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A REVIEW OF FUNGAL INFECTIONS OF ORAL CAVITY AND ITS EMERGING TRENDS

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ABSTRACT Oral commensal flora consists of a wide range of micro-organisms that include eubacteria, archaea, fungi, mycoplasmas and protozoa. From oral commensals, fungi are classified as eukaryotes. Fungal species that are present as commensal inhabitants in the oral cavity but can lead to a very serious infection with broadcast to various parts of body in patients with immune-suppressed state and therefore are referred to as opportunistic pathogenic fungi . Mucor and Cryptococcus too are etiological agents of significant number of oral infections. Clinical presentations of the fungal infections vary from pseudo-membranes, purulent swellings, erosive lesions, pustules to widespread destruction due to necrosis that may extend upto bone.(1,2)

Despite advances in treatment modalities, the frequency of deaths associated with invasive candidiasis remains high and is about one-third to one-half of affected patients. (3)

The candida species, adhere utilizing both specific and nonspecific mechanisms including dimorphism with direct tissue invasion & extra-cellular enzymes. (7)

Oral superficial candidiasis may occur in various clinical forms. Also, besides candida, the fungi that can cause deep-seated fungal infections in humans are : Aspergillus fumigatus, Cryptococcus neoformans, Histosplasma capsulatum, Blastomyces dermatitidis, Zygomycetes class, Coccidioides immitis, Paracoccidioides brasiliensis, Penicillium marneffei, Sporotrix schenckii and Geotrichum candidum. Detection of invasive fungal disease cannot be done from isolation and identification of fungal DNA alone. At present, treatment of candidiasis, of any type, relies only on a limited arsenal of antifungal agents. (2, 7)

KEYWORDS : Non candida albicans candida(NCAC), Proteinases, Haemolysins, Opportunistic pathogens.

INTRODUCTION

Daily, humans are exposed to enormous quantity of fungal spores, but, in a large majority, this exposure does not produce any damaing health effects, because of, the numerous defense mechanisms of the body, particularly in the respiratory tract, that effectively eliminate the fungal spores. (1)

Commensal flora of oral cavity includes a vast spectrum of organisms and includes various species of eubacteria, archaea, fungi, mycoplasmas and protozoa. Fungal oral commensals are eukaryotes with the genus Candida alone including upto about 200 species. Morphologically, yeasts are imperfect, unicellular, dimorphic fungi and they multiply by shedding off of their buds which are fungal cells which are on their surface and thus they form hyphal is structures. Among yeasts, the Candida species is, often recovered from oral cavity of about one-third of the normal individuals, as, they are normal inhabitant flora of oral and gastrointestinal tract. Sometimes, non- Candidal fungi such as Rhodotorula glutinis and Saccharomyces cerevisae are occasional commensal fungi of oral environment. (2, 6)

Candida, *Aspergillus*, *Rhizopus* and *Fusarium* fungal species are opportunistic pathogenic fungi and hence they are non-virulent in the oral cavity of healthy individuals, yet, can be a cause of deep-seated &/or widespread as well as fatal infections in patients with suppressed or altered immune status. Similarly, *Mucor* and *Cryptococcus* species too are etiological agents of significant number of oral infections, while *Geotrichum, Fusarium, Rhodotorula, Saccharomyces* and *Penicillium marneffei* are rarely pathogenic and so very infrequently cause infections of the oral cavity. Among all the fungal species that can cause infection, *Cryptococcus neoformans* is seen to cause both true as well as opportunistic infections that may occur in individuals of any immune status. All the above-mentioned fungi, tend to cause infections with variable clinical manifestations that may be of pseudo-membrane or abscess formation, denudation of surface mucosa causing ulcers, or at times pustule formation or widespread tissue necrosis foci that may even involve the bone.(1)

In humans, fungal infection is now emerging as a major public health situation. Infective fungi are likely to be responsible for causing all types of fungal infections. Infection is referred to as primary when it infects healthy population who are exposed to endemic fungi for the first time, whereas opportunistic infections are those that occur in immune-suppressed individuals.(11)

Opportunistic yeast infections may occur as superficial or systemic infections, and in either case they often tend to prove fatal. Also, in recent years, a surge in the etiological role of these pathogens in development of hospital-acquired infections has been identified as important in the disseminated form of these infections. This is attributed to the over-zealous healthcare methods, to an increase the life-span of even extremely ill patients from either premature neonates or elderly people with immune-compromised status and immuno-compromised patients due to other reasons. However, as there is extensive use of hospital equipment-based and prolonged medication based life sustainance which promotes the spread of opportunistic yeasts from their commensal colonization areas to non-commensal areas where they prove to be pathogenic.(3) Some researchers have found, a genetic predisposition to invasive fungal infection which is believed to be a consequence of alterations like: altered NADPH oxidase activity, alterations in production of tumor necrosis factor- α, interleukin 10 and other cytokines.(1) In spite of this being true, however, the depletion of CD4T is the primary host-associated cause whereby candidal infections are often the end-result as commonly seen in HIV-infected

individuals. Owing to, a depletion in CD4 T cells, such patients become predisposed to the occurrence of oro-pharyngeal candidosis or neutropenia, and, they both in turn, tend to be high risk characteristics for making the patients prone to development of systemic fungal infection. (5)

In invasive infections like Oral/esophageal, vulvovaginal, bloodstream and even systemic fungal infections of the abdomen they are the most commonly responsible agents for actuating the infection.(3)

The most virulent among pathogenic *Candida* spp. is *C. They are albicans*, which is also the commonest pathogenic species of oral mycotic infections. *C. albicans* can change its phase from blastospore to the hyphal phase and also has the ability to form germ tubes thereby, marking the onset of hyphal growth of the organism. Hyphal growth (mycelia phase) of *C. albicans* is important as it is the most probable causative factor of candidiasis. Another notable feature of Candidal lifecycle is its reproduction by multiple buddings from its lateral aspect, thereby, forming a mycelial structure, made up of elongated septae or filaments with numerous divisions, or it may even develop into spherical or ovoid yeast cells. (2, 7)

Despite advances in treatment modalities, the death rate due to invasive candidiasis is quite notably high, and one of the reasons for this is that the treatment for this disease is very often complicated by the increasing resistance to antifungals, and the other reason is the emergence of novel pathogenic candidal species which are the non-*albicans Candida* species.

