

MYCOTIC LESIONS IN SURGICAL PATHOLOGY



Dissertation submitted in
Partial fulfillment of the regulations required for the award of
M.D. DEGREE
in
PATHOLOGY – BRANCH III



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DECLARATION

I hereby declare that the dissertation entitled "**MYCOTIC LESIONS IN SURGICAL PATHOLOGY**" was done by me in the Department of Pathology at Coimbatore Medical College & Hospital, Coimbatore during the period from June 2007-June 2009, under the guidance and supervision of **Dr.C. LALITHA, M.D.**, Additional Professor, Department of Pathology, Coimbatore Medical College, Coimbatore. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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CERTIFICATE

This is to certify that the dissertation entitled "**MYCOTIC LESIONS IN SURGICAL PATHOLOGY**" is a record of bonafide work done by **Dr.S.Kalyani**, Post graduate student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore, under the supervision of **Dr. R. VIMALA, M.D.**, Professor & Head, Department of Pathology, Coimbatore Medical College and Hospital, and under the guidance of **Dr. C. LALITHA, M.D.**, Additional professor, Coimbatore Medical College and Hospital, in partial fulfillment of the regulations of the Tamilnadu Dr. M.G.R. Medical University towards the award of M.D. Degree (Branch III) in Pathology.

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INTRODUCTION

The fungal diseases are grouped arbitrarily into four broad categories based on the predominant location of infection within the body as superficial, cutaneous, subcutaneous and systemic infection. Superficial mycoses are those in which the fungus is usually confined to the keratinized layer of the skin and its appendages. The cutaneous and subcutaneous mycoses are a polymorphic group of diseases caused by a variety of fungi. Systemic mycoses usually have a pulmonary inception from which they disseminate to other organs.

Most fungal infections occur because a person is exposed to a source of fungi such as spores on surfaces or in the air, soil or bird droppings. Usually there is a break or deficiency in the body's immune system defences and the person provides the "right environment" for the fungi to grow. Any one can have a fungal infection but certain populations are at increased risk of fungal infections and recurrence of the infection. These include HIV–AIDS spectrum, malignancy, multidrug resistant Tuberculosis, diabetes mellitus, organ transplant recipients, those who are on chemotherapy or immunosuppressants, prolonged intake of steroids for chronic disorders like bronchial asthma, rheumatoid arthritis and inborn immunological deficiencies. Neutropenic patients are prone to develop invasive mycosis. Persons who have undergone abdominal or cardiac surgery and those who received repeated intravenous injections are at risk of mycoses. In the macroscopic evaluation of tissue specimens, mycotic infections are frequently mistaken for neoplasms or other diseases and a mycosis often may not be considered until the histopathological examination is complete².

There are four basic approaches to the diagnosis of mycotic diseases: (1) clinical (2) mycologic (3) immunologic (4) pathologic. Diseases caused by fungi may be difficult to distinguish both clinically and pathologically from those caused by other microbial agents. Because serological tests have certain limitations and have not been developed for some fungal diseases, a definitive diagnosis of mycotic disease often rests on direct microscopic demonstration of a fungus in tissues and exudates, or on isolating and identifying it in culture. Histopathology should not be a substitute for mycologic culture; rather the two should complement each other whenever possible. Histopathologic evaluation provides indisputable evidence of tissue invasion and therefore can confirm the pathogenic significance of a cultural isolate that belongs to the body flora or that is usually encountered as an environmental contaminant in culture. Histopathology can also confirm the presence of coexisting infections by other fungi, bacteria, viruses and protozoans thus guiding the clinician in the selecting the most appropriate therapy and management for the patient. Although some fungi and related organisms can be detected in hematoxylin and eosin stained tissue sections, special histochemical stains are usually necessary to demonstrate their morphology in detail. Fungi can be adequately detected in cytologic specimens obtained by fine needle aspiration biopsy, brushing, washing or scraping. A drawback of cytologic specimens however may be the inability to distinguish invasive fungal infections from fungal colonization¹.

The three common fungal stains used are Gomori methenamine silver (GMS)^{5,36}, the Gridley fungus (GF), and the Periodic Acid – Schiff (PAS) procedures. GMS procedure is considered to be the best of the special fungal stains for screening a tissue section because it provides better contrast and stains fungal cells that are refractory to the GF and PAS procedures.

