Candida species in periodontal disease: A literature review

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ABSTRACT

Periodontal disease is one of the earliest human diseases recognized to be associated with mixed-species biofilms. One of the microorganism involved in this biofilm include Candida species which have been invariably found in large quantities in subgingival area of patients having periodontal disease. This paper reviews the prevalence of Candida species in periodontal disease as well as their virulence factors and identification methods.

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1. Introduction

Periodontal disease is characterized by inflammation of the gingiva that results in periodontal pocket formation with loss of the supporting periodontal ligament and alveolar bone around the teeth. It has a multifactorial etiology. One of the main etiological factors includes dental plaque. This dental plaque is considered to be a diverse microbial community which is synergistic as well as dysbiotic in nature with keystone pathogens such as Porphyromonas gingivalis which instigate interruption of tissue homeostasis.2 It comprises a massive amount of micro-organisms that include bacteria, yeasts and possibly viruses.2 Yeasts are present in the subgingival area in 10 — 30% of healthy subjects and in up to 52% of diabetics.3,4 There is still a disagreement if the yeasts are transient members of oral biofilms, or if they are definite members of the oral microbiome.

Among these yeasts, Candida species (spp.) are more prevalent in the oral cavity which is also a part of the normal commensals in the oral cavity. This normal commensal is also a well-known human and animal pathogen which is capable of initiating polymicrobial disease, probably because of its capacity to form multi- spp. biofilms.5

Immunocompromised patients (e.g. Diabetics, AIDS patients) are more prone to Candida colonization and infection compared to healthy adults.6 Candida spp. is most commonly found on the dorsal surface of the tongue, followed by the palatal and buccal mucosa in the oral cavity. Candida albicans (C.albicans) has a preponderant role among other spp. of its genus in periodontal disease. In fact, the presence of its hyphae was found in association with highly invasive anaerobic bacteria such as Porphyromonas gingivalis, Prevotella intermedia and Aggregatibacter actinomycetemcomitans in the connective tissue of periodontal patients.7

There are numerous studies with reported increased subgingival colonization by yeasts, predominantly C.albicans, in chronic periodontitis patients compared to periodontally healthy subjects. Apart from C.albicans, the prevalence and importance of other spp., such as Candida glabrata, Candida tropicalis, Candida krusei and Candida dubliniensis are increasing which may due to the increased usage of broad spectrum antibiotics. These are called the Non Candida Albicans Candida (NCAC) spp. which were thought to be present in increased quantities in immunocompromised subjects, but recently they were identified in healthy subjects as well.9
2. Prevalence of Candida spp. in oral diseases

It was observed that a higher prevalence of Candida spp. was found in the oral cavity, and particularly in the subgingival biofilm, of Human Immunodeficiency Virus (HIV)-seropositive patients. Also a higher prevalence of these were found in chronic periodontal disease patients with diabetes than in those without diabetes.  

Candida-Associated Denture Stomatitis (CADS) is an eminent oral infection in which Candida coexists with oral bacteria like S. aureus, E. coli and Klebsiella spp. Another lesion with polymicrobial etiology involves Angular stomatitis. Major fungi involved is Candida spp. mainly C. albicans with co-infecting agents like S. aureus, streptococci, enterococci, E. coli, Klebsiella and Pseudomonas. Dental implant failure is commonly associated with the overgrowth of atypical periodontal microbiota such as Staphylococci, E. coli, Pseudomonas, Prevotella and Candida.  

3. Prevalence of Candida spp. in periodontal diseases

The evidence regarding yeast involvement in periodontal disease is scarce. Candida spp. are mainly seen in association with periodontal pathogens like pophyromonas gingivalis, tannerella forsythia and aggregatibacter actinomyctecomitanis which are considered to be the most prevalent periodontal pathogens. Among these Candida spp., C. albicans was alleged to enhance Porphyromonas gingivalis invasion of human gingival epithelial and fibroblast cells in vitro signifying that it may contribute to initiation or exace rbation of periodontal disease. C. albicans, have been recovered from periodontal pockets in 7.1% to 19.6% of patients with chronic periodontitis. This is mainly achieved by the virulence factors present in these which aid them to colonize and proliferate in the oral mucosa as well as in periodontal pockets.  

