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Oral Fungal Infections - An Insight Into Its Microbiological Laboratory Diagnosis

Medical Science

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ABSTRACT

Oral cavity, the gateway to the body is occupied by lot of microbes which act as commensals and maintain an oral biome. Recent advances in medical & dental science, with the help of microbiological tools have helped us to identify a lot of these microbes and also to distinguish between commensals and pathogens. This has helped the oral health care professionals (OHCPs) all over the world in better diagnosis, management and higher patient satisfaction rate. The diagnosis of fungal disease is a multidisciplinary approach requiring cooperation and collaboration of many people with diverse expertise. Due to its difficulty in sample collection, poor sensitivity and prolonged time required for conventional procedure, attention has been drawn to the non culture based diagnostic techniques. The main goal of a clinical mycology laboratory is to isolate efficiently and to identify accurately the suspected etiological agent of fungal infection. OHCPs play a crucial role in the diagnosis and management of oral fungal diseases. Therefore, an adequate knowledge is pivotal in recognizing and diagnosing the various kinds of oral fungal infections for better management of patients.

Introduction

An oral health care professional (OHCP) comes across variety of challenges in managing a patient with oral infections. Oral cavity homes commensal microbes that aid in maintaining an oral biome. It is also susceptible to different kinds of opportunistic & nosocomial infections, which pose great challenge. Establishing a proper diagnosis is the key in its efficient management.

Oral fungal infections are relatively rare and unfortunately, there are very few articles that give emphasis on laboratory diagnosis pertaining to fungal infections of the oral cavity. The oral mycosis has emerged as a fatal and life threatening event in patients suffering from HIV and Tuberculosis (TB), where it is often severe, rapidly progressive, and difficult to diagnose or treat.[1] A thorough appreciation and understanding of fungal infections, including diagnostic and therapeutic modalities are needed to provide better patient care. A lot of fungi invade the oral cavity, the commonest being the Candia species. A majority of fungal infections are caused by Candida species[2,3,4] followed by Aspergillus species. Few Candida species that are commonly encountered are Candida albicans, Candida tropicalis, Candida krusei, Candida glabrata, Candida parapsilosis and C. lusitaniae. Numerous reports have documented increased incidence of C. parapsilosis, C. lusitaniae, C. tropicalis, and C. glabrata among hospitalized patients.[5,6,7]

Apart from Candida and Aspergillus, Zygomycosis species like Rhizopus, Mucor, Absida etc. are also seen. Infections with these organisms are almost always a consequence of some form of immunocompromised states such as neutropenia accompanying leukemia or chemotherapy, diabetic ketoacidosis, burns, immunosuppressive therapy especially corticosteroids and the acquired immunodeficiency syndrome (AIDS). A recent study characterized the oral mycobiome of twenty healthy individuals showing that Candida species were the most frequently isolated fungi (present in 75% of participants), followed by Cladosporium (65%), Aureobasidium, Saccharomycetales (50% for both), Aspergillus (35%), Fusarium (30%), and Cryptococcus (20%).[8]

The diagnosis of fungal diseases is a multidisciplinary approach requiring cooperation and collaboration of many people with diverse expertise. Due to its difficulty in sample collection, poor sensitivity and prolonged time required for conventional procedure, attention has been drawn to the non culture based diagnostic techniques. For conventional techniques, basic bacteriological techniques may be used when working with most of the fungi. However, cultures may be kept longer and provisions may be made to prevent media from excessive drying.[9]

1. Sample Collection and Processing

The main goal of a clinical mycology laboratory is to isolate efficiently and to identify accurately the suspected etiological agent of fungal infection. Specimen should be collected aseptically in a clean, sterile and properly sealed container. It should be delivered to laboratory within 2 hours. Swabs are not satisfactory, since they allow drying of specimen and loss of viability. All specimens sent to mycology laboratory must be clearly labelled with the patient's name, age, sex and history of predisposing factors if any, distinct patient identification number.[9] A proper collection of specimen and their transport to clinical laboratory is very important for recovery of fungi. Therefore, it is important to transport the specimen to the laboratory as early as possible, as the growth of contamination is not so uncommon. The different samples that could be collected are discussed. (Table 1)

2. Direct examination of smear / Microscopy & Staining Methods

In Potassium hydroxide (KOH) Mount, concentration of 10-20% KOH used, acts as a cleansing agent to observe fungi in a specimen. One drop of KOH is placed on a dry slide and small quantity of specimen is placed. This is incubated at room temperature for 20 to 30 minutes. Stains like methylene blue or Parker blue black fountain ink are used along with KOH as a modification of KOH.[9] Periodic acid shiff (PAS) stain is used to stain Candida species, in which PAS stain preferentially stains glycogen in the fungal cell wall and thus renders the Candidal organisms magenta colour under the microscope.[10] A chair side diagnostic procedure can be performed by placing a drop of 10% KOH onto the fixed slide. KOH lyses the keratinocytes, thus allowing the Candidal organisms to be more readily seen under the microscope. It is also used in case of Aspergillosis.[11]

