisors of patient aged 20-40 years having no visible cracks, root caries or anomalies were collected and stored in normal saline. After removal of external debris by ultrasonic scaler, access cavity was prepared using a high-speed round carbide bur (Dentsply, Maillefer) with water spray. Bio-mechanical preparation done upto Protaper file F3 using Endo-motor device and irrigated with 0.9% Normal saline. The external surface of each tooth root was covered with nail polish and root tip was closed by Glass Ionomer cement. Each tooth with apendroff tubes was autoclaved properly. A suspension of standard strain of Candida albicans (MTCC227) in Sabouraud’s Dextrose agar broth was obtained and adjusted to a specific turbidity (0.5 McFarland’s turbidity). 1ml of turbid suspension of Candida albicans was inoculated in each tooth root canal by micropipette and into the apendroff tubes to submerge the teeth. Now each tooth with tube was incubated separately in incubator for 72 hrs. Now sample teeth was equally divided into four groups and irrigated with corresponding irrigants using 5ml glass syringe and 30G 25mm side vented needle. All teeth were irrigated with a speed of 2ml/min

Group A - Teeth was irrigated by 2ml of 3% Sodium hypochlorite twice in seven minutes interval followed by 2ml of 0.9% normal saline at least 30 mins after the last 3% Sodium hypochlorite irrigation.

Group B - Teeth was irrigated by 2ml of 17% EDTA twice in seven minutes interval followed by 2ml of 0.9% normal saline at least 30 mins after the last 2% Chlorhexidine Gluconate irrigation.

Group C - Teeth was irrigated by 2ml of Fluconazole twice in seven minutes interval followed by 2ml of 0.9% normal saline at least 30 mins after the last Fluconazole irrigation.

Group D - Teeth was irrigated by 0.9 % Normal saline only.

Now 1ml of fresh Sabouraud’s Dextrose Agar broth was inoculated in each root canal including apendroff tubes to submerge the teeth, then incubated for 72 hrs. Aliquots from each root canal generated by Protaper F4 filing was collected and cultured on S.D. Agar plate for 72 hrs. Presence of colonies from each group of teeth was examined along with CFU count.

III. RESULT AND STATISTICAL ANALYSIS

![CFU count when 3% NaOCl used as irrigant](image)
Statistical Analysis was performed with help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC).

Descriptive statistical analysis was performed to calculate the means with corresponding standard deviation (s.d.). Also, One Way Analysis of variance (ANOVA) followed by post hoc Tukey’s Test was performed with the help of Critical Difference (CD) or Least Significant Difference (LSD) at 5% and 1% level of significance to compare the mean values. p<0.05 was taken to be statistically significant.

Table 1: Mean (mean± s.d.) number of Candida albicans colonies in S.D Agar plates for different intracanal irrigants

<table>
<thead>
<tr>
<th>Descriptive Statistics (in ml.)</th>
<th>Sodium hypochlorite</th>
<th>Fluconazole</th>
<th>17% Ethylene diamine tetraacetic acid</th>
<th>Normal saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean± s.d.</td>
<td>63.60±8.79</td>
<td>0.00±0.00</td>
<td>98.80±8.16</td>
<td>3774.20±7.42</td>
</tr>
<tr>
<td>Median</td>
<td>68</td>
<td>0</td>
<td>100</td>
<td>3773</td>
</tr>
<tr>
<td>Range</td>
<td>51 – 72</td>
<td>0 - 0</td>
<td>87 - 108</td>
<td>3764 – 3784</td>
</tr>
</tbody>
</table>

One way ANOVA showed that there was significant difference in mean number of Candida Albicans colonies in S.D Agar plates for different intracanal irrigants (F3,16 = 347529.69;p<0.0001)
As per Critical Difference (CD) the mean number of colony for normal saline was significantly higher than that of others followed by EDTA and sodium hypochlorite (p<0.001). No growth was found for Fluconazole.