AIM OF THE STUDY

- To find out the incidence of fungal lesions in surgical pathology particularly when inflammatory reaction dominates histopathologically.
- To study various types of fungi and their histological reactions in tissues.
- To study predisposing factors if any in these fungal infections.
- To study the clinicopathological correlation.

NEED FOR THE STUDY

The incidence of fungal lesions is on the increase, probably due to the increasing incidence of immunosuppressed state. This could be due to varied factors like malignancy, HIV – AIDS spectrum, multidrug resistant TB, Diabetes mellitus, organ transplantation, prolonged intake of steroids for chronic disorders like bronchial asthma, rheumatoid arthritis etc.

With the advent of sophisticated and potent antifungal drugs, rapid and accurate tissue diagnosis would pave way for early institution of specific therapy, thereby reducing serious morbidity and suffering. Also the presence or absence of tissue invasion diagnosed by histopathology would help in formulating apt treatment protocols. When fungal infections present as mass lesions, prompt diagnosis alleviates the need for unnecessary radical surgical intervention.

REVIEW OF LITERATURE

HISTORY:

The branch of biology, which deals with the study of fungi, is known as “Mycology”. The term is derived from ‘mykes’, a Greek word for mushroom³. The ‘fungus’ is a Latin word that also means mushroom.

The history and development of mycology are characterized by several stages³. In 1835 Augustino Bassi in Italy observed that a fungus, *Beauveria bassiana*, was the cause of disease in silkworms (*Bombyx mori*) called muscardine. He predicted on the basis of these findings that the fungi could also cause infections in man. Shortly thereafter Schoenlein⁴ (1839) recognized the fungal nature of the disease known as favus. In the days of Gruby, Malmsten and Schoenlein around 1940, there was a wave of excitement in this new field and investigators were determining the fungal etiology of several dermatologic diseases, such as tinea and thrush.

The history of the majority of the fungal diseases has the following chronological order: Dermatomycosis, Schoenlein (1839), Aspergillosis, Sluyter (1847), Candidiasis, Robin (1853); Actinomycosis, Harz (1877), Nocardiosis, Eppinger (1890), Coccidioidomycosis, Posadas (1892); Cryptococcosis, Busse (1894), Blastomycosis (N.A), Gilchrist (1896); Sporotrichosis, Schenck (1898), Histoplasmosis, Darling (1906) and Blastomycosis (S.A), Lutz (1910)-(4,5,6,7,8,9,10,19).

Aspergillosis was one of the first fungal diseases of man recognized. The ‘Aspergillum’ (Latin: to sprinkle) referred to a perforated globe used to sprinkle holy water during religious ceremonies (aspergite). *Aspergillus* pneumomycosis was

described by Sluyter in 1847⁶. In 1897, Renon published a book containing an excellent review of the field and also of the association of the disease with certain occupations in addition to pigeon handlers such as wig cleaner¹⁶. The allergic form of Aspergillosis and the colonizing type of Aspergillosis are now realized to be common and frequently encountered diseases.

In 1910, the French dermatologist Raymond Jacques Sabouraud³ published his monumental work on dermatophytes, "Les Teignes". He has rightly been called as the "Father of Medical Mycology". Soon after this however, the literature became cluttered with numerous synonyms for almost every fungal infection. A group of Latin American scientist clinicians is responsible for a large portion of our knowledge. This group includes Gonzalez Ochoa, F.Almedia, Machinnon and others.

Hippocrates in his 'Epidemics' described aphthae or thrush in debilitated patients. In 1839 Lagenbeck described a fungus in aphthae. In 1847 Robin placed it in the name of *Candida albicans*. Presently *Candida* is recognized as one of the most frequently encountered opportunistic fungal infections. In 1900, Guillermo Seeber published the first case of Rhinosporidiosis in a 19 year old agricultural worker. Ashworth made a very detailed analysis of the organism and its development in tissues¹⁵. Most of the early studies were made in India and Sri Lanka where the disease occurs frequently. Karunaratne in 1964 published a detailed account of the disease in man and reviewed the literature¹⁶.

