2. Cutaneous mycoses

Cutaneous mycoses extend deeper into the epidermis, and also include invasive hair and nail diseases. These diseases are restricted to the keratinized layers of the skin, hair, and nails. Unlike the superficial mycoses, host immune responses may be evoked resulting in pathologic changes expressed in the deeper layers of the skin.

Clinical manifestations of ringworm infections are called different names on basis of location of infection sites:

- tinea capitis - ringworm infection of the head, scalp, eyebrows, eyelashes
- tinea favosa - ringworm infection of the scalp (crusty hair)
- tinea corporis - ringworm infection of the body (smooth skin)
- tinea cruris - ringworm infection of the groin (jock itch)
- tinea unguium - ringworm infection of the nails
- tinea barbae - ringworm infection of the beard
- tinea manuum - ringworm infection of the hand
- tinea pedis - ringworm infection of the foot (athlete's foot)

Etiological agents are called dermatophytes - "skin plants". Three important anamorphic genera, (i.e., ***Microsporum, Trichophyton,*** and ***Epidermophyton***), are involved in ringworm.

- **Diagnosis**: Microscopic examination of slides of skin scrapings, nail scrapings, and hair. Often tissue suspended in 10 % KOH solution to help clear tissue. Slides prepared this way are not permanent. These degrade rapidly due to presence of base. **Treatment:** azoles, inhibits cytochrome 450 dependent enzyme systems at the demethylation step from lanosterol to ergosterol. Hair- Griseofulvin, oral , affects microtubular system

3. Subcutaneous mycoses: These are caused by fungi that grow in soil and on vegetation and are introduced into subcutaneous tissue through trauma. Clinical manifestations of subcutaneous mycoses are: chromoblastomycosis, sporotrichosis, rhinosporidiasis, mycetoma, subcutaneous phaeohyphomycosis and lobomycosis .

Chromoblastomycosis:

This is a slowly progressive granulomatous infection that is caused by several soil fungi **(*Fonsecaea, Phialophora, Cladosporium*, etc**.) conidia or hyphae are dark-colored, either gray or black. Wart like lesions with crusting abscesses extend along the lymphatics. The disease occurs mainly in the tropics and is found on bare feet and legs. **In the clinical laboratory**, dark brown, round fungal cells are seen in leukocytes or giant cells. Treatment with oral **flucytosine** or **thiabendazole**, plus local surgery.

Sporotrichosis:

Etiology*: Sporothrix schenckii* is a **dimorphic** fungus. Sporotrichosis occurs most often in **gardeners, especially those who prune roses,** because they may be stuck by a rose thorn. **In the clinical laboratory**, round or cigar-shaped budding yeasts are seen in tissue specimens. In culture at room temperature, hyphae occur bearing oval conidia in clusters at the tip of slender conidiophores (resembling a daisy). **The drug** of choice for skin lesions is **itraconazole (Sporanox).** It can be prevented by protecting skin when touching plants, moss, and wood.

4. Systemic (deep) mycoses

These infections result from inhalation of the spores of dimorphic fungi that have their mold forms in the soil. Within the lungs, the spores differentiate into yeasts or other specialized forms, such as spherules. Most lung infections are asymptomatic and self-limited. However, in some persons, disseminated

disease develops in which the organisms grow in other organs, cause destructive lesions, and may result in death. Infected persons do *not* communicate these diseases to others. Systemic fungi are also called endemic fungi because they are endemic (localized) to certain geographic areas. Systemic mycosis include: Coccidioidomycosis, Blastomycosis, Histoplasmosis and Paracoccidioidomycosis.

Coccidioidomycosis "valley fever":

Etiology*: Coccidioides immitis* is a **dimorphic** fungus that exists as a **mold** in soil and as a **spherule** in tissue (Stages of *Coccidioides immitis*. **A:** Arthrospores form at the ends of hyphae in the soil. **Endemic** in arid regions of the **southwestern United States** and **Latin America**.In tissue specimens, spherules are seen microscopically. Cultures on Sabouraud's agar incubated at 25°C show hyphae with arthrospores. No treatment is needed in asymptomatic or mild primary infection. Amphotericin B, (Fungizone) or itraconazole is used.

5. Opportunistic mycoses

Opportunistic fungi fail to induce disease in most immunocompetent persons but can do so in those with **impaired** host defenses. There are five genera of medically important fungi are : *Candida* (Candidias),*Cryptococcus* (Cryptococcosis), *Aspergillusn*(Aspergillosis), *Mucor* and *Rhizopus*(Mucormycosis).

Laboratory diagnosis of mycoses:

Specimen collection: specimen collection depends on the site affected. Different specimens include hair, skin, scrapings, nail clippings, sputum, blood, CSF, urine, corneal scraping, discharge or pus from lesions and biopsy.

• All specimens must be transported to the laboratory without any delay to prevent bacterial overgrowth. Incase of delay specimens except skin specimen, blood and CSF may be refrigerated for a short period.

• Infected hairs may be plucked using forceps. Those hairs that fluoresce under Wood's lamp may be selectively plucked. Hairs may be collected in sterilized paper envelopes.

• Surface of the skin must be disinfected with spirit before specimen collection. The advancing edge of the lesion is scraped with the help of a blunt forceps and collected in sterilized paper envelopes.

• Discoloured or hyperkeratotic areas of nail may be scraped or diseased nail clipping may be collected in sterilized paper envelopes.

• Specimens from mucus membranes (oral) must be collected by gentle scraping and transported to laboratory in sterile tube containing saline. Swabs may be collected from vagina.