The novel pathogenic non-*albicans Candida* species likely to cause oral infections which have been recently identified are *Candida dubliniensis*, *Candida glabrata*, *Pichia kudriavzevii* (syn. *Candida kruseis*, *Candida parapsilosis* and *Candida tropicalis*. Additionally, *Candida auris* is also now a globally emerging multidrug-resistant species. Thus, the non-albicans candida species that may be responsible for oral candidiasis in descending order of incidence are: *C. parapsilosis*, *C. tropicalis, C. krusei*, *C. glabrata*, *C. dublieniesis*, , *C. kefyr* and *C. guilliermondii*. Currently, more than two and a half dozen different *Candida* spp. have been identified as pathogenic for candidiasis. The incidence of infection with the non-albicans candida species has become more common over the last few decades. Furthermore, whenever, hybridization occurs among pathogenic and non-pathogenic lineages, it results in the evolution of a new virulent species. According to phylogeny, the term '*Candida*' is used for all imperfect fungal species most of which are diploid (no clearly defined sexual cycle) and may show parasexual activity. Thus, various *Candida* species are spread throughout the Saccharomycotina tree, but, owing to the common usage, the species name *Candida* is still being used in medical mycology. (1, 3, 10)

Since 1997 the SENTRY Antifungal Surveillance Program has been functioning to monitor the worldwide statistical data of invasive Candida infections along-with the data of the infection causing species and their resistance to antifungals and has recently published its documented data of the fungal infections of the past 20 years, wherein data of a large clinical sample size with samples through passive surveillance from 39 countries worldwide were analyzed.

According to their data also, the most prevalent species causing invasive candidiasis was undoubtedly *C. albicans*, however, what changed was the proportion of total infections attributable to C. albicans which according to the study had shown a decrease from an initial quantity of a little more than half of infections to just about 45% over the 20-year surveillance period.(4)

The most significant virulence factors of *C. albicans* include:

1. adhesins (e.g., Hwp1 and the Als family),
2. extracellular enzymes (e.g., the secreted aspartyl proteinase (Sap) family and phospholipases), and
3. the ability to change form from unicellular yeast to filamentous hyphal forms of growth and vice-versa as, both morphological forms are essential for virulence. Hyphae, according to researchers, plays a major role in adhesion, invasion, and biofilm formation while yeast cells are likely to be important for dissemination and initial colonization of host surface.

On analysis of *C. albicans* and *C. dubliniensis* to compare their phenotypes it was found that the *in vitro* isolates of *C. dubliniensis* had a higher proteinase activity and greater adherence ability to the buccal epithelial cells, along-with ability to experience phenotypic form change to either type at a higher rate than *C. albicans*. Additionally, *C. dubliniensis* is the only *Candida* species, other than *C. albicans* that is able to produce hyphae. (5)

Isolated species like C. inconspicua, C. lusitaniae, C. norvegensis and C. rugosa are a cause of infection in very less number of cases. These species are unyielding to the usually prescribed azole antifungal drugs. (1, 10)

Candidiasis due to NCAC species is of low virulence & severity because, these species lack, either completely or partially, some of the virulence factors that the virulent species of *C. albicans* usually manifest.(6) The virulence factors consistently seen in the virulent *C. albicans* but not in NCAC species are:
the ability to form hyphae and to experience phenotypic form change to either type.

low attachment capacity to buccal epithelial and vascular endothelial surfaces.

Secretion of lower quantity of proteinases.(1)

Non-candida Albicans Candida Species (NCAC) & Their Infections:

The NCAC species are a composite class of fungi inspite of which, they are different from each other and also from *C. albicans*, yet, they tend to give the same presenting clinical features of infections and so are usually indistinguishable on the basis of clinical manifestations. Notable is the fact that, several NCAC species are having an inherent resistance to commonly used antifungal drugs or sometimes they acquire resistance, or both. (6,8)

1. C. dubliniensis : *In the* oral microflora, *C. dubliniensis* is not a common constituent and is recovered from very few healthy individuals only, As a pathogenic species, *C. dublieniesis* is important because it is often found in oral lesions in HIV-infected individuals. Interestingly, both *C. albicans* & *C. dublieiesis* produce germ tubes and chlamydospores. Thus, it is structurally and by genetic makeup closely related to *C. albicans*. Other than *Candida albicans, C. dublieniesis* is also the only *Candida* species that forms true hyphae. Unlike *C. albicans* however, *C. dubliniensis* species show decreased susceptibility to fluconazole. Depite the close relationship between the two species and their extreme phenotypical similarity, yet, *C. albicans* has evolved and therefore is a better commensal and opportunistic pathogen. There are also genetic differences between the two species for the same reason. *C. dubliniensis* occurs in many diverse locations on earth land mass, including Europe, North and South America and Australia (1, 5, 6)

2. C. glabrata: It commonly inhabits the oral cavity of HIV infected individuals and often leads to the development of severe oropharyngeal candidiasis that additionally tends to

be resistant to flucanozole treatment. It is also identified as a major infective agent of both superficial and deep infections and also in cancer patients. In cases where it is a major pathogen, the disease is usually more fatal in its presentation and hence more demanding with a tendency to a rapid onset of resistance to fluconazole by this species. Even the newer azoles fail to have adequate effect owing to an ability of cross-resistance to them in *C. glabrata*. Resistance can be both innate and acquired. Because of tendency of resistance development, *C. glabrata* infections are averse to various treatments and are thus, can lead to fatal systemic infections with a greater mortality rate. However, as *C. glabrata* exhibits a lower oral keratinocyte adherence capacity compared to C. albicans, and hence, the probability of oral infection with this species is much lesser than that by albicans. (1,6)