Actinomycosis was undoubtedly observed early in the 19th century, as actinomycotic tumours were described erroneously in 1826 by Leblanc as osteosarcomas. It was first recognized as a specific parasitic disease in 1876 by Bollinger. Harz described the disease in cattle and called the etiologic agent

Actinomycosis bovis. Modern concepts of the organism and disease were summarized by Erikson¹⁸.

Mycetoma as a medical entity was first reported by Dr. Gill in 1842. The first cases were from India. Brumpt in 1905 stressed that several fungi were capable of eliciting the same clinical disease and Langeron applied the term 'mycetoma' to cases involving actinomycosis/nocardia species^{19,20}.

Sanfelice in 1894 isolated from peach juice an encapsulated yeast like fungus which he named *sacharomyces neoformans*²¹. At about the same time Busse and Buschke reported isolation of the same fungus from a sarcoma like lesion of tibia^{22, 23}. Von Hansemann in 1905 appeared to be the first to see this fungus in a case of meningitis. In 1901 Vuillemin transferred the fungus to the genus *Cryptococcus* because he did not find ascospores typical of *saccharomyces*²⁴. An excellent monograph by Littman and Zimmerman summarises the literature of Cryptococcosis up to 1956²⁵.

Histoplasmosis was discovered in Panama in 1905 by Darling while searching for *Leishmania donovani* (LD) bodies in necrotic material. In 1934, Dodd and Tompkins made the first diagnosis of Histoplasmosis during life²⁶. Palmer (1946) demonstrated remarkable geographic differences in rates of hypersensitivity to histoplasmin²⁷. An excellent review of the biology of Histoplasmosis is found in Domer and Moser, Goodwin et al, have summarized the state of understanding of the various clinical manifestations^{28,29}.

Historically, Palmer (1885) is credited with the first histologic description of generalized Mucormycosis in a 52 year old patient³⁰. In 1901 Lucet and Costantin

described a woman with respiratory distress who coughed up strands of fungal mycelium³¹. In 1903, Barthelat produced accurate line drawings of non-septate mycelium in tissues³².

Puckett in 1953 and Zimmerman in 1954 were the first to emphasize the use of selective tissue stains for the staining of resected granulomatous tissues for an accurate diagnosis of fungal diseases^{33,34}. A first review on the immunology of human mycoses by Kligman and De Lamater in 1950 listed 200 references. Eight years later, in 1958 Seeliger had collected over 500 pertinent reports and by the end of 1960 almost 700³⁵.

In present day medicine, the advent of cytotoxic drugs, long term steroid treatment and immunosuppressive agents has markedly increased the number and severity of diseases in this category. The diverse array of organisms being isolated from these cases emphasizes that probably all fungi may be considered potential pathogens when normal defences are sufficiently abrogated. Fungi are particularly remarkable for their ability to adapt and propagate in a wide variety of environmental situations; thus their invasion of debilitated patients is not surprising.

DIAGNOSIS OF FUNGAL INFECTIONS¹

There are four basic approaches to the diagnosis of mycotic diseases:

1. Clinical.
2. Mycologic.
3. Immunologic.
4. Pathologic.

CLINICAL DIAGNOSIS³

Fungal sinusitis should be considered in all patients with chronic sinusitis.

The clinical diagnosis of fungal infections is aided by the appearance of the lesion. Mycologic identification of fungi is valuable for those organisms that are morphologically similar in tissue sections.

The clinical criteria may give a presumptive diagnosis of the fungal infection. Superficial and subcutaneous mycoses often produce characteristic lesions that strongly suggest their fungal etiology but they closely resemble other diseases. It is not unusual to find that the appearance of the lesions has been considerably modified and rendered atypical by prior therapy with topical steroid or antifungal medications.

Patients with non invasive forms have intractable sinusitis that fail to respond to antibiotics. Invasive fungal sinusitis usually occurs in immunocompromised patients with acute onset of fever, cough, nasal mucosal ulceration, eschars, epistaxis or headache.

In case of systemic mycosis there is no sign or symptom that specifically suggests a fungal disease. Early diagnosis considerably increases the chances of successful treatment. It is important that the possibility of fungal involvement should be considered from the outset. Recently, the clinical importance of fungal infections has been better recognized mainly due to increased awareness among the medical personnel.

The modern imaging techniques for patient's evaluation has improved the accuracy and speed of diagnosis.