India ink stain, a negative stain is generally used to observe encapsulated organisms like Cryptococcus species. Nigrosine stain can be used as an alternative for India ink which is also a negative stain. It is a much better option than India ink as it contains formalin as an antimicrobial agent and there is no appearance of carbon particles in stain solutions. A water soluble, colourless, fluorescent whitener, Calcoflour white stains which binds to the cellulose and chitin of the fungal cell wall can also be used. This gives a bluish white fluorescence when observed under UV light. Lacto Phenol Cotton Blue (LPCB) Stain is used to study the morphology of fungal isolates. One drop of Lactophenol Cotton Blue Stain (LPCB) is placed on a clean slide and then the fungus to be identified is placed on the stain. This is tease mounted with the help of needle and observed under microscope.[10]

Gram Stain is used to observe fungi like Candida, Malassezia, Sporothrix, while modified gram stain is used for Nocardia, Actinomycetes. Giemsa stain, a compound formed by mixing methylene blue and eosin is commonly used to demonstrate intracellular yeast of Histoplasma capsulatum and other fungi.[10] Gomoris Methanamine silver stain is the most useful stain for visualizing fungi in a tissue which works on the principle of liberation of aldehyde group and their subsequent identification by reduced silver method. Generally, it is used to demonstrate polysaccharide content of fungi in tissue section. Fungal elements are sharply delineated in black against a pale green or yellow background. To identify Cryptoccous neoformans and Rhinosporidium seeberi from other fungi of similar size and shape, Mayer's Mucicarmine stain is used. The capsule stains deep red, tissue yellow while, nuclei are black.[9,10]

3. Culture techniques

The growth of fungi is usually slow and culture should be kept incubated for 4-6 weeks before declaring it as negative. Usually positive cultures are obtained within 7-10 days. All the fungal cultures should be handled in bio safety cabinets.[10] On the basis of growth characteristics; they can be classified into following: (Table 2)

It must be noted that growth rate also varies with type of media and incubating conditions. Materials from infected cases of mycolic infection should be cultured, even though direct examination of the material fails to reveal the presence of a fungus. The use of variety of culture media at incubation temperature of 25-37oC is likely to increase the recovery of fungal pathogens. Sabourauds Dextrose Agar (SDA) is used generally for primary isolation of fungi. Further, Cycloheximide/Actidione can inhibit a large number of saprobes/opportunistic fungi as well as certain medically important fungi. No one specific media or combinations of media are adequate for all specimens.[9] Potato dextrose agar may be used for primary isolation of fungi from clinical specimens, as the combination of potato and dextrose helps in luxuriant growth of fungus. The media contains dextrose and peptone. The media may be supplemented with antibiotics such as gentamicin and chloramphenicol to minimize bacterial contamination and cyclohexamide to inhibit saprophytic fungi.[10] There are various other culture media used for isolation of fungi. (Table 3)

Non Culture methods of diagnosis of fungal infections includes a variety of antibody and antigen based assay, including detection of glucan, mannan, enolase or proteinase and have also been used for diagnosis of fungal infections. Some of the metabolic detection assay that have been studied includes those of mannose and arabinitol. Molecular identification using PCR for the amplification of fungal DNA from tissue is applied to fungal diagnosis more frequently. On the horizon, investigational modalities, including bundling together antigenic panels or immunodiagnostic panels, use of DNA or RNA probes, and exoantigen testing to detect metabolites and antigens diffusing out in agar, have potential for rapid diagnosis.[9,10] Various biochemical tests for fungal infections are listed: (Table 4)

4. Serodiagnosis

For more rapid non culture method of fungal diagnosis, the serodiagnosis method is though of low sensitivity is employed. Generally, these tests are performed in highly specialized laboratories or reference laboratories. In case of Histoplasmosis or Coccidioidomycosis complement fixations test titre of greater than 1:32 may be diagnostically significant, whereas lower titre represents an early infection, cross reaction or a residual antibody from previous infection. Precipitation can be found in 90% patients with aspergilloma. Commercially available ELISA test for antigen detection in Cryptococcus, detects greater than 90% of cryptococcal meningitis and ~70% disseminated cryptococcal infection.[9] Detection of circulating antigen galactomannan (GM), a component of the cell wall of Aspergillus species, and/or specific antibodies in the serum are useful markers for diagnosis particularly in cases of invasive aspergillosis.12 The serum immunodiffusion assay that detects antibodies against the H and M antigens of H. capsulatum is reported to be a reliable diagnostic method.[13] Serodiagnosis for various fungal infections are discussed: (Table 5)

