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Original Research

Presence of *Candida albicans* in Root Canals of Teeth with Apical Periodontitis and Evaluation of their Possible Role in Failure of Endodontic Treatment

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Abstract:

Background: Candidal organisms are commensals of the oral cavity and their opportunistic infection depends on the interplay of local and systemic factors. Endodontic treatment failure has been associated with the persistence of microbial flora following therapy in the root canal system, and this includes fungi like *Candida,* which are resistant to conventional root canal irrigants.

Materials and Methods: A total of 20 mandibular molars were included in the study of which 15 were primary randomized controlled trial cases, whereas 5 were retreatment cases. The patient's saliva, as well as the root canal flora, were examined for the presence of *Candida* organisms and further confirmed for the presence of *Candida albicans*.

Results: Of the 20 samples comprising the study group, the saliva culture revealed 11 of them to have a colony forming unit (CFU) >400 and 9 of the 20 canals microbiology showed CFU >400. Among the 15 first-time root canal treatment cases, saliva examination these revealed 8 of them to have CFU >400 and the canal microbiology of these showed 6 of them to have CFU >400. Among the retreatment cases, 3 of the 5 cases showed CFU >400 in both the saliva DN canal culture.

Conclusion: Candidal contamination of the root canals could be the cause of endodontic treatment failure and thus emphasizes the importance of rubber dam isolation and the use of irrigants with antifungal properties for sufficient duration during treatment.

Key Words: *Candida albicans*, randomized controlled trial, root canal irrigants, rubber dam isolation

Introduction

Candidial organisms are ubiquitous in the environment, and symptom-free oral carriage of *Candida* has been recognized for many years. Their opportunistic pathogenicity is dependent on local and systemic predisposing factors affecting the host. Endodontic failure is a major cause for concern and the major factors associated with endodontic failure are the persistence of microbial infection in the root canal system.

An important consideration in endodontic treatment is the elimination of microorganisms, including fungi, from the complex three-dimensional root canal system. Certain bacteria, such as *Enterococcus faecalis*, and certain fungi, such as *Candida albicans*, have been shown to be resistant to antibiotics present in the irrigating solutions causing endodontic failure.

Aim

To examine the presence of *C. albicans* in teeth which are indicated for root canal treatment or endodontic retreatment following recurrent infection and to identify the role, if any, of candidial contamination of root canal in treatment failure.

Materials and Methods

Subjects for the study were selected from the patients visiting private dental clinics in Bengaluru. Twenty teeth were included in the study that included mandibular molars that were indicated for root canal treatment and symptomatic, endodontically treated, but failed mandibular molars. Subjects with a history of antibiotic treatment were excluded from the study.

Method of collection

After pulp extirpation was done and the bleeding from the canal controlled, sterile paper points were introduced into root canals and were transferred to Staurt's transport medium. 2 ml of whole unstimulated saliva was collected from each patient into sterile plastic containers by the "spitting method." In the case of symptomatic endodontically treated teeth, the root canal filling was removed, and sterile paper points were introduced into the canal and transferred to the transport medium. The saliva and the root canal culture samples were inoculated within 4 h onto Sabouraud's agar plates and were incubated for 48 h under

anaerobic conditions. The totals colony forming units (CFU) were counted using the colony counting machine. Obtained colonies were stained with periodic acid-Schiff stain to examine the organisms. "Germ tube test" was done to confirm that the fungal organisms were indeed *C. albicans*.

Identification of Candida species

Germ tube formation test

This is a rapid screening procedure for differentiating *C. albicans* from other *Candida* species. The yeast is lightly inoculated into approximately 1 ml of sterile serum and incubated for 2-2.5 h at 37°C. After incubation, suspensions are placed on a glass slide, mounted under a cover slip and examined for the presence of germ tubes.

Results

Of the 20 samples comprising the study group, the saliva culture revealed 11 of them to have CFU >400 and 9 of the 20 canals microbiology showed CFU >400 (Table 1).

Among the 15 first-time root canal treatment cases, saliva examination these revealed 8 of them to have CFU >400 and the canal microbiology of these shoed 6 of them to have CFU >400 (Table 2).

Among the retreatment cases, 3 of the 5 cases showed CFU >400 in both the saliva DN canal culture (Table 3).