3. C. guilliermondii : The candidal infections in patients that undergo surgical procedures, suffer endocarditis and are intravenous drug users or immunocompromised patients with fungaemia the cause often is *C. guilliermondii* . These species infections are resistant to amphotericin B.(1)

4. C. krusei : It does not cause candidemia commonly. However, in patients with severe neutropenia when they are critically ill they become prone to infection by *C. krusei*. Also,owing to the widespread use of fluconazole prophylaxis & itraconazole in HIV patients, it has lead to their increased susceptiblility to candidiasis due to *C. krusei* infection . Resistance to amphotericin B has also occurred in some cases. (1,6)

5. C. lusitaniae : This species primarily infect those patients who are having a compromised immunity for variable reasons like the prolonged administration of antibiotics with or without hospitalization or administration of cytotoxic drugs or corticosteroids. It has also been reported in patients with granulocytopenia and in low-birthweight babies.

6. C. parapsilosis : The patients at high risk with the infection by *C. parapsilosis* are usually critical and prematurely born neonates and infants who are in intensive care units due to low birth weight. Poor infection control practices coupled with its propensity to harbor the medical devices like intravascular catheters and prosthetic devices, have lead to a rise of *C. parapsilosis* infections. *C. parapsilosis* is a commonally present on the skin of healthcare workers and hence, it is easily transmitted within healthcare settings with another concern of its often being resistant to antifungals. The fungemia or endocarditis in intravenous drug users is also believed to be caused by *C. paralopsilosis* and it may also cause infections of hard body tissues. Hematogenous *C. parapsilosis* infections have also increased. *C. palapsilosis* isolates from blood culture manufacture an extracellular polysaccharide, that increases its adherence and biofilm formation ability on plastic surfaces. (1, 3, 6)

7. C. tropicalis : It is an extremely pathogenic non-*albicans Candida (NCAC)* species. It has high virulence, owing to its affinity to attach to epithelial cells as seen *in vitro* and also because it secretes a moderate level of proteinases that assist its adherence to epithelial cells. It is a commensal of oral mucosa and skin. It leads to oesophageal fungal disease in individuals who have a systemic disease or diseases.(1)

Candidal Biofilms:

In the oral cavity, structured microbial communities, particularly of candida, are seen as candida encompassed in a network of extracellular polymers and thereby forming biofilms particularly, when they get tightly attached to a surface like those of acrylic dentures and dental implants. For the Candida species, biofilm formation is an important virulence attribute owing to an increase in its tolerance to the defence mechanisms of host and also due to a notable increase in its persistance against an antifungal therapy. Also, Candidal biofilm helps to establish a reservoir for continuing infections.

As a result of the biofilm formation, the strains that form a biofilm persist longer and are more pathogenic leading to more number of patients with the disease that also may sometimes be fatal. The generation of a mature biofilm with subsequent production of extracellular matrix imparts a proportionate pathogenecity and is variable in presence of each candidal species, strain, and their environmental conditions.(8)

Studies on Candida biofilms have highlighted that biofilm formation gets initiated by the mutual adherence among cells. Once the biofilm is formed there is formation of germ tube and extracellular matrix by the candidal species. Yeast cells form hyphae and pseudohyphae in the third phase. This leads to formation of a mature biofilm often several microns deep and it has a three dimensional structure. Structurally, the biofilm is jelly-like and retains large quantity of water and so the micro-organisms in it are almost immobilized. The 3-D structure of biofilm of *C. dubliniensis* and *C. albicans* is similar whereas that of *C. parapsilosis* biofilm is different from the biofilm of *C. albicans*. *C. parapsilosis* biofilm has patches of mushroom-shaped biofilm communities whereas *C. albicans* biofilm has a biphasic arrangement of discrete layers. As mentioned earlier, biofilms reduce the susceptibility of candidal organisms to all the targeting agents whether it is immune cells or antifungal drugs. This is proven by the fact that, all of the commonly used antifungal agents are reported to have a marginal effect only on the biofilms of candida species.(6)

The factors that contribute to an emergence of fungal infections include the adaptations by the fungi of demographic changes, microbial environment adaptation, appearance of inherently resistant fungal species as a human pathogen as well as excessive hospital equipment usage, travel and commerce. (11)

Oral Candidiasis:

The yeasts of normal oral flora which also includes Candida in a large majority of individuals may also cause Oral candidiasis. However, as part of the normal oral flora candida is kept under control by means of various immune components of oral environment along-with the encounters that occur with the organisms of the normal flora and give them a low profile. Therefore, oral yeast carriage does not mean infection. (7)

Candidiasis occurrence as an opportunistic infection was first highlighted by Hippocrates, who referred to it as "a disease of the diseased". (9)

The most important criteria of clinical symptoms of mucosa candida infection is mucositis. Elderly people particularly those using a dental prosthesis for multiple teeth and the AIDS patients are most often susceptible to oral candidiasis.