Among the Candida spp., Urzúa et al. observed that C. albicans and C. dubliniensis were capable of colonizing periodontal pockets in patients with chronic periodontitis, while only C. albicans was identified in the subgingival microflora of healthy individuals and patients with aggressive periodontitis. C. albicans was found in 57%, C. dubliniensis in 75%, C. tropicalis in 16%, and C. glabrata in 5% of periodontal pockets in the case of diabetic patients. C. albicans and C. dubliniensis were present in 20% and 14% of periodontal sites, respectively; there was no evidence of C. tropicalis or C. glabrata colonization in nondiabetic patients. Thein et al. evaluated the effects of oral bacteria, including the periodontopathogens Prevotella nigrescens and Porphyromonas gingivalis, on the development of Candida albicans biofilm in vitro. They observed a reduction in yeast counts when these microorganisms were cocultured with Candida biofilm, possibly because metabolites produced by anaerobes interfere with biofilm physiology or because the physical presence of bacteria inhibits biofilm growth.  

4. Pathogenecity mechanisms

The capability of Candida spp. to infect these varied host environments can be attributed to its miscellaneous virulence factors as well as fitness traits. Virulence factors include the morphological transition between yeast and hyphal forms, the expression of adhesins and invasins on the cell surface, thigmotropism, formation of biofilms, phenotypic switching and the secretion of hydrolytic enzymes. Fitness traits include rapid adaptation to fluctuations in environmental pH, metabolic flexibility, powerful nutrient acquisition systems and robust stress response machineries.  

4.1. Adhesion

These are a specialized set of proteins (adhesins) which mediate adherence of Candida spp. to other microorganisms, abiotic surfaces or host cells. Examples for adhesins are agglutinin-like sequence (ALS) protein 3, hypha-associated GPI-linked protein (Hwp1).  

4.2. Dimorphism

It can be defined as the transition between yeast and hyphal form. Both these forms are important for its pathogenicity. Yeast form is mainly involved in dissemination while the hyphal form is believed to be involved in invasion as well as adhesion.  

4.3. Thigmotropism

It is defined as directional hyphal growth. This takes place on surfaces with particular topologies (such as the presence of ridges). It is regulated by extracellular calcium uptake through the calcium channels Cch1, Mid1 and Fig1 or polarisome Rsr1/Bud1-GTPase module.  

4.4. Invasion

Invasion into host cells depends on two likely complementary mechanisms. First, induced endocytosis is mediated by Als3 and Ssa1. Secondly, active penetration mediated by some undefined molecular mechanisms.  

4.5. Secreted hydrolases

Active penetration of Candida spp. to the cells is mediated by certain secreted hydrolases. Proteases, phospholipases and lipases are the 3 different classes of secreted hydrolases. These are also thought to enhance the efficiency of extracellular nutrient acquisition.
4.6. Biofilm formation

One of the major virulence factors of Candida spp. is formation of biofilms in biotic (mucosal cell surfaces) or abiotic (catheters and dentures surfaces). Mature biofilms are more resistant to antimicrobial agents and host immune factors when compared to planktonic cells. The complex architecture of biofilms, the biofilm matrix, increased expression of drug efflux pumps and metabolic plasticity are considered to be the reason for this increased resistance property. The process of biofilm formation occurs in a sequential manner. First step is adherence of yeast cells to the substrate, then proliferation of these yeast cells, then formation of hyphal cells in the upper part of the biofilm, then accumulation of extracellular matrix material and, finally, dispersion of yeast cells from the biofilm complex. Moreover, recent studies indicate that Candida spp. biofilms are resistant to killing by neutrophils and do not trigger production of reactive oxygen spp. (ROS). This may be due to the presence of β-glucans in the extracellular matrix which protects it from this attacks.