IV. DISCUSSION

Endodontic treatment success depends on the depletion or elimination of microorganisms including fungi from the complex three-dimensional root canal system. Yeasts can be detected in 7-17% of infected root canals. (1) Amongst the yeast, C. albicans is the most common and the most resistant to endodontic procedures and showed an ability to colonize canal walls and invade dentinal tubules (3). Baumgartner found Candida albicans by PCR method in 21% of samples taken from infected root canals. Molander et al found Candida albicans in three of 68 teeth failed endodontic treatment with chronic apical periodontitis. (4) Candida albicans is the most infective and invasive yeast among the candida species. Candida albicans has a series of features that allow them to survive in the treated root canals. These include resistance to drugs and adaptation in ecologically harsh conditions in the canal (Phenotypic alteration), ability to form biofilm and Colonization of dental hard tissues (Adherence), invasion to dentinal tubules (Hyphal formation and Thigmotropism) and long survival without substrate (Protease secretion). Sequera et al in a study showed dentinophilic nature of Candida albicans.

Sodium hypochlorite is most commonly used root canal irrigant with high tissue dissolving capacity and antimicrobial efficacy. According to Waltimo et al, sodium hypochlorite showed the highest efficacy against C. Albicans in therapeutic concentrations. Concentrations below the minimum inhibitory concentration of sodium hypochlorite may be effective on potentially pathogenic traits of Candida species (Webb et al., 1995). However, in the environment of necrotic root canal, the significance of this finding is questionable. (4)

The primary mode of action of Fluconazole is the inhibition of fungal cytochrome P-450-mediated 14 alpha-lanosterol demethylation, an essential step in fungal ergosterol biosynthesis. Ergosterol serves as a bioregulator of membrane fluidity and asymmetry and consequently of membrane integrity in fungal cells. Integrity of the cell membrane requires that inserted sterols lack C-4 methyl groups. Several lines of evidence suggest that the primary target of azoles is the heme protein, which cocalytizes cytochrome P-450-dependent 14α-demethylation of lanosterol. Inhibition of 14a-demethylase leads to depletion of ergosterol and accumulation of sterol precursors, including 14a-methylated sterols (lanosterol, 4,14-dimethylzymosterol, and 24-methylenedihydrolanosterol), resulting in the formation of a plasma membrane with altered structure and function. The accumulation of these sterol precursors correlates with the subsequent loss of ergosterol in the fungal cell wall and may be responsible for the antifungal activity of Fluconazole.

EDTA, a chelating agent has shown to have the most effective antifungal activity. It has anticolonisation, anti-growth and anti-collagenolytic properties against C.albicans. By chelating calcium ions in the medium, EDTA prevents binding of C.albicans to the proteins in a dose-dependent manner. In the second process, EDTA reduces the growth of C.albicans by removing calcium from the cell walls and causing collapse in the cell wall and by inhibiting enzyme reaction. Using the agar diffusion method, Sen et al demonstrated that 17% EDTA had the highest antifungal activity in comparison with routine antifungal drugs and other test solutions.

According to the findings of Ruff et al. effectiveness of 2% CHX and 6% NaOCl against C. albicans was equal and was superior to 17% EDTA and MTAD. (5) In this study, Fluconazole showed best antifungal efficacy as no CFU seen in culture, followed by 3% NaOCl and 17% EDTA. One minute of contact time was chosen in this study because less than 1 minute of contact time was enough to eliminate Candida albicans when sodium hypochlorite was used. (Himani Lau et al.). In this study, it was proved that Fluconazole has greatest antifungal efficacy among three irrigants. NaOCl shows lesser efficacy may be due to the difficulty of penetration of NaOCl into root canal irregularities. Similar results were obtained by Siqueira et al. Ethylene diamine tetraacetic acid is recommended for removing the smear layer in root canal treatment. However, disinfection of the dentin surface and dentin tubules may still be questionable.

V. CONCLUSION

Fluconazole shows greatest antifungal efficacy. 3% Sodium hypochlorite and 17% EDTA exhibited antifungal efficacy with lower level. Further studies with larger sample size and in vivo studies might through more like in this subject.

REFERENCES