The common causative agents of clinically identical candidal mucositis tend to be *C. albicans, C. tropicalis, C. krusei or C. dubliniensis*. The difference seen for different candidal species caused mucositis is in the invasiveness of infection and its susceptibility to antifungal agents. (7)

Predisposing Factors:

1. Changes in physical properties of saliva and a decrease in its quantity, smoking, persistent mucositis of any cause, decrease in flow of blood to the oral tissues as a consequence of irradiation of the area, are all local factors that predispose to the occurrence of candidiasis.
2. Drugs with side effect of xerostomia(dry mouth) often lead to

Oral Candidiasis. The drug groups under this spectrum include drugs like antiadrenergics, antidepressants, anticholinergics, antipsychotics and antihypertensives. Their effect is indirect as decrease of quantity of saliva leads to a lack of its cleansing action and also a reduction in the quantity of impotant salivary enzymes such as lactoferrin, lysozyme, histatins and also immunoglobulins thereby promoting the extravagant colonization by *Candida* in individuals taking drugs that may lead to xerostomia.(2)

3.In one-third to one-half of oral lichen planus (OLP) cases Candidal infection has been reported to occur. The corticosteroids that are used for the treatment of OLP further promote inhibition of normal oral microbial flora and also lead to a consequent fungal infection,which may complicate the treatment of OLP. (7)

4.Many common hormonal diseases and autoimmune diseases of salivary glands besides oral cancer and malnutrition, particularly, Protein-energy malnutrition, Vitamin C and possibly Vitamin A deficiency, are all implied as pathogenic agents or conditions predisposing to Oral Candidiasis. Interestingly, a rich sugar diet may also lead to an increase in the incidence of Candidiasis of oral cavity. (2)

5. All these predisposing factors mainly reduce salivary flow leading to a decrease in its molecules and therefore also cause a decreased level of immunoglobulins in the saliva which primarily guard against fungal infections when present. The decrease in salivary immunoglobulins indicates a lower than normal competence of the antibody-mediated defence mechanisms with a reduced effectiveness also and so, in such cases, management of *Candidal* infection becomes difficult. Therefore, oral candidiasis is commonly present in Hereditary myeloperoxidase deficiency, Chediak-Higashi syndrome, DiGeorge syndrome and human immunodeficiency virus (HIV) infection which are the immunological disorders with compromised humoral immunity.(2)

6.The fungal infection reported to occur when HIV-infected individuals are considered, is Oral Candidiasis. Oral candida infection is seen in at some point in the course of HIV infection in a large majority of them. Therefore, oral candidiasis is taken as a milestone clinical symptom serving as an indicator of the underlying deficiencies of cell-mediated immune surveillance and hence can be used as a prognostic indicator for the HIV infected people as, C. albicans is retrieved from almost all of the AIDS patients in significantly higher quantities than among the healthy individuals. Other frequent species *are C. dubliniensis ,C.glabrata & C.krusei*. (7)

7.In patients with advanced cancer there is altered host defense often with mucositis and additionally a prolonged neutropenia.

All these, lead to an increased oral yeast carriage and may cause oral candidiasis. Colonization of more than 90% individuals and infection of up to 29% have been reported among patients under radiation therapy for head and neck cancer. The incidence rate of oral candidiasis in the patients with advanced cancer can therefore, range from few to near 100%. (2,7)

8.Those people suffering from chronic diseases, medication, poor oral hygiene and reduced salivary flow together with the consumption of various types of drugs including antimicrobial agents cause selective constraint on the commensal flora and may facilitate yeast hyper-colonisation thus such people are a risk group to yeast infections. The acrylic material of dentures also acts as a reservoir of many disease causing microbes and therefore, also promotes the inhabitation by pathogenic strains of Candida in the denture-bearing areas. (7)

In the current scenario, wherein best treatment modalities are accessible to many individuals, even the immunocompetent patients who have been subject to abdominal surgeries many a times are at a higher risk of non-albicans candidal infections. Also, those patients who were subject to a prolonged use of broad-spectrum antibiotics, or, have blood sugar imbalances, cancer, severe renal function problems are at a higher risk of non-albicans candidal infections.(10)

Pathogenesis Of Oral Candidiasis:

Candida organisms can **adhere** to any surface, living or non-living and that proves to be a pioneering quality in its pathogenecity as a result of which the oral yeast infections occur. The candida species, adhere utilizing both specific and nonspecific mechanisms. Against their adherence abilities, in the oral cavity, the key role players in preventing yeast adherence & colonization are, the local defence mechanisms of both, the epithelium that by its presence creates an obstacle to invasion and of saliva with dual contribution by its physical properties and by its many immunity boosting molecules.

There are certain enzymes elicited by candida organisms in their immediate mileu which augment the ability of candida to adhere and/or to penetrate into the tissue.The enzymes are broadly grouped as: Secreted aspartic proteinase (SAP), secreted phospholipases and lipases. SAPs facilitate adherence of candida by actively degrading extracellular matrix and host surface proteins (laminin, fibronectin, and mucin) and thus allowing candida to invade the host tissue ultimately leading to nutrient acquisition. Owing to SAPs C. albicans are able to evade host defenses as they are able to degrade the antibodies and antimicrobial peptides generated by the host tissue in order to compromise the invading organisms. Candidal hyphae are able to damage the epithelial tissue by secretion of a cytolytic peptide toxin called candidalysin, encoded by the hyphal-specific gene ECE1. Besides encoding for candidalysin toxin, the *C. albicans, C. dubliniensis, C. tropicalis and C. parapsilosis* are known to also possess SAP genes. (6, 9)

Other enzymes that promote candida pathogenecity substantially by similar mechanisms as explained so far, are the extracellular hydrolases. Production of extracellular hydrolases varies with the species type, the source of infection &/or site of infection. The most prominent damage is done to the cell membranes of host tissue cells by phospholipases and they bring that about in two ways. Firstly, by hydrolyzing the cell membrane phospholipids into fatty acids and then by exposing the cell surface receptors of host cell in order to encourage adherence. As a consequence, Candida biofilms usually have a significant extent of phospholipase activity. Thus, if the biofilms of Candida species are tested for their extent of phospholipase activity it would probably be an important criteria of differentiating invasive strains of candida from noninvasive colonisers. (8)

Another pathogenecity enhancing factor of candida species is dimorphism, which, not only exaggerates the clinical problems of diagnosis of their infection but also the treatment of fungal infections. Candida species can cause infection by inducing a hypersensitivity reaction in the affected tissues or as a result of effect of potent Candida toxins. The toxins, in turn lead to development of oral candidiasis. Disturbances or imbalance of the micro- environment of host tissue causes a hypersensitivity state of the tissue and that in turn promotes an overgrowth of Candida organisms thus allowing establishment of oral candidiasis. (2, 7)

Further disruption &degradation of important structural and immunological defense proteins of host cells is facilitated by Candida proteinases, as a consequence of which, Candida invasion and colonization of host tissue can occur.

