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Endodontic Mycology: A New Perspective of Root Canal Infection.

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Review Article

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ABSTRACT

An important consideration in endodontic treatment is the elimination of microorganisms, including fungi, from the complex three-dimensional root canal system. Yeasts can be detected in 7-17% of infected root canals. *Candida albicans* (CA) has a major role in endodontic treatment failure as the most important fungus isolated from the root canal system. C. albicans has been associated with root canal infections resistant to non-surgical therapy and a potent pathogen to infect periapical lesions. They are commonly associated with persistent cases of apical periodontitis, but yeasts can also be isolated in primary apical periodontitis. A variety of virulence factors enable C. albicans to adhere to and penetrate into dentine. Yeasts do not respond favourably to conservative root canal therapy, due to resistance against a commonly used medicament, calcium hydroxide. Thus, alternative therapeutic approaches and other intracanal medicaments are required to treat persistent cases of apical periodontitis.

INTRODUCTION

Fungi are chemoorganotroph eukaryotic microorganisms, ubiquitous in the environment constitute a small part of the oral microbiota [1].The largest proportion of the fungal oral microbiota is made up of Candida species [2]. *C albicans*, one of the most well-studied fungal species. Fungal infections are usually ''diseases of the diseased,'' and some predisposition has to be present for the host to be affected [3]. Species of candida have been found as commensal microorganisms in the oral cavities of 25% of healthy adults, 50% of hospitalised patients, and approximately 90 % of immunocompromised patients [4].

In last decade, incidence of *Candida albicans* in endodontic infection has received attention and fungi were observed in primary and refractory endodontic infections [5,6] .Fungi have been detected in infected root canals, but the number of yeast cells in the root canal is usually lower than that of bacteria [7]. 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 [8]. Clinically important candida species grow well in vitro over a pH range of 3.0-8.0 [9].

It is a unique parasite capable of colonizing, infecting, and persisting on mucosal surfaces, like dorsum of the tongue, the primary oral habitat of *C albicans*, whereas other sites may be colonized secondarily [10], such as the mucosa and supragingivae [10], the dentin [11], the root [12], the subgingivae [13], and the periodontal pockets [14]. It stimulates mucosal immune responses leading to the induction of immunity or tolerance [15]. The ability of C. albicans to persist in infected tissues or to behave as a commensal may involve primarily down regulation of host cell-mediated adaptive immunity [16].

Prevalence of Yeast in Root Canal

The prevalence of yeasts in saliva was 32.7%. There was a significant association between the presence of yeasts in saliva and root canal but the effect of previous root canal treatment and restoration leakage on the recovery of yeasts from root canal was equivocal [17].

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Table 1: Prevalence of yeast in root canal system by data collected from various studies:-

S.No.	Authors	Year	Prevalence of yeast in root canal(treated /untreated)
1.	Grossman et al [18]	1952	17% (In untreated root canals)
2.	Slack et al [19,20]	1953,1957	7% (treated cases)
3.	MacDonald et al.[21]	1957	2.2%
4.	Jack & Halder [22]	1963	26% (treated cases)
5.	Wilson & Hall [23]	1968	1.9%(untreated cases)
6.	Matusow et al [24]	1981	1 case report
7.	Nair et al. [25]	1990	22%
8.	Najzar-Fleger et al.[26]	1992	55% (untreated root canals)
9.	Sen et al. [27]	1995	40% (untreated cases)
10.	Waltimo et al.[32]	1997	7% (untreated root canals)
11.	Molander et al.[6]	1998	4.2%
12.	Baumgartner [28]	2000	21% (untreated root canals)
13.	Egan et al. [17]	2002	10%(16%- yeast in untreated root canals,5.7%- treated root canals)
14.	Ferrari et al. [29]	2005	4% (infected root canals)
15.	Ahsraf et al.[30]	2007	25%(36.7%-yeast in root canals with periapical lesions, 13.3%- without periapical lesions)
16.	Gomes et al.[31]	2010	28.3% (filamentous fungi in untreated root canal)

Pathway of Yeast to Root Canal System

Oral candidiasis has recently been a growing concern about yeast infections of the root canal [32]. Common dental sites for yeast infections are dental plaque, dental caries, and periodontal pockets [33]. The most common pathway for bacteria to enter the pulp is probably through open dentinal tubules [34].

