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Conventional and Recent Diagnostic Aids in Oral Candidal Infections: A Brief Overview

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ABSTRACT

Candidiasis refers to multiplicity of diseases caused by yeast like fungus candida. Candida is a normal commensal inhabitant of the oral cavity and gastrointestinal tract, and Candida albicans is the commonest species demonstrated in the oral cavity. C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, C. dubliniensis are some other species isolated from oral cavity. Candida proliferates if there is a change in the local ecology or suppression of the immune system. As C. albicans exhibits a dimorphic pattern (yeast and mycelial phase), the physicians and dentists encounter diagnostic and treatment challenges for the disease. Diagnosis of candidal lesions is essentially based on clinical manifestations and supplemented by smear and culture. Species differentiation can be done by morphological features such as germ tubes and chlamydospores and a variety of biochemical techniques. Immunological and genetic techniques are also employed for the diagnosis of candidal infections.

Keywords: Oral candidal infections, Diagnosis, hyphae, Germ tube, biochemical tests.

INTRODUCTION

A wide variety of organisms ranging from eubacteria, archaea, fungi, mycoplasmas and protozoa are the normal inhabitants of the oral cavity[1] Fungi are eukaryotic organisms, and genus Candida is the most significant to oral cavity[2] The term candida has a origin from a Latin word candid, meaning white[3] Although around one hundred and fifty species of the genus candida have been isolated from the oral cavity, majority of the isolates (80%) were found to be candida albicans[4] C. tropicalis, C. glabrata, C. parapsilosis, C. guillermondii, C. krusei and C. kyfer and, in recent times, C. dubliniensis are some other species isolated from human infections[5,6] Candida species are considered normal microflora of oral and gastrointestinal tract and may be isolated from up to one third of the oral cavity of healthy human being[7] It has a dimorphic nature, and exist in both a yeast phase (blastospore) and a hyphal (mycelial) phase. This dimorphism poses diagnostic and treatment challenges for the Candidal lesions[8] An accurate and prompt diagnosis is essential for specific treatment of a fungal infection and may prove lifesaving or stave off the complications produce there in[9] This paper provides an overview on the conventional and recent trends in the diagnosis of oral candidal infections.

Etiopathogenesis

The transformation of this commensal micro-organism to the pathogenic entity may be linked with factors other than the pathogenic attributes of the organism. This is a unique feature, in contrast to most of the other infections, where the

virulence of the organism is considered as the principal cause in the pathogenesis. Candidiasis is an opportunistic infection, and an underlying pathology is essential for both the superficial and systemic forms of Candida infections[10]

The etio-pathogenesis of candidiasis is attributable to three factors: host, fungus and oral microenvironment-modifying factors. [TABLE 1]

Classification

Samaranayake [29] classified oral candidal lesions into two main groups:

Group I, or primary oral candidiasis (limited to oral cavity without skin / other mucosal involvement)

Group II or secondary oral candidiasis (widespread involvement of oral cavity and other extraoral sites such as skin)[30] [TABLE 2]

Diagnosis of oral candidiasis

The essential aspect in the diagnosis of oral candidiasis is based on the clinical identification of the lesions, and confirmatory diagnosis is made by the microscopic detection of Candida in the oral samples and/or isolation in culture. As candida is a normal commensal organism inhabiting the oral cavity, hence, candidal detection in the oral cavity is not indicative of infection. However, tissue invasion by Candidal organisms is essential for a definitive diagnosis, thus, underscoring the significance of the clinical diagnosis of the disease[11]

Candida may be isolated from the oral cavity by a variety of techniques such as the use of a smear, a plain swab[31] an imprint culture[32] collection of whole saliva[33] the concentrated oral rinse, [34] and mucosal biopsy. A direct sample collection (use of a swab or an imprint) is more acceptable in cases of accessible and defined lesions. An indirect sampling (culturing saliva specimens or an oral rinse) is given a preference for cases where the lesion is difficult to access or where there are no obvious lesions[31]

Direct microscopic examination

Evaluation of various morphological characteristics of Candida species is essential for identification[35] Differentiating between yeast and hyphal forms can be made by smear, although, it is less sensitive than cultural methods[36] A representative sample from the infected site is usually taken by exfoliative cytology and transferred on glass slide for microscopic assessment. Ideally, it is treated with potassium hydroxide (KOH), Gram stain or periodic acid–Schiff (PAS) stain[37] The most frequently used stain to identify fungi or yeast cells is Potassium hydroxide solution (10-20% KOH and 10% glycerine). KOH digests keratin and glycerine prevents yeast degradation[38] The KOH clears organic material and imparts a clear blastoconidia, hyphae or pseudohyphae appearance to the fungi[37] Nonpigmented septate hyphae with distinctive dichotomous branching (at an angle of approximately 45æ%) is usually appreciated on Potassium hydroxide (KOH) preparation[39] Fungal features such as hyphae, yeast cells, and other fungal elements will show fluorescence on KOH-Calcofluor fluorescent-staining[40]