Investigators have therefore found high proteinase activity in candida causing oro-pharyngeal candidiasis.(8)

Haemolysins act by targeting iron contained in the haemoglobin. This enzyme functions by activating the complement pathway owing to which there is opsonization of surface of red blood cells. Erythrocytes get disintegrated and that in turn facilitates hyphal invasion through bloodstream in systemic candidiasis. Therefore, haemolysins are responsible for enhancing the infecting capacity of the fungi of Candida species as they enable the organism to endure and last with perseverence in the host. (8)

According to Deorukhkar et al. the production of enzyme coagulase is highest in Candida albicans The cascade of reactions leading to clotting of plasma are consequence of activation of cascade when enzyme coagulase binds to plasma fibrinogen. (8)

Clinical Presentation:

The most common area of yeast colonization in the oral cavity is the tongue dorsum, with the palate and buccal mucosa being the other favored colonization areas. Thus, it is imperative that oral candidiasis also, when it occurs would show a similar predilection of affecting tongue dorsum in the oral mucosa. Associated symptoms of candidal infection are very much dependent on the effect of infection on the oral mucosa and, so, these infections are either totally symptomless or they present with severely painful erosions or lesions associated with burning sensation and in either cases interfering with the ability to eat and swallow. (7)

The symptoms associated with oral Candida infection can range from none to a painful burning sensation that may interfere with the ability to swallow (dysphagia) and ability to take in nutrition

Oral superficial candidiasis may occur in various clinical forms like:

erythematous candidiasis, pseudomembranous candidiasis, median rhomboid glossitis, angular cheilitis and candidal leukoplakia. Noteworthy, is the fact that these clinical variants of oral candidiasis can occur simultaneously in numerous foci of mucosa of mouth and also at one time in an individual patient. The oral manifestations of candidiasis due to different candidal species are identical, irrespective of the causative species.(7,9)

More recently a revised classification system that stratifies Candida lesions into 2 subgroups, group I (localized) and group II (secondary), has been proposed.

According to it, the Group I lesions which appear in foci only in the mucosa of the mouth could occur in any of the three clinical forms from pseudomembranous, erythematous, and hyperplastic candidiasis and may have an acute or chronic course. When oral candidiasis occurs as a secondary infection in an existing primary lesion like Angular cheilitis, central papillary atrophy, denture-associated stomatitis, and keratinized lesions are other disorders than also those primary lesions are referred to as group I Candida-associated lesions.

Group II lesions also known as secondary lesions show a persistent association with very uncommon genetically transmitted abnormalities of the surveillance sytem of the body and hence, may manifest in the form of infection at any site of the body. (7)

A more simplistic classification which is used commonly for oral manifestations of candidiasis classifies it into three broad categories according to the course & site involvement of the disease ie. (1) acute candidiasis, (2) chronic candidiasis, and (3) chronic mucocutaneous candidiasis syndromes.(9)

From the candidaemias that occur in the general patient population, NCAC species cause 35-65% of the infections. They commonly affect the patients who have had bone marrow transplants or may be seen in patients with hematological malignancies. Other less commonly affected patients include those in intensive care or post- surgical care (35-55%).Children or HIV-positive patients are only infected in 35% of cases . The proportion of NCAC species that tends to cause infections is on the rise. Along with it, the mortality due to NCAC species infections is similar to C. albicans infections, ranging from 15% to 35%. (10)

Acute Pseudomembranous Candidiasis:

Clinical presentation: Acute pseudomembranous candidiasis is often referred to as "thrush" and its usual presenting feature is a multifocal, curdy, yellow-white plaque anywhere in the oral mucosa. As and when the plaques are scraped off by gentle rubbing, the scraping serves as a diagnostic feature, as plaques are usually made up of the flakes on mucosal surface and inflammatory cells in addition to, both candidal forms and often leave behind an underlying red erosive base. These lesions may be either discrete or confluent.

This form of candidal infection is common in neonates who have some or the other form of immunodeficiency coupled with treatment by inhaled steroids which further increase their proneness to this form of candidiasis. The most common subset of primary disease sufferers likely to develop this acute infection with pseudo-membrane formation are patients with HIV infection and malignancy, and individuals who are subject to therapies that nullify their immune response. In the AIDS patients, persistent and repetitive infection is common, and may eventually result in candida infection of the esophagus thereby further complicating the infection owing to an accompanying malnourishment and difficulty in consuming anything across infected oral mucosa.