Yeast particularly *Candida albicans*, have been isolated from infected dental pulp and root canals. The root canal systems of teeth had some communication with the oral cavity, via poor asepsis during endodontic treatment procedures, coronal restoration leakage, deep fracture line, sinus tract, oro-antral communication, incision/drainage of a swelling associated with the tooth some months earlier, and a deep vertical bony defect that communicated with the periapical lesion [17].

Dental caries were reported as the main source of fungus presence and the only part of entry of fungi into the root canal system [19]. Interestingly, yeasts have also been isolated from an intact non-vital tooth after trauma [35].

Candida Species In Root Canal System

The most regularly encountered species of opportunistic oral fungal pathogens are members of the genera Candida and Aspergillus, both belonging to the Deuteromycetes group. *Candida albicans* (CA) is the fungal species most commonly detected in the oral cavity [36].

Egan et al.[17]carried out a study on the presence of CA in the root canals with apical periodontitis concomitant with a positive saliva test for CA was 13.8 times higher than a situation in which the saliva test was negative for CA.

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Seven Candida species were identified in oral mucosa(*C. albicans, C. dubliniensis,C. guilliermondii, C. krusei, C. parapsilopsis, C. tropicalis* and *C. glabrata*). They were all identified from oral mucosa samples, while two of them (C. parapsilopsis and C. glabrata) were not found in the periapical zone of root canals with pulp necrosis [37]. C. dubliniensis was recently identified by Sullivan et al.[38] and is believed to be strongly associated with human immunodeficiency virus infection [39]. Other species such as *C. glabrata, C. guillermondii ,C. incospicia* and *Geotrichum candidum* were also isolated by Waltimo et al [32].

Various filamentous fungi in the root canals of teeth with pulp necrosis and periapical lesions were isolated in situ from 17 of 60 samples (28.3%). Four species of aspergillus were identified: *Aspergillus ustus, A. granulosus, A. niger,* and *A. sydowii. Emericella quadriluniata,* sexual form of Aspergillus, was isolated from one sample. Penicillium species (*Penicillium implicatum, P. micsynvisk, P. lividum,* and *P. citrionigrum*) were isolated from four samples (24%). Fusarium species (*Fusarium moniliforme* and *F. melanochorum*) were isolated from two samples (12%). The species *Aureobasidium pullulans, Exophiala jeanselmei, Eurotium amstelodame*, and *Cladosporium sphaerospermum* were isolated from one sample each [31].

Candida albicans is adaptive oral yeast that can occasionally be isolated from the root canal in cases of persistent apical periodontitis both in pure culture and together with bacteria [32]. Yeasts were isolated most often together with facultative Gram positive bacteria, while Gram negative isolates were rare. The dominance of accompanying facultative Gram positive bacteria may be due to the harsh ecological conditions prevailing in the root canal in prolonged treatment.

C. albicans has been associated with root canal infections resistant to non-surgical therapy and a potent pathogen to infect periapical lesions. They are commonly associated with persistent cases of apical periodontitis, but yeasts can also be isolated in primary apical periodontitis. *C.albicans* do not seem to occur in perapical granuloma [40].

Virulence Factors Of Yeast

C albicans has a number of virulence factors required for tissue penetration. It was hypothesized that *Candida albicans* may survive and infect the periradicular tissues. The occurance of *Candida albicans* in persistent root canal infections and the number of factors that contribute to the virulence and invasiveness are:-

- Tolerance to harsh environmental conditions [41].
- Thigmotropism (contact sensing) for penetration [42].
- Production of proteolytic enzymes [41].
- Phenotypic switching phenomenon [43] .
- Biofilm formation [44,45].
- Evasion and immunomodulation of the host defense [46].

C albicans adapt to an extremes range of pH, low oxygen and nutritional environment. Candida is polymorphic fungus that exists in blastophores, germ tubes, true hyphae, pseudohyphae and chlamydospores depending on environmental conditions which helps in survival. Provides ability to penetrate dentinal tubules via hyphal adherance and able to bind to collegen types I and IV. Candida species has ability to produce secreted aspartyl proteases, collegenases, hyaluronidase, acid and alkaline phosphatases help to degrade variety of host dentinal collagen and other extracellular proteins. Oral candidiasis is a prevalent disease in immunocompromised patients. A poorly functioning immune system might increase the risk of fungal infection in the root canal system.