5. Host immune response to Candida spp.

Host immune response to Candida spp. involves both innate and adaptive immune responses. Phagocytic cells recognize pathogens by means of a variety of pattern recognition receptors (PRRs), including toll-like receptors (TLRs). These are abundantly expressed on innate immune cells – such as macrophages, dendritic cells (DCs), monocytes, neutrophils. Complement activation is mainly important for chemotaxis and opsonization in C. albicans which is mediated by alternative pathway. Membrane bound receptors like dectin 1, dendritic cell-specific intercellular adhesion molecule-3- grabbing non-integrin (DC-SIGN), and mannose receptor (MR) which is located in innate immune cells contribute to the phagocytosis of Candida spp. Adaptive immune responses to C. albicans are crucial to the successful eradication of infecting fungus. Recognition of C. albicans by the immune system triggers the production of the Th1 and Th2 cytokines, mainly by CD4+ T cells. The immune response to Candida spp. is related mainly to the different cytokines and chemokines produced by Th1 or Th2 cells; however, concomitant humoral immune responses to C. albicans specific IgA and IgG antibodies have been observed. The exact mechanisms by which these antibodies protect against Candida infection are unknown, but are likely to include inhibition or germ tube formation, opsonization, neutralization of virulence-related enzymes, and direct yeast activity.

6. Isolation of Candida species

Numerous candida spp. are isolated till now. (Table 1) A variety of techniques are available for the isolation of Candida spp. from blood stream, mucosal surfaces etc.

Techniques used in oral cavity include the use of:
1. A smear
2. A plain swab
3. An imprint culture
4. Collection of whole saliva
5. The concentrated oral rinse
6. Mucosal biopsy
7. Subgingival plaque

7. Identification of Candida species

7.1. Based on morphology

The standard laboratory method used for identification of C. albicans & C. dubliensis is germ-tube test. The procedure of this test involves the induction of hyphal outgrowths (germ tubes) when subcultured in horse serum at 37 °C for 2 – 4 hours.

7.2. Biochemical tests

Biochemical identification of Candida spp. is largely based on carbohydrate utilization. On a basal agar lacking a carbon source, test isolates will be isolated in the case of traditional testing. Carbohydrate solutions would then be placed within wells of the seeded agar or upon filter paper discs located on the agar surface. In the vicinity of a carbon source, growth would indicate utilization of this carbon. On the basis of this same principal, commercial systems are available but test carbohydrates are housed in plastic wells located on a test strip. Growth in each well is read by changes in turbidity or colour changes in certain kit systems. Test organisms are identified by numerical codes obtained from the test results based on database comparison.

7.3. Serology

Serological tests are frequently used to determine the clinical significance of Candida spp. isolates. Rising titers of IgG antibodies to C. albicans may reflect invasive candidiasis in immunocompetent individuals. Tests like enzyme linked immunosorbent assay (ELISA) and radio immuno assay (RIA) for detection of candidal antigen, either cell-wall mannan or cytoplasmic constituents are now available in developed countries. Serologic tests are normally not a diagnostic tool for oral candidosis.

7.4. Molecular-Based identification Methods

Identification by analysis of genetic variability is a more stable approach than using methods based on phenotypic criteria. Electrophoretic karyotype differences and restriction fragment length polymorphisms (RFLPs) using gel electrophoresis or DNA-DNA hybridization are used for the identification of Candida based on genetic variation. A new detection technique is Fluorescence in situ hybridization.
Table 1: Principal Candida spp.

| Candida spp.        | Candida guillermondii | Candida utilis          | Candida lipolytica        | Candida famata            | Candida haemulonii        | Candida rugosa           |
|---------------------|-----------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Candida albicans    |                       |                         |                          |                          |                          |                          |
| Candida dubliniensis|                       |                         |                          |                          |                          |                          |
| Candida parapsilosis|                       |                         |                          |                          |                          |                          |
| Candida tropicalis  |                       |                         |                          |                          |                          |                          |
| Candida glabrata    |                       |                         |                          |                          |                          |                          |
| Candida kefir(pseudotropicalis) |           |                          |                          |                          |                          |                          |
| Candida lusitaniae  |                       |                         |                          |                          |                          |                          |
| Candida krusei      |                       |                         |                          |                          |                          |                          |

with Peptide Nucleic Acid method (PNA Fish) which targets highly conserved species-specific sequences in the abundant rRNA of living C. albicans. This technique achieves a sensitivity of 98.7–100%, with a specificity of 100%, allowing for the discrimination of C. albicans from the phenotypically similar C. dubliniensis. Molecular-based technology can also be used to identify strains of Candida spp. although the use of techniques such as Pulsed Field Gel Electrophoresis (PFGE), Random Amplified Polymorphic DNA (RAPD) analysis, and Repeat sequence amplification PCR (REP).