Diagnosis:

The presence of distinctive clinical features of Pseudo-membranous candidiasis is essentially diagnostic. Alternatively, the surface flakes can be removed and sent for microscale analysis to identify Candida organisms or they can be sent for fungal cell culture by various methods to identify the Candida species, if present. (9)

Acute Erythematous Candidiasis :

Acute erythematous candidiasis is also referred to as "antibiotic sore mouth" as it usually occurs following long-term use of broad-spectrum antibiotics which leads to an alteration in both the quantity & quality of the commensal bacteria in oral microflora and thus, also facilitates overgrowth of Candida. The normal homeostatic balance of the microbial community gets gradually restored once the antibiotic consumption is stopped and the re-established oral microflora in turn helps to resolve the candidal infection often without the need for therapeutic intervention. Usually, the symptomatic variant of oral candidiasis occurs as reddened lesions preferentially affecting the palate and tongue dorsum but may be seen throughout the oral cavity. Lesions of this form of candidiasis, can either arise after the pseudomembrane from lesions of acute pseudomembranous candidiasis has shed off or may arise as an erythematous form only. In either case, it may run an acute or chronic course. (9)

Chronic Erythematous Atrophic Candidiasis:

Chronic erythematous atrophic candidiasis usually is a consequence of mildening of acute form and hence has a similar presentation. In HIV+ individuals it is commonly encountered. The most frequent presentation, however, is as Candida-associated denture stomatitis (DS), with a very common feature of an erosive change of the mucosa of palate covered by denture. This form of candidiasis is seen in more

than three-fourth of the denture wearers and, it may be often symptomless. It is so common due to the poor denture associated habits &/or prolonged use of a denture for many years, as, because of an associated decrease in quantity & flow of saliva, in the area covered by the prosthesis that provides a perfect place for the fungal habitat. The non-biotic acrylic denture material gives an environment which is more caustic than other parts of oral mucosa and also facilitates a non-aerobic condition below the denture by its porous nature. In addition, this type of place also serves as an undisturbed accumulation that facilitates non-stop nucleation focus of the Candida organisms upon the mucosa of palate. The excessive candida colonization elicits hypersensitivity by a strong reaction by the native immune cells of body that elicit both an erythema in that area and also hyper-proliferation of the tissues. Ill-fitting dentures also cause frictional irritation that damages the mucosal barrier and that paves way for facilitating ingress of Candida organisms into the mucosa there by leading to development of an infection. It is considered a true Candida colony-associated infection. Therefore, for clinical management of this form of candidiasis it is required and essential to remove the candidal colony present on the acrylic of prostheses to avoid a relapse of formation of the organism habitat and hereby recurrence of infection. (9)

Angular Cheilitis:

When the corners of the mouth present a commonly bilateral occurence of erythema, maceration, fissuring, crusting, or a combination of any of them, it is referred to as angular cheilitis. Commonly denture stomatitis or some other initial presentation of oral candidiasis in which increased quantity of the fungal organisms are present on the mucosa of mouth permitting a straight-forward dissipation and self-implantation with self-infection of the corners of the oral cavity and leading therefore to angular cheilitis. These lesions often show a mixed infection of bacteria & fungi and, therefore, the precise participation of Candida in the consequent pathology is hard to confirm. The lessened upright jaw closure measurements in the senior citizens who are toothless also makes them more susceptibile to angular cheilitis. (9)

Cheilocandidiasis:

A chronic candidiasis with crusting and ulcerations of the lips is referred to as Cheilocandidiasis and it often develops secondary to vigorous and repeated use of petrolatum-based products on lips or due to persistent wetting of lips or due to thumb-sucking habit. In all these cases, the presence of wetness, allows Candida species to thrive in the moist environment. (9)

Chronic Hyperplastic Candidiasis:

Chronic hyperplastic candidiasis commonly affects the buccal mucosa in proximity to the commissures of mouth and the lateral aspect of tongue. It may also affect buccal mucosa near angle of mouth, palatal mucosa or mucosa of tongue dorsum in that order. The presenting feature is a well-demarcated leukoplakia or raised fissured white plaque which is non-scrapable and therefore it is also referred to as Candidal leukoplakia. It is a rare form of oral candidiasis that is commonly seen in middle-aged male smokers. Most notable fact about this form of candida infection is that it has a higher rate of malignant transformation and hence can lead to oral squamous cell carcinoma. (9)

Median Rhomboid Glossitis :

Median rhomboid glossitis manifests as a midline area of an ovoid or rhomboid outline with depapillation and subsequent redness of the midline area usually of dorsal surface of tongue immediately in front of the circumvallate papillae. It is believed to develop in people with a habit of repeated use of steroid inhalers and/or smoked tobacco and so, men are affected more frequently than women. (9)

Chronic Mucocutaneous Candidiasis Syndromes:

Chronic mucocutaneous candidiasis has signs & symptoms of candida infection in various organs/sytems besides oral cavity. Clinically, it presents with persistent or recurrent mucocutaneous candidiasis that usually shows involvement of oral mucosa in almost all of the affected patients while other mucosae, skin and nails are not involved very often.

These are rare heterogeneous immunologic disorders but the manifestations are due to a common pathogenic immune deficiency. The harshness of the clinical manifestations thus, correlates with the intensity of the elicited host defense deficiencies. Chronic mucocutaneous candidiasis is seen as a manifestation of a large number of syndromes. They may occur as a sporadic disease or as a secondary disease to some primary immune disorder or disease or it may be seen as an autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED). A major problem of this candidiasis is that, it tends to be refractory to standard antifungal therapies or to long-term anti-fungal therapies and so, they have an increased susceptibility to carcinomatous changes of the affected mucosa. (9)

Laboratory Diagnosis of Candida species:

Superficial Candida infections usually are diagnosed based on their clinical features and accompanying symptoms of local oral burning, if present. However, diagnostic tests that may be used in the detection of oral candidiasis include identification of candida organisms with PAS stained exfoliative cytology smears, imprint culture, swab culture, salivary assays, and mucosal biopsy. (12)

Therefore, whenever the tests are done they are broadly categorized into direct microscopic examination, macroscopic examination, morphological criteria, biochemical methods, immunological & genetic methods. (13) Exfoliative cytology aids differentiation between the two forms of Candida, but with a drawback that it is less sensitive than culture methods. The procedure requires cytology sample on the microscopic slide to be stained with potassium hydroxide (KOH), Gram stain, or periodic acid-Schiff (PAS) stain before examination of the cytology. Observation of candida hyphae is as clear tubes with KOH staining and thus elicting the fungi in a more easily appreciable manner. With Gram staining, hyphae and yeast stain dark blue when stained with Gram's stain in contrast to, reddish purple when stained with PAS stain.