Gram's staining is a routine laboratory method as it is rapid and economic. Gram's technique not only facilitate the identification of the morphologic forms of Candida species (hyphae and yeasts will appear dark blue), but also aid in the detection and the distinction of gram-positive and gram negative bacteria in the sample[41] Candidal species are inadequately stained by Hematoxylin and Eosin stain. The special stains generally used for demonstrating fungi in the tissues (fungi color strongly with these stains) are PAS stain, Gridley stains, and Grocott Gomori's methenamine silver (GMS)[42] The PAS stain preferentially stains glycogen in the fungal cell wall and imparts a magenta appearance to the candidal organisms[43]

Hyperplastic candididal lesions may mimic squamous cell carcinoma, hence, a biopsy is highly recommended for subsequent invasion by candida. Fungal elements within tissues may be identified by PAS or Grocott Gomori's methenamine silver (GMS) stain. Candidal species may be identified by the demonstration of blastospores and hyphae or pseudohyphae, and accompanied with other histopathological features, a diagnosis of chronic hyperplastic candidosis can be arrived at[44]

Macroscopic examination

Macroscopic inspection is carried out on culture plates. Sabouraud dextrose agar (SDA) is

the preferably used culture medium. Due to its low pH, it permits the growth of candida and suppresses the growth of many species of oral bacteria. Chloramphenicol 0.05 g/l or gentamycin 0.5 g/l is generally incorporated to this medium to hamper bacterial growth. It is also possible to add cycloheximide 0.05 g may also be incorporated as it will prevent the overgrowth of other associated fungi[17,23] Candida forms cream, smooth, pasty convex colonies on SDA (after aerobic incubation at 37æ%C for1-2 days), however, distinction between species is rarely possible[39]

Morphological criteria

Germ tube test is named as Reynolds-Braude Phenomenon after Reynolds and Braude who first reported it. This is a hallmark laboratory criteria for demonstration of C. albicans and C. dubliniensis by its capacity to produce short, slender, tube like structures called germ tubes after incubation in serum at 37°C for 2 hours. A positive germ tube test implies demonstration of at least five germ tubes in the preparation. The test may be established as negative after examining a minimum of 10 high power fields for the demonstration of germ tubes[45] Distinction of C. albicans and C. dubliniensis from other species can also be made on their capacity to produce morphological characteristics known as chlamydospores. Chlamydospores are retractile, round structures produced at the end of hyphae following culture of isolates on a nutritionally poor medium such as

Host Predisposing Factors	Oral Microenvironment-modifying Factors
endocrine alterations (diabetes mellitus, pregnancy, renal failure and hyperthyroidism) [11,12]	Ill fitting prosthesis [24-27]
Immune suppression (patients on chemotherapy and organ transplants, agammaglobulinemia or cellular immune defects) [13-18] acquired immunodeficiency syndrome (AIDS), hematological and immune disorders such as agranulocytosis (neutropenia).	loss of vertical dimension, prolonged antiseptic use, poor oral hygiene [26]
Other predisposing conditions- malignancies (lymphomas or leukemias), aplastic anemia, drug therapies (prolonged use of broad spectrum antibiotics, corticosteroids, antidepressants, antineoplastic drugs and immunosuppressants) [19,20,21] hyposialia (Sjögren's disease, drugs or radiotherapy), terminal/end-stage systemic diseases. [20,22,23]	smoking and alcoholism [28]

Table 2: Classification of Oral Candidiasis

1.Primary Oral candidosis (Group I) The "Primary triad"	2.Secondary Oral Candidiasis (Group II) condition
• Pseudomembranous (mainly acute)	• Familial chronic mucocutaneous candidosis
• Erythematous (acute/chronic)	• Diffuse chronic mucocutaneous candidosis
• Hyperplastic (mainly chronic)	1. Candidosis endocrinopathy syndrome
1. Plaque-like	2. Familial mucocutaneous candidosis
2. Nodular/speckled	3. Severe combined immunodeficiency
• Candida associated lesions	4. Di George syndrome
Denture stomatitis	5. Chronic granulomatous disease
Angular cheilitis	• Acquired immunodeficiency syndrome
Median rhomboid glossitis	
Linear gingival erythema	

cornmeal agar[31] Cornmeal Tween 80 highlights the lipolytic function of varied clinically important candidal species, and established that their temporal responses to Tween opacity is a useful method that complements the standard morphologic and physiologic tests employed for isolation of various species of candida[46]

Biochemical methods

Various biochemical techniques such as enzyme techniques, nutrient assimilation methods and mixed techniques (combination of enzymatic and nutrient assimilation tests) are employed for evaluation of other candidal species.