8. Association of Candida spp. in periodontal disease: literature review

A Jarvensivu et al in 2004 conducted a study to evaluate the frequency of Candida in periodontal tissues of chronic periodontitis patient and the extent of Candida penetration into the gingival tissues. Sample collected were tissue specimens and subgingival plaque. They concluded that C.albicans could have a role in the infrastructure of periodontal microbial plaque and in its adherence to the periodontal tissue and also indicates that hyphal germination starts in the gingival pocket.

A study was done by B Urzua et al in 2008 to analyse the composition of the yeast microbiota present in the mucosa and subgingival sites of healthy individuals, patients with aggressive and chronic periodontitis. It was noted that only C. albicans and C.dubliniensis were capable of colonizing the periodontal pockets in patients with chronic periodontitis, while only C.albicans was identified in healthy individuals and patients with aggressive periodontitis.

Previous studies had reported an increased prevalence of Candida spp. in subgingival sites of HIV positive patients compared to healthy adults. A study was conducted by Jewtuchowicz et al in 2008 to determine the prevalence of different yeast species in periodontal pockets from immunocompetent subjects through a comparison of phenotypic and molecular assays. It was found that C. albicans is the most frequently isolated species of yeast (24.4%), followed by C.parapsilosis (5.6%) and C.dubliniensis (4.4%).

Melton et al in 2010 conducted a pilot study to determine the prevalence of C. dubliniensis and other Candida spp. from saliva samples and from subgingival plaque samples at periodontally healthy and periodontally diseased sites in subjects with type 2 diabetes and periodontitis. In this study, C. dubliniensis was not found in oral samples of diabetic patients & for other Candida spp. no significant differences were found.

Machado et al in 2011 conducted a study to analyse the adherence to epithelial cells of C. albicans isolated from subgingival plaques sample of patients with chronic periodontitis in comparison to healthy patients. The result of this study suggested a higher Candida adherence of samples isolated from patients with chronic periodontitis.

Mcmanus et al in 2012 conducted a study to investigate the prevalence and cell density of Candida spp. in periodontal pockets, healthy subgingival sites and oral rinse samples of patients with untreated periodontitis. It was concluded that the distribution of sequence between both groups were significantly different and indicated an enrichment of C.albicans isolates in periodontal pockets.

A study was conducted by Canabarro et al in 2013 to analyze the relationship between the subgingival colonization by Candida albicans and other yeasts with the severity of chronic periodontitis. In this study, it was concluded that subgingival colonization of some yeasts, especially C.albicans was associated with severity of chronic periodontitis.

De-La-Torre et al. in 2018 conducted a study to analyze the oral Candida carriage in patients suffering from chronic periodontitis (CP) and its correlation with the severity of this condition. Microbiological samples were taken from 155 patients using the oral rinse (OR) technique and by using paper points in the periodontal pockets (GPP). It was concluded that colonization by Candida was present in the samples of patients without CP, and with both moderate and severe CP. Nonetheless, patients with severe CP had a higher rate of Candida colonization, especially by C. albicans.

9. Conclusion

Candida spp. are ubiquitous fungal organisms that often colonize the oral mucosa of normal individuals, without
causing disease. These opportunistic microorganisms might influence the inflammatory process, as they possess several virulence factors by which they invade tissues and evade host defense mechanisms, thereby facilitating proliferation and release of exoenzymes that promote tissue degradation. All these virulent mechanisms of this opportunistic fungus might add to increased destructive property of periodontopathic bacteria in periodontal disease. Even though there is a lot of evidence about the presence of yeasts in periodontal pockets, their role in pathogenesis of periodontitis is still to be elucidated. However, additional studies are needed to reveal the pathogenic potential of this opportunistic fungus.

10. Source of funding
None

11. Conflict of Interest
None

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