• Corneal scrapings may be collected using a fine needle and inoculated at bedside.

• Pus may be collected by aspiration; use of cotton swabs may give false positive microscopic results.

• Clean catch urine may be collected in a sterile wide-mouthed container.

• Biopsy specimens must be transported in saline.

In certain cases, pus or exudates must be looked for presence of granules.

Microscopy: Microscopy is used to observe clinical specimens for the presence of fungal elements or to identify the fungus following culture. In the latter case, lactophenol cotton blue is stain of choice, which stains the fungal elements blue. Direct examination of clinical specimens could be stained or unstained.

• Wet mount: *Candida* may be observed in urine wet mounts

• 10-20% KOH mount: Several specimens are subjected to KOH mount for direct examination. The material is mixed with 20% KOH on a slide and a cover slip is placed. The slide is then gently heated by passing through the flame 2-3 times. The slide is observed on cooling. KOH serves to digest the protein debris and clears keratinised tissue and increases the visibility. Addition of Dimethyl sulphoxide (DMSO) permits rapid clearing in the absence of heat.

• Calcofluor white: This is a fluorescent dye, which binds selectively to chitin of the fungal cell wall. The specimen then can be observed under fluorescent microscope.

• India Ink: Capsules of *Cryptococcus neoformans* can be demonstrated by this negative staining technique.

• Periodic Acid-Schiff (PAS) stain: On staining by this stain, fungal elements appear bright magenta coloured while the background stains green. It is useful in staining tissue specimens.

• Giemsa's stain: It is particularly useful in the detection of *Histoplamsa capsulatum* in the bone marrow smears.

• Haematoxylin and Eosin (H&E) stain: Useful for staining tissue sections.

• Gomori's methenamine silver nitrate (GMS) stain: Outlines of the fungi are black, internal parts stain pinkblack while the background stains light green. Candida and Aspergillus may be missed in H&E stained sections, therefore GMS stained sections are essential for tissue pathology.

• Gridley's stain: It stains hyphae and yeasts dark blue-pink, tissues deep blue and background yellow.

• Meyer mucicarmine stain: Capsules of *C. neoformans* and inner walls of *Rhinosporidium seeberi*'s sporangium are stained pink.

• Gram stain: Candida is best demonstrated in clinical specimen by Gram stain.

• Masson-Fontana stain is helpful in staining phaeoid (dematiaceous) fungi in tissue.

• Immunofluorescence: Monoclonal antibody labelled with fluorescent dyes can be used to detect several fungi in the clinical specimens.

Culture: One of the most common media used to culture fungi in laboratory is Sabouraud's Dextrose Agar (SDA). It consists of peptone, dextrose and agar. High concentration of sugar and a low pH (4.5-5.5) prevents growth of most bacteria and makes it selective for fungi. Emmon's modification of SDA contains 2% dextrose and has pH of 6.8. Other basal media to grow fungi include Potato Dextrose Agar, Malt Extract Agar etc. Most fungi are able to grow at room temperature while few pathogenic fungi (e.g, Cryptococcus, dimorphic fungi) can grow at 37oC. Saprophytic fungi grow much quickly than pathogenic fungi (e.g, dermatophytes). In such situations the saprophytic fungi can be inhibited by the addition of cycloheximide (actidione) to the SDA. Addition of antibiotics such as Chloramphenicol,

Gentamicin or Streptomycin to SDA serves to inhibit bacterial multiplication. An example of SDA with cycloheximide and Chloramphenicol is Mycosel agar. Other specialized media used for different fungi include:

• Brain Heart Infusion Agar general isolation of fungi and conversion of dimorphic fungi.

• Inhibitory Mould Agar, an isolation medium with Chloramphenicol to suppress most bacteria.

• Caffeic Acid Agar and Birdseed Agar for isolation of *Cryptococcus neoformans*.

• Corn Meal Agar: Enhances production of chlamydospores in *Candida albicans* and formation of conidia in fungi.

• Trichophyton Agars: Used for selective identification of *Trichophyton* species.

• Dermatophyte Test Medium: Used for recovery of dermatophytes from clinical specimens.

• Sabhi Medium: Isolation of *Histoplasma capsulatum*.

• 'CHROM agar Candida' is useful in identification of Candida species.

Conversion of mould to yeast phase must be demonstrated in vitro for identification of dimorphic fungi. Since some fungi grow slowly cultures should not be discarded for 4-6 weeks. Fungi are identified on the basis of colony morphology (including pigmentation) and microscopic observation by tease-mount preparation or slide culture technique.

Serology: Detection of anti-fungal antibody is helpful in diagnosis of sub-cutaneous and systemic mycoses, prognosis and response to anti-fungal drugs. Different serologic techniques that are used include agglutination,

immunodiffusion, counter-immunoelectrophoresis, complement fixation test, immunofluorescence, RIA and ELISA.

Antigen detection: It is particularly useful in the diagnosis of cryptococcal meningitis from CSF specimens. The test is performed by Latex Agglutination or immunodiffusion tests. It is also helpful in the detection of *Aspergillus* and Candida antigens in systemic infections.

Skin tests: Delayed hypersensitivity reactions to fungal antigens can be demonstrated by skin tests. A positive skin does not necessarily indicate an active infection; it only indicates sensitization of the individual.

Molecular techniques: Newer techniques such as DNA hybridization, PCR are useful in diagnosis of mycoses in a shorter period as well as detect those fungi that are difficult or dangerous to cultivate in vitro.

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