5. Molecular Methods

PCR has gained popularity in diagnosis of fungal infection, with high sensitivity and specificity. There have been various studies of DNA fragment as primer for PCR as a diagnostic tool for invasive Aspergillosis.[14-16] In case of Candidal molecular diagnosis a specific DNA probe has been developed for faster myceclial identification.[17] Hybridization based detection methods is a technique which uses a probe with sequence homology to the target DNA. It can be used to detect fungal specific sequence in DNA or RNA sample. The various Hybridization based detection methods are Fluorescent insitu Hybridization (FISH), microtitre Hybridization Assay, Reverse Hybridization line probe Assay and Hybridization on DNA chips.[10]

Conclusion

Dentists play a crucial role in the diagnosis and management of oral fungal diseases. A sufficient awareness is crucial in identification of many kinds of oral infections, which could be markers of immune deterioration. The possibility of systemic mycoses may be considered in cases where chronic oral ulcerations or unusual mouth lesions are observed particularly in the immunocompromised patient population.Awareness of the myriad presenting signs and symptoms of oral fungal diseases and knowledge of various sample collection, processing and laboratory diagnostic techniques aids in early diagnosis, proper treatment and prevention of disease dissemination, thereby decreasing morbidity.

Table 1: Sample collection in oral fungal infections[9]

Oral samples encountered in Mycology laboratory	Collection Method	Unacceptable specimens
SUPERFICIAL AND CUTANEOUS MYCOSIS		
Throat and mucous membrane	Ideally scrapping with blunt scalpel Moist swab to be collected in a sterile container	Dry swab
SUBCUTANEOUS MYCOSIS		
Pus	Aseptically with needle and syringe from undrained abscess. Pus expressed from abscess opened from scalpel/scraping or crust collected from superficial lesion transported to laboratory in a sterile container/syringe and needle.	Swab and material from open wound
SYSTEMIC MYCOSIS		

Sputum	5-10 ml early morning prior to eating Mouth rinse before collection. Induce sputum when unproductive. Collected in a sterile wide mouthed container.	Saliva, Nasal secretion, throat swab, 24 hours collections
Bronchial brush / washing / broncheo alveolar lavage	Collected in sterile container using fiber optics bronchoscopes	Dried Specimen

Table 2: Classification of fungi based on their growth characteristics[10]

Growth of fungus	Time duration
Rapid Grower	2 -5 days
Intermediate grower	6-10 days
Slow Grower	2 -3 weeks

Table 3: Various media for isolation of fungus[9]

BASAL MEDIA	NUTRITIONALLY DEFICIENT MEDIA	ENRICHED & SELECTIVE MEDIA	MEDIA USED FOR STIMULATION OF ASCOSPORES
Sabourad Dextrose Agar	Rice Starch Agar	Brain Heart Infusion Agar	Alphacel Yeast Extract Agar
Neutral Sabourad's Dextrose Agar	Corn Meal Agar/ Corn Meal Tween Agar	Biphasic Medium	Soil Extract Agar
Sabourad Dextrose Agar with antibiotics		Cystein Heart and Hemoglobin agar	
		Bird Seed Agar	
		Sunflower Seed Agar	
		Dermatophyte medium	
		Blood Agar	
		Czapek Dox Agar	
		PYG Agar Medium	
		Malt Extract Agar	
		Oat meal Agar	
		Potato Dextrose Agar	

Table 4: Various biochemical tests for fungal infections[9]

MEDIA FOR BIOCHEMICAL TEST	
A. Terazolium Reduction Medium (TRM) – Used to differentiate between various Candida spp	
Candida Spp	Colour on TRM
1. C. albicans	Pale pink
2. C. tropicalis	Orange pink
3. C. pseudotropicalis	Salmon pink
4. C. parapsilosis	Rose pink
5. C. guilliermondii	Pink and pasty
6. C. krusei	Pink and dry
7. C. glabrata	Pale pink
B. Carbohydrate Fermentation Media	
C. Carbohydrate Asssimilation Media	
D. Urease Medium – helps in identification of Cryptococcus, Trichosporon, Rhodotorula spp from Candida and Geotrichum and Trichophyton mentagrophyte from Trichophyton rubrum	
E. Rapid Urease Test	
F. Carbohydrate Fermentation Media	

Table 5: Serodiagnosis for various fungal infections[10]

	Histoplasmosis	Blastomycosis	Coccidiodomycosis	Cryptococcosis	Candidiasis	Aspergillosis	Zygomycosis
Precipitation reaction	Positive	Positive	Positive	Negative	Variable	Positive	Positive
Agglutination	Variable	Positive	Negative	Positive	Positive	Negative	Negative
Complement Fixation	Positive	Variable	Positive	Negative	Negative	Negative	Negative
Immunofluo-rescence	Negative	Negative	Negative	Positive	Negative	Variable	Negative
ELISA	Positive	Negative	Negative	Negative	Negative	Positive	Negative
Radio Immunoo Assay	Positive	Negative	Negative	Negative	Negative	Negative	Negative

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