MAVEN

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF EFFECTIVENESS OF 3% SODIUM HYPOCHLORITE, 17% ETHYLENE-DIAMINE- TETRA-ACETIC ACID (EDTA) AND VORICONA- ZOLE ON CANDIDA ALBICANS IN ROOT CANAL TREATMENT – AN IN VITRO STUDY

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Abstract

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 microorganism from the root canal poses a special challenge by routine instrumentation and conventional irrigation methods.

Objective: Compare the anti-fungal efficacy of Voriconazole, 3% Sodium hypochlorite and 17% EDTA through specific methodology.

Method: Twenty extracted human maxillary central incisors were selected and biomechan- ical preparation was done up to Protaper F3. Teeth were randomly divided into three test groups (n=5) and a positive control group. After that all root canals were contaminated with Candida albicans and incubated for 72 hours. Then the root canals were irrigated with the antifungal solutions and enlarged by Protaper F4. Generated aliquots were cultured on Sabouraud's dextrose agar plate and numbers of colony-forming units (CFU) were counted and statistically analyzed.

Result & Conclusion: One way ANOVA showed that there was significant difference in mean number of Candida albicans colonies in S.D. agar plates for different intracanal irri- gants ($F_{3,16} = 347529.69$; $p<0.0001$) and Voriconazole showed greatest and EDTA exhib- ited lowest level of antifungal efficacy out of the three agents tested. (2018, Vol. 02; Issue 01: Page 1 - 6)

Keywords: Candida albicans, Voriconazole, Sodium hypochlorite, EDTA.

Introduction

Candida albicans is one of the most re- sistant pathogens found in root canals of treatment failure cases. In last decade, in- cidence 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 environ- mental 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 chal- lenge for endodontists to eliminate can- dida albicans from root canal system to avoid recurrent root canal treatment fail- ure. Residual pulpal tissue, bacteria, fungi and dentine debris may persist in the ir- regularities of root canal systems, even af- ter meticulous mechanical preparation. Therefore, irrigant solutions should be used in combination with canal prepara- tion (2).

Numerous root canal irrigants have been recommended out of which Sodium hypo- chlorite is the irrigant of choice univer- sally. But undesired extrusion of sodium hypochlorite beyond the root apex can cause mild to severe degree of tissue reac- tions.

Ethylene-diamine-tetra-acetic 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 pro- longed exposure.

Voriconazole is a potent anti-fungal agent. It is a Triazole anti-fungal drug used in different topical, invasive fungal diseases including Candidiasis. Thus the aims and objectives of this study were to compare the anti-fungal efficacy of Voriconazole, 3% Sodium hypochlorite and 17% EDTA through specific methodology (1, 3).

Material and method

A total of 20 extracted maxillary central in- cisors 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 ultra- sonic scaler, access cavity was prepared using a high-speed round carbide bur (Dentsply, Maillefer) with water spray. Bio- mechanical preparation done upto Prota- per file F3 using Endo-motor device and irrigated with 0.9% normal saline. The ex- ternal surface of each tooth root was cov- ered with nail polish and root tip was closed by Glass Ionomer cement. Each tooth with eppendorf tubes was auto- claved properly. A suspension of standard strain of Candida albicans (MTCC227) in Sabouraud's Dextrose agar broth was ob- tained and adjusted to a specific turbidity (0.5 McFarland's turbidity). 1ml of turbid suspension of Candida albicans was inoc- ulated in each tooth root canal by micropi- pette and into the eppendorf tubes to sub- merge the teeth. Now each tooth with tube was incubated separately in incubator for 72 hrs. Now sample teeth was equally di- vided into four groups and irrigated with corresponding irrigants using 5ml glass syringe and 30G 25mm side vented nee- dle. 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 minutes 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 minutes after the last 2% Chlor- hexidine Gluconate irrigation.

Group C - Teeth was irrigated by 2ml of Voriconazole twice in seven minutes inter- val followed by 2ml of 0.9% normal saline at least 30 minutes after the last Garlic Extract 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 ca- nal including eppendorf tubes to sub- merge the teeth, then incubated for 72 hrs. Aliquots from each root canal gener- ated 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 (Fig 1 to 4).

Result and statistical analysis

Statistical Analysis was performed with help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Con- trol and Prevention (CDC).

Descriptive statistical analysis was per- formed to calculate the means with corre- sponding standard deviation (s.d.). Also One Way Analysis of variance (ANOVA) fol- lowed by post hoc Tukey's Test was per- formed with the help of Critical Difference (CD) or Least Significant Difference (LSD) at 5% and 1% level of significance to com- pare the mean values. $p<0.05$ was taken to be statistically significant (Table 1).