The enzyme techniques demonstrates the functioning of certain yeast enzymes through the specific hydrolysis of a chromogenic substrate in the presence of an enzyme indicator[47] Pagano-Levin agar or commercially available chromogenic agars, namely, CHROMagar Candida, Albicans ID, Fluroplate, or Candichrom albicans are frequently used[36] Pagano-Levin agar discriminates a variety of Candida species based on reduction of triphenyltetrazolium chloride. C. albicans forms pale-coloured colonies on this medium in contrast to other Candida species which produce pink colored colonies. Pagano-Levin agar has a comparable sensitivity to SDA but is superior for the demonstration of more than one species in the sample[48] CHROMagar Candida identifies C. albicans, C. tropicalis, and C. krusei based on colour and appearance of colonies[49] whilst Albicans ID and Fluroplate have proven valuable for the presumptive identification of C. Albicans[50] The reported specificity of identification for CHROMagar Candida and Albicans ID and Fluroplate agars is 95% for 98.6% respectively[51] CHROMagar Candida facilitates distinction between the newly described C. dubliniensis[6] and C. albicans. C. dubliniensis produce slightly darker green colonies on this agar as compared to colonies of C. albicans[52] However, after subsequent subculture and storage of isolates, the distinction between these two species using CHROMagar tend to decline. C. dubliniensis donot show growth at the incubation temperature of 45æ%C[53] Staib agar test is another test employed to delineate C. albicans and C. Dubliniensis. Grey-white shiny colonies with smooth entire edges are suggestive of C. albicans produces and C. Dubliniensis produces rough, grey-white colonies with a fringe or hyphal halo[54]

Nutrient assimilation tests assess fungal ability to use different sugars as exclusive carbon source. Media with all the elements necessary for growth (except a carbon source) are used. A certain sugar is subsequently added to the medium, and the growth of the fungus in the culture medium indicates the ability of the fungus to assimilate that sugar[28] Candidal species possess the ability to metabolize carbohydrates both aerobically (assimilation) and anaerobically (fermentation). Yeasts capable of fermenting a given carbohydrate may also assimilate it, but not necessarily vice versa. [55] The assimilation and fermentation of carbohydrates is the basis for biochemical identification of Candida species. Simpler and rapid auxanographic methods have replaced the conventional assimilation tests of Wickerham and Burton[56]

The most convenient and popular methods for candida species identification include a variety of commercially accessible strips or plates for carbohydrate assimilation and/or enzyme detection[57] API 20C AUX and API ID 32 tests are reasonably useful for demonstrating the commom germ tube negative candida species. Disadvantages with API 20C system are- set up requires time, provide results after 72 hours of incubation and interpretation of test results is difficult. [58] ID32C strip system evaluates the assimilation of 30 carbon sources and the growth of yeasts in the presence of cycloheximide. Because of widespread database and precision, ID32C have been used as a reference method. However, the interpretation of test results is difficult and requires experience[59]

Candifast identifies candida based on sugar fermentation reactions, urease production and resistance to actidione. It is simple to use and provides results after 24-48 hour incubation at 37^0 C. Interpretation of color changes within test wells is subjective. Sensitivity of candida to seven antifungal agents is incorporated into the kit.[60]

Immunological and genetic methods

Diagnosis of invasive candidiasis and

delineation between C. albicans and C. Dubliniensis may be made by various immunologic (ELISA) and genetic techniques (PCR).

Immunological methods

Cell-mediated immunity to C. albicans antigens can be established both by the appearance of delayed skin hypersensitivity to Candida antigens (ID reaction) and by in vitro tests of cellular immunity such as inhibition of leukocyte migration or stimulation of lymphocyte transformation to Candida antigens. The important candidal antigens used for the serological tests include- whole nonviable yeast cells, cell wall polysaccharides or glycoproteins, Candida culture filtrates, and cytoplasmic antigens from mechanically disrupted yeast cells. Diagnosis of candidal infections is essentially based on clinical assessment and by smear or culture, and serological tests care not of much diagnostic value[61] Serological methods donot provide a prompt diagnosis, the tests lack sensitivity and specificity, and produce a inconsistent antibody production in immunosuppresed individuals[62]

Molecular tests (genetic analysis)

Genotypic techniques have been widely used for the demonstration and typing of candida strains but have been used less often for species differentiation[63] For candidal molecular diagnosis, a specific DNA probe has been developed which facilitate rapid mycelial identification[64] Hybridization based detection method is a technique which uses a probe with sequence homology to the target DNA. The various hybridization based detection methods are Fluorescent in situ Hybridization (FISH), microtitre Hybridization Assay, Reverse Hybridization line probe assay and Hybridization on DNA chips[65]

Use of peptide nucleic acid (PNA) oligonucleotides to identify target sequences in chromosomal DNA using fluorescent microscopy and analysis software is a new technique which targets highly conserved species-species sequences in the abundant rRNA of living C. albicans[66] This technique achieves a sensitivity of 98.7-100%, and a 100% specificity allowing for accurate diagnosis of C. albicans which is phenotypically similar C. dubliniensis[67] For identification of yeast, multiplex PCRs are usually employed as they can directly identifiy yeast without the need for nucleic acid extraction.

CONCLUSION

Candida is a normal commensal inhabitant of the gastrointestinal tract and becomes opportunistic in immunocompromised states. The existence of candida species in yeast and hyphael forms poses diagnostic and therapeutic challenge to the oral physicians. Early diagnosis and manangement is essential to combat the dreaded complications of this multisystemic disesase.

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