Formalin fixed mucosal biopsy should be stained with PAS stain after processing and section cutting to ascertain Candida infection. The suspected diagnosis and history of patient should be given to the concerned pathologist for special staining of fungi to be done.

Most fungi are readily demonstrable with the common special stains such as Gomori's methenamine silver (GMS), Gridley's fungus (GF), and periodic acid-Schiff (PAS), also referred to as "broad spectrum" fungal stains. Gomori's methenamine silver and PAS are the stains usually employed to identify fungi both in tissue slide and in cytology specimen, but, fungal morphology is better demonstrated by the PAS stain. Even when the routine H & E stain cannot detect candida the PAS stain can stain them positive and thus make them identifiable. However, the detection of candida by methods like swab cytology or culture of organisms along-with tissue histology analysis simply ascertain that, Candida species organisms are present in the oral milieu and they do not prove the pathogenic role of these organisms. (12, 7)

Imprint culture technique requires the use of a sterile foam

pad treated with growth media at the focal area of infection. The pad is then subjected to a special agar medium for culture. A mop collection of the lesion can also be transposed onto growth media specific for fungal organisms and the growth culture can be studied and analyzed. For wash techniques, a sterile phosphate-buffered saline rinse solution is given to the patient to rinse and the expectorated rinse is centrifuged, supernatant discarded and the sample is streaked on a culture media growth plate for fungi. Both these methods can be used to quantify the Candida, and thus can help to differentiate between normal and increased levels of the candida organisms, thereby indicating the likelihood of their pathogenic role in oral infection of the patient.

On microscopic examination of the specimen itself, the morphological features most often noted include: budding yeast cells (eg, blastoconidia), pseudohyphae, and hyphal structures. Of these features, budding yeast cells and pseudohyphae are the hallmark features of most of the Candida species organisms and also occur in a few other genera, such as Trichosporum and Geotrichum. Yeast cells that show budding but do not show pseudohyphae formation are typical of C glabrata, Cryptococcus species, and Histoplasma capsulatum. (7)

When fungal culture which grows and matures rapidly is observed in which the growth of organisms appears to be of various shades from cream to yellow, it is indicative of candida species culture. The texture of the colony varies and may be either pasty, smooth, dry, wrinkled or dull, depending on the species. Candida species is usually seen as, a single cell yeast, but, it can also occur as a multicellular mold. It is possible to observe specific features of yeasts by observing their morphology. Specimen from exudates, sputum, urine and cerebrospinal fluid should be examined under reduced-light bright field microscope or by phase-contrast microscope to identify the fungal organisms.

Many organisms of Candida species are detectable by observing the changes of the indicator color subsequent to utilization of 1% carbohydrates, such as glucose, maltose, sucrose, trehalose and raffinose by the yeast colonies of culture. These tests are now available as commercial kits such as API 20C or API 32C. Other than carbohydrates, hydrolysis of 1% fatty acid ester, 0.05% aryl-substituted glycosides, 0.3% urea and 0.01% acrylamide substrates can be detected with RapID Yeast Plus system. Currently, this is the fastest commercial method for the identification of yeasts which requires a 4 h incubation period only. API 32C is also useful in differentiating C. albicans from C. dubliniensis strains as this two species are phenotypically alike and require differentiation. (10)

Thus an apt protocol implementation in the oral pathology laboratory is a pre-requisite for a definitive diagnosis.(7)

Among the virulent fungi, virulence and antifungal resistance varies between species, and even between strains of the same species, therefore, from a diagnostics perspective, providing resolution at the species level (or even beyond) is important to help in therapy decisions of the infection for appropriate antifungal therapy, especially in patients suffering from life-threatening candidiasis. As mentioned already, at present, availability of advanced techniues to identify fungi facilitate the recognition of genetic and phenotypic diversity among fungal pathogens and thus ensure a better treatment along-with the avoidance of the deteriorating effects of the fungal infections.

Deep Seated Oral Fungal Infections:

Deep-seated fungal infections of oral & other tissues are usually caused by *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, Zygomycetes class, Coccidioides immitis, Paracoccidioides brasiliensis, Penicillium marneffei, Sporotrix schenckii or Geotrichum candidum. Although infection due to some of these fungi are rare yet their incidence has markedly risen in the past few years due to an increase in the prevalence of diseases that cause lack of immunocompetent state in patients, as in HIV infection. In the oral cavity they tend to cause occasional & isolated lesions but usually these oral lesions are inadvertently detected simultaneously with fungal infections of the respiratory tract or of multiple organ systems. Infections like aspergillosis, cryptococcosis, histoplasmosis, geotrichosis, blastomycosis, and mucormycosis are usually seen in the oral mucosa and their diagnosis needs to be confirmed through biopsy. (2, 7)

Aspergillosis:

Aspergillosis is the second most common fungal infection and the specific reasons that predispose to aspergillosis infection include enhanced consumption of high-dose corticosteroids, a global spike in solid organ and bone marrow transplantation, and an augmented implementation of immunosuppressive regimens for autoimmune diseases to ameliorate the host response system. It tends to present as an acute invasive pulmonary aspergillosis, particularly in the immune-compromised host. The aspergillosis spore is commonly present in decaying vegetation, air and environmental surfaces of hospital settings, or in areas undergoing structural renovation. Therefore, hospitalized immune-suppressed individuals are at an increased risk for acquiring the disease. Aspergillosis organisms have affinity for blood vessels and cause thrombosis and infarction of the peri-vascular tissues of blood vessels. Less commonly, they invade the maxillary sinuses as they are in the inhaled air and in such cases they cause oral & palatal lesions subsequent to invasion of the underlying soft tissue and bone. The oral lesions are characteristically black or yellow necrotic lesions of the soft tissue. (7)