Will Yeast Persist on Dentine?

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 [47]. *Candida albicans* is the most infective and invasive yeast among the candida species [48], and it has a particular affinity to dentin and smear layer [49]. They were also observed in the apical end of root canals and in dentine tubules, being considered to be dentinophilic microorganisms [25]. Candida must adhere to a surface for colonization to occur and infection to persist [4]. The mechanism of this attachment to any substratum is believed to involve the interaction of cell surface protein receptors of *C.albicans* with the target surface [50]. Once Candida species enter the root canal, they may further penetrate into root canal dentinal tubules by adoption of a range of growth patterns (blastospores and hyphae) ,use dentin as a source of nutrition for a long period and interact with other microorganisms to form a complex biofilm [44,45]. According to Cannon et al. [51], *C albicans* adheres poorly to clean tooth surfaces and always requires a pellicle of proteins.

The dentinal tubules are tapered structures measuring approximately 2.5 um in diameter near the pulp, 1.2 um in the midportion of dentine, and 0.9 um near the dentinoenamel junction [52]. The small diameter of

bacteria (0.3-0.8 um for most species) allows them to invade the pulp through the dentinal tubules. Yeast blastoconidia are 3-8 x 2-7 um [53] in size, and hyphae, when cultured in serum, are 1.9-2.6 um in diameter [54]. Therefore, the possibility that Candida spp. could penetrate into dentinal tubules cannot be excluded.

Dentinal smear layer is composed of organic (collagen) and inorganic(ca++ ions) materials [55]. Candida has a specific affinity for dentinal collagen type I and IV, significantly enhances candidal adherence [50]. The presence of calcium ions has a critical role in the control of candida morphogenesis [56] and the adherence potential of CA to various extracellular matrix proteins [57]. The increased adhesion in the presence of smear layer is the result of the availability of disintegrated organic structure of dentin and availability of calcium ions as a source of growth and adhesion [58].

Siqueira et al. [48] investigated the pattern of radicular dentin colonization by five fungal species: *Candida albicans*, *Candida glabrata, Candida guilliermondii, Candida parapsilosis*, and *Saccharomyces cerevisiae*. *C. albicans* showed the ability to colonize dentin as found in most of the specimens whereas the other four fungal species did not. This can explain why *C. albicans* is the fungal species most often found in endodontic infections. *Candida albicans* is able to invade dentinal tubules to a variable extent. Within tubules, the microorganism may be protected from lethal action of endodontic medicaments by inactivating effects of dentin [59].

Will Yeast be responsible for Endodontic Treatment Failure??

Endodontic treatment success, to great extent, depends on the depletion or elimination of microorganisms including fungi, from the complex three-dimensional root canal system .*Candida albicans* as a major role in endodontic treatment failure as the most important fungus isolated from the root canal system. To this end, the use of irrigating solutions with proper anti-microbial and anti fungal properties during canal debridement and preparation are considered enormously important [60].

Waltimo et al. introduced fungi as microorganisms resistant to endodontic treatment in apical periodontitis. Fourty-eight fungal types were isolated from 47 specimens (7%) out of 692 chronic apical periodontitis cases resistant to endodontic treatment[32]. Root canals function as an incubator since they are closed spaces with low oxygen concentration and therefore they promote the growth of microorganism [60]. Studies have demonstrated that fungi are also present in infections resistant to conservative root canal treatment and despite proper cleaning and irrigation of the root canal system play a role in failure of periapical lesions treatment [25].

Peciulene et al. isolated fungi as resistant microorganisms in the obturated root canals of teeth with chronic apical periodontitis [61]. CA was found in 25% of root filled canals. Proper isolation of teeth during endodontic treatment may prevent microorganisms, including CA from entering the root canal system [62].

How Medicaments Help In Eliminating Yeast From Root Canal System??

Endodontists have been long aware of the need to use proper antimicrobial strategies that include fungi elimination from infected root canals. This can be attested by the statement of Grossman [63]: ''One of the problems in endodontic treatment is the presence of Candida organisms in infected root canals; it is necessary to eliminate these organisms to maintain the periapical tissue in a normal state or to restore it to a state of health.'' He proposed the use of antifungal agents as intracanal medication.

C.albicans is frequently associated with root canal treatments failures and has the ability to form biofilms on different surfaces. This property is one of the reasons why this species is considered to be more pathogenic than species that are less able to form biofilm[64].