Discussion

The present study was aimed to evaluate the presence of *C. albicans* in root canals of teeth with apical periodontitis and in cases where endodontic treatment has failed. Culture medium used was a selective media and allowed only candidial organisms to grow, and the presence of *C. albicans* was confirmed by the germ tube test. Only mandibular molars were included in the study to minimize variations in root canal morphologies and position of the tooth in the oral cavity.

The results indicate that all the cases showed presence of *Candida* both in saliva and root canals and of those cases 11 were positive for *C. albicans* with CFU more than 400/ml of saliva. The CFU/ml count was high compared to other studies. A possible reason may be that all the cases were done without the use of rubber dam isolation and possible contamination with saliva cannot be overlooked.

Through the past decades, it has been well-known fact that fungi like *Candida* can be isolated from infected root canals.[1,2-14] The occurrence of yeasts in infected root canals varies between 1% and 17%.[3,4,15]

In this study, colony culture with counting of colonies was carried out along with germ tube test method of identification of *C. albicans*. Diagnostic procedures using molecular techniques have a higher sensitivity than conventional culturing techniques. Higher numbers of microbial species, including novel taxa, have been identified from root canals using polymerase chain reaction based molecular detection techniques.[16] The choice of methods will depend on the goal of the investigation, identification of causative agents would warrant molecular methods of identification whereas culture methods would be preferred for effective treatment planning.[17]

In this study, there was a steady increase in CFU obtained from root canal samples with salivary candidal counts. This may be due to improper isolation during randomized controlled trial (RCT) and lack of antifungal efficacy of irrigating solutions. In retreatment cases, the root canal samples showed increased CFU that was equal to or more than that of salivary samples. Attention is required towards

Table 1: Distribution of cases.

	Saliva	Root canal
Number of patients in study	20	15 - Indicated for root canal treatment 5 - Retreated root canal treatment
No of samples obtained	20	20
Number of samples with presence of candida	20	20
Number of patients with candida positive samples (more than 400 CFU)	11	9

CFU: Colony forming unit

Table 2: Primary RCT cases.

Patient	Yeast from saliva CFU/ml	Yeast from root canal CFU/ml
1	102	72
2	472	276
3	325	506
4	426	406
5	122	86
6	480	286
7	502	412
8	180	60
9	220	88
10	416	312
11	486	436
12	134	58
13	516	489
14	460	434
15	274	164

RCT: Randomized controlled trial, CFU: Colony forming unit

Table 3: Retreatment cases.

Patient	Yeast from saliva CFU/ml	Yeast from retreated root canal CFU/ml
1	192	182
2	166	194
3	472	432
4	444	426
5	485	452

CFU: Colony forming unit

sufficient treatment strategies including selection of medicaments potentially efficacious against microorganisms residing in the root canal system. In this context, it should be noted that extended exposure times to microbiota increase the killing efficacy of all antiseptic agents.[18] Adequate time of the irrigants, or intracanal dressings is a significant factor to achieve proper disinfection.

Caries has been considered as the portal of entry for fungi to enter the root canal system. In the present study where cases with endodontic failure were included shows more fungal growth compared to saliva suggesting that the retained candidal organisms from caries or by salivary contamination during initial treatment may be the cause of failure of the endodontic treatment.

The candidal organisms may have entered the root canal during primary treatment or through microleakage due to improper restorations or those, which were lodged in the dentinal tubules. There is a significant increase in CFU in root canals with *P* value 0.04.

Removal of the smear layer appears to improve the antiseptic action of irrigants, and intracanal medications.[19,20] Ethylenediaminetetraacetic acid (EDTA) is the most frequently used chelator in root canal therapy.[21] EDTA is found to have significant antifungal activity and may be an adjunct in antimicrobial agents used in canal sterilization.[22,23]

Conclusion

Isolation using rubber dam is a crucial step in preventing contamination of root canals by saliva in addition to sufficient contact duration of the canal irrigants. In private dental practice, this step is frequently and perhaps inadvertently skipped or avoided due to paucity of time and patient comfort. Isolation, a major step during RCT could avoid candidal infection and increase the success rate of endodontic treatment to a greater extent. Commonly used irrigation solutions by practitioners include - saline, sodium hypochlorite, and chlorhexidine. None of these solutions have significant antifungal action but providing sufficient contact time has found to provide adequate disinfection. EDTA is one of the regularly used chelating agents with known antifungal activity. Incorporation of antifungal agent may be preferred to reduce failure of RCT caused due to candidal infection.

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