Cryptococcosis-

Similar to aspergillosis, the non- immunocompetent individuals are prone to this infection. Initially it affects the lungs and then progresses to cause meningitis. When cutaneous lesions occur, the face, neck', and scalp are the common sites. Oral lesions do not occur frequently and they appear as superficial ulcerations, nodules, granulomas or may also mimic carcinoma-like presentation when they occur. The pathogen, Cryptococcus neoformans, is often found in pigeon's and other birds' droppings. (7)

Histoplasmosis:

Infection with H capsulatum leads to a rapid or prolonged disease of the skin and mucosa or of the organs involved in breathing of gases. The mucocutaneous form involves both the skin and mucosa even of the oral cavity where it occurs as ulcerative or erosive lesions on the tongue, palate, and buccal mucosa. According to Samaranayake, more than half of the patients with the dispersed form of histoplasmosis are likely to have oral lesions. In this disease, oral lesions are usually noted as the presenting sign of infection or alongwith pulmonary infection. The disease usually manifests as a long duration ulcer of the oral mucosa, which may or may not be symptomatic. The lesions are very likely to be misdiagnosed as a malignant neoplasm, owing to its clinical presentation as an ulcer with firm and rolled borders. The sites most commonly affected are the tongue, palate, and buccal mucosa. (7)

Geotrichosis:

This organism is a commensal of cutaneous, oral and gastrointestinal tract tissues. Geotrichum candidum is responsible for geotrichosis, which may have oral, bronchial,

intestinal, or pulmonary manifestations due to an inoculum from endogenous or exogenous source. Geotrichosis is a sporadic, infectious, non-contagious and opportunistic mycotic disease. It commonly occurs as a super-infection or as a co-infection in the immune-comromised individuals. Transmission of the disease occurs due to inhalation of fungal spores from the saprobic environment. Geotrichosis infections on the mucosa may show presence of a pseudomembrane and therefore may produce a chronic inflammatory reaction in the mucosa in response to the surface pseudohyphae similar to that seen in pseudomembranous candidiasis, and thereby, it may be presumed to be candidiasis. Other clinical presentations of oral geotrichosis may be as a hyperplastic or an ulcerative form. Geotrichosis should therefore be included in the differential of ulcerative inflammatory mucosal lesionsIt tends to affect the immunocompromised individuals in a disseminated form. Geotrichosis is a disease of immunocompromised individuals. It has a poor prognosis with high mortality rate of more than 50%. (7, 14)

Blastomycosis:

It is a rare infection. However, subsequent to the onset of infection from a point source, it tends to be an epidemic or usually causes sporadic endemic infection. It may occur in immune-competent as well as immune-compromised people with more likelihood of dissemination in the later. Oral manifestations are seen in the disseminated form of disease and causes ulcerative mucosal lesions. It is endemic to certain parts of North America (7).

Mucormycosis:

Most individuals are exposed to mucorcae on a routine basis but infection is rare. Although rare, yet, it is the second most common invasive mould infection that presents as a rapidly spreading opportunistic infection caused by a saprophytic fungus commonly found in soil, bread mold, decaying vegetation, and animal manure. Infection of the paranasal sinuses or nasal cavity when unresolved leads to secondary involvement of the oral cavity and usually presents as palatal necrosis or ulceration. Early diagnosis and improved treatment has now decreased the morbidity and mortality due to this disease which was high so far. Greater mortality in this disease is attributable to individual host-based risk factors and a unique immune-pathogenesis of the disease with extensive angio-invasion, increased virulence and use of chelators by the fungus as siderophores coupled with a delay in diagnosis. The disease culminates in extensive tissue necrosis owing to its typical behavior of extending into adjacent structures and may extend into the brain also. It may occur in both the immune-competent and immune-suppressed individuals but with a greater fatality in the immune-compromised patients. (7)

Laboratory Diagnosis Of Deep-seated Fungal Infections:

Biopsy is the standard procedure used to determine oral deep-seated fungal infections. However, invasive fungal disease, particularly invasive yeast infection, is often first detected by a positive blood culture. Sensitive assays for detection of fungemia are a major development that has helped rapid diagnosis of invasive mycoses. Among the newer methods, fungal DNA and/or antigen detection has given a great hope to the early and rapid detection of invasive fungal disease. However, currently only culture methods are used for diagnostic purpose and Most other methods are currently investigational and include polymerase chain reaction, galactomannan antigenemia, Western blot to detect antibodies, and detection of fungal metabolites (eg, D-arabinitol and 1,3-b-D-glucan). (7)

New Approaches In Antifungal Treatments:

The antifungal agents which are mainly categorized into polyenes, azoles, and echinocandins are commonly relied upon for the treatment of any form of candidiasis. Thus, there is a narrow spectrum of the antifungal pharmacological groups available and this paucity is enhanced further by the shortcomings of the available therapeutic agents making it difficult to overpower the causative organisms of the fungal infections. The shortcomings of antifungal agents with the most marked impact are their suboptimal selectivity, their very high toxicity, and a significant amount of probability to develop resistance. The inherent high toxicity to all eukaryotic cells, fungal or mammalian and lack of selectivity makes Amphotericin B, less effective even though it is referred to as the "gold standard" of antifungal therapy. On the other hand, Azoles, are not highly toxic to human cells but their therapeutic effect only leads to stasis of the targeted agents of fungal infection and thus often causes drug resistance. Echinocandins can only be used for systemic candidiasis and, they also have shown emergence of resistance.

A strategy that can be developed toward accomplishing the goal of an effective, rapid and non-toxic anti-fungal agent is possible by targeting specific virulence factors. For eg.: Preventing filamentation and biofilm formation of C.albicans and thereby preventing its becoming an opportunistic pathogen.(9)

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