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

Descriptive Statistics (in ml.)	**Sodium hypochlorite**	**Voricona- zole**	**17% Ethylene diamine tetraacetic acid**	**Normal saline**
Mean± s.d.	63.60±8.79	0.00±0.00	98.80±8.16	3774.20±7.42
Median	68	0	100	3773
Range	51 – 72	0 - 0	87 - 108	3764 – 3784

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 ($F_{3,16} = 347529.69$; $p<0.0001$)

As per Critical Difference (CD) the mean number of colony for normal saline was significantly higher than that of others fol- lowed by EDTA and sodium hypochlorite ($p<0.001$). No growth was found for Voriconazole.

Discussion

Endodontic treatment success depends on the depletion or elimination of microor- ganisms including fungi from the complex three-dimensional root canal sys- tem.Yeasts can be detected in 7-17% of in- fected root canals (1). Amongst the yeast, Candida albicans is the most common and the most resistant to endodontic proce- dures 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 (4). Molander et al found Candida albicans in three of 68 teeth failed endodontic treatment with chronic apical periodontitis (5). According to Aysin Dumaniet et al (2012), Candida albicans genome was identified in 20% and 11% of the necrotic and retreated root canal infections, respectively, by PCR (6). 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 alter- ation), ability to form biofilm and coloniza- tion of dental hard tissues (Adherence), in- vasion to dentinal tubules (Hyphal for- mation and Thigmotropism) and long sur- vival without substrate (Protease secre- tion). Sequera et al in a study showed den- tinophilic nature of Candida albicans (7). Sodium hypochlorite is most commonly used root canal irrigant with high tissue dissolving capacity and antimicrobial effi- cacy. According to Waltimo et al., sodium hypochlorite showed the highest efficacy against Candida Albicans in therapeutic concentrations. Concentrations below the minimum inhibitory concentration of so- dium hypochlorite may be effective on po- tentially pathogenic traits of Candida spe- cies (Webb et al., 1995) (8). However, in the environment of necrotic root canal, the significance of this finding is questionable (9).

The primary mode of action of Voricona- zole is the inhibition of fungal cytochrome P-450-mediated 14 alpha-lanosterol de- methylation, an essential step in fungal ergosterol biosynthesis. Ergosterol serves as a bioregulator of membrane fluidity and asymmetry and consequently of mem- brane 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 co-catalyzes cytochrome P-450-dependent 14a-demethylationof lanosterol. Inhibition of 14a-demethylase leads to depletion of ergosterol and accumulation of sterol pre- cursors, 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 er- gosterol in the fungal cell wall and may be responsible for the antifungal activity of Voriconazole.

EDTA, a chelating agent has shown to have the most effective antifungal activity (10). It has anti-colonisation, anti-growth properties against Candida albicans. By chelating calcium ions in the medium, EDTA prevents binding of Candida albi- cans to the proteins in a dose-dependent manner. In the second process, EDTA re- duces the growth of Candida albicans by removing calcium from the cell walls and causing collapse in the cell wall and by in- hibiting enzyme reaction (11). Using the agar diffusion method, Sen et al demon- strated that 17% EDTA had the highest antifungal activity in comparison with routine antifungal drugs and other test so- lutions (12).

According to the findings of Ruff et al. ef- fectiveness of 2% CHX (Chlorhexidine) and 6% NaOCl (Sodium hypochlorite) against Candida albicans was equal and was su- perior to 17% EDTA and MTAD (a mixture of tetracycline isomer, an acid and a deter- gent) (13).

In this study, Voriconazole showed best antifungal efficacy as no CFU seen in cul- ture, followed by 3% NaOCl and 17% EDTA.

According to Sen BH et al, in the absence of the smear layer, 5% NaOCl alone started to show antifungal activity after 30 minutes (14).

According to Harrison JW et al, NaOCl ex- hibits complete antifungal activity in a range from 15 seconds to 5minutes (15). In this study, teeth were irrigated by 2ml of specific irrigant twice in seven minutes interval followed by 2ml of 0.9% normal saline at least 30 minutes after the last ir- rigation by specific irrigant, to achieve maximum contact time of 30 minutes as mentioned by Sen et al (1999) (14).

In this study, it was proved that Voricon- azole has greatest antifungal efficacy among three irrigants. NaOCl shows lesser efficacy may be due to the difficulty of pen- etration of NaOCl into root canal irregular- ities. Similar results were obtained by Si- queira et al. Ethylene-diamine-tetra-acetic acid is recommended for removing the smear layer in root canal treatment. How- ever, disinfection of the dentin surface and dentinal tubules may still be questionable.

Fig 1: CFU count when 3% NaOCl used as irrigant

Fig 2: CFU count when 0.9% NaCl used as irrigant

Fig 3: CFU count when 17% EDTA used as irrigant

Fig 4: CFU count when Voriconazole used as irrigant

Conclusion

Within the experimental conditions and results of the present study, it could be concluded that Voriconazole having great- est antifungal efficacy than 3% Sodium hypochlorite and 17% EDTA. But further studies with larger sample size and in vivo studies are required for substantiate the facts.

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