Since mechanical instrumentation and irrigation may not eliminate all microorganisms, it has been emphasized that antimicrobial agents should be used in the root canal between visits [65]. Common root canal microorganisms are readily killed after contact with a wide variety of intracanal disinfectants [66]. Residual microorganisms are likely to play a role in treatment failures [6]. To leave the canal empty is an opportunity for endodontic infection or reinfection [67]. However, when the root canals were left empty, it allows surviving bacteria in the root canal to multiply [68].

Mechanical instrumentation of canals may help to disrupt and expose biofilm organisms to irrigating solutions but at the same time may produce a smear layer that may enhance the growth of surviving yeasts cells. EDTA not only remove smear layer from the root canals but also remove the microorganisms entrapped in the smear layer [69]. Furthermore, EDTA has been shown to have a potent antifungal effect [70], possibly due to its ability to chelate calcium ions which have a critical role in morphogenesis and pathogenicity of *C. albicans*. Therefore it may be expected that other chelating or calcium binding agents would also have antifungal potential. EDTA and TiF4 may be recommended as an alternative irrigating solution particularly in persistent root canal infections and in

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root canals of patients having a high incidence of oral candidiasis [71]. EGTA is also considered as a specific calcium ion chelator and it has been recommended as an alternative smear layer removal agent [72].

Fungi, unlike most bacteria, are highly resistant to routine root canal irrigants such as sodium hypochlorite[73] (NaOCl) and medication (calcium hydroxide) [74]. CA was resistant to killing by NaOCl in the presence of a smear layer [73].Thus, it has been demonstrated that sequential use of EDTA and NaOCl limits the growth and adhesion of *C.albicans* to dentin [58]. Thus antifungal potential of EDTA will make it very valuable as an endodontic irrigating solution.

Ruff et al.[75] showed that 6% NaOCl and 2% CHX were equally effective and statistically superior to BioPure MTAD and 17% EDTA in antifungal activity. MTAD was significantly better than 17% EDTA as a final rinse on C. albicans in vitro. 17 % EDTA had the highest antifungal activity compared to 5% NaOCl and Savrolin(1.5% CHX, 15% cetrimide) [70].

The best interappointment medicament available in endodontics is calcium hydroxide (Ca(OH)2) [76], but it doesnot effectively kill candida species [74]. Sen et al. hypothesized that if there is a yeast infection in the root canal, the use of common intracanal medicaments during endodontic therapy may favour the overgrowth of yeasts between visits [73].

Combinations of the medicaments were equally or less effective against CA than the more effective component. Calcium hydroxide inhibited the effect of chlorhexidine and iodine potassium iodide whilst the combinations were more effective than calcium hydroxide alone. Combination of calcium hydroxide and sodium hypochlorite was an effective as pure sodium hypochlorite. CHX inhibited the effect of iodine potassium iodide and sodium hypochlorite. Iodine potassium iodide and sodium hypochlorite inhibited each other. Sodium hypochlorite, iodine potassium iodide and CHX were all clearly more effective on CA than calcium hydroxide. These disinfectants can be useful as irrigants or local medicaments in cases of persistent apical periodontitis with yeasts. The high pH of calcium hydroxide is unaffected in combinations with CHX acetate or iodine potassium iodide. Thus combination of calcium hydroxide with another disinfectant may be useful in treating persistent cases of apical periodontitis [77].

Recently it has been shown that a direct correlation existed between MTA concentration and its inhibition effect on CA growth. Both gray-colored and white colored MTA in concentrations of 50 mg/ml and 25 mg/ml are effective in killing CA for periods of upto 1 wk. Lower concentrations of grey-colored MTA may still be effective while lower concentrations of white MTA may not be effective.

CONCLUSION

CA is an opportunistic microorganism and is becoming increasingly more prevalent in modern medicine. Presence of fungi in root canals more than expected and the knowledge of persistent periapical infections may advocate the use of antifungal agents in modern endodontic therapy. More effective antifungal methods could possibly become more important in endodontic therapy. The presence of CA in the microbial population of root canals and in persistent lesions has prompted researchers to test new (intracanal) medications and methods to eliminate these fungi from the root canal system. However, further investigations are needed to evaluate the antifungal activity associated with various intracanal medicaments used routinely.

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