FUNGAL CULTURE^{3,34}

The solid media are employed for fungal culture, as the broths are not usually recommended except for fungal blood cultures where bi-phasic medium is used. The

medium commonly employed is Emmon's modification of Sabouraud dextrose agar. The media may be supplemented with antibiotics, such as gentamicin and chloramphenicol to minimize bacterial contamination and cycloheximide to inhibit saprophytic fungi.

Fungi grow relatively slow. Culture should be retained for atleast four weeks and in some cases upto six weeks before being discarded as sterile. Usually positive results of culture are obtained within 7 to 10 days. In *Candida* and *Aspergillus* species, the growths appear within 24 – 72 hours. Therefore cultures should be examined for growth daily for the first week and twice a week for subsequent period.

HISTOPATHOLOGICAL DIAGNOSIS^{1,2}

Based on the morphologic distinctiveness of their etiologic agents in tissues, the mycoses are grouped as follows¹.

1. Those caused by fungi that can be identified because they have a distinctive morphology in tissue.
2. Those caused by any one of several species of a genus that are morphologically “similar” and therefore can be identified only to the genus level.
3. Those caused by any of a number of fungi belonging to various genera that appear similar if not identical to one another in tissue.
4. Mycetomas, which are special cases that constitute a group by themselves. Because most agents of mycetoma form their own distinctive type of granule, the etiologic agent can be identified by the size, shape, architecture and colour of a granule.

In the macroscopic evaluation of tissue specimens, mycotic infections are frequently mistaken for neoplasms or other diseases (2). It is preferable to do cultural examination in conjunction with Histopathological studies.

Because of their size, characteristic morphology, and tinctorial properties, fungi can be studied satisfactorily in tissue. For some diseases, e.g., Lobomyosis and Rhinosporidiosis, microscopic examination of histological material either in the form of sections or smears is the only way to establish a diagnosis, because the aetiologic agents of these diseases have not yet been grown in culture.

Histological studies make it possible to detect the presence of fungi and to confirm tissue invasion. Histopathologic procedures are also rapid and relatively inexpensive. It often results in an immediate diagnosis or at least an immediate presumptive diagnosis of mycotic infection. Histologic examination of fungi enables the microscopist to select the appropriate battery of fluorescent antibody (FA) reagents when they are needed.

The accuracy of a histopathologic diagnosis of a mycotic or actinomycotic disease depends upon the following factors ²:

- a. Agents involved.
- b. Adequacy of staining procedures.
- c. Use of proper stains.
- d. Expertise of the microscopist.

Using a battery of special staining procedures and immunofluorescence techniques, an accurate diagnosis of the common mycotic diseases can be made. The range of inflammatory responses to mycotic agents is wide and more than one type of

reaction may be elicited by a single fungal species. The type of inflammatory response depends on which reproductive stage of a fungus is in contact with host tissues.

Haematoxylin and eosin (H&E) is a versatile stain that is useful for the histological diagnosis of fungal diseases. With this stain the tissue response can be visualized and a fungus can be characterized as hyaline or dematiaceous. Some fungi such as the *Aspergillus* and *Zygomycetes* stain well with H&E, but many fungal agents are not stained or stain poorly.

The three special fungal stains most commonly used in the histological study of mycotic diseases are ^{2,33}:

- a) Gomori methenamine silver stains (GMS).
- b) Gridley fungus stains (GF).
- c) Periodic acid-Schiff stains (PAS).

The staining reactions are based on the principle that in the presence of chromic acid or periodic acid, adjacent hydroxyl groups of the complex polysaccharides in fungal cell walls are oxidized to aldehydes. In GMS procedure, the aldehydes reduce the methenamine silver nitrate complex, resulting in the brown-black staining of fungal cell walls due to the deposition of reduced silver wherever aldehydes are located. The depth of the colour produced depends on the amount of aldehyde present.

In the GF and PAS procedures, the aldehydes react with Schiff's reagent, colouring fungi reddish-purple and pinkish-red, respectively. The PAS procedure can be preceded by diastase digestion to remove glycogen. This will eliminate some of the nonspecific staining of normal tissue components and cellular debris.

The GMS procedure is considered to be the best of the special fungal stains for screening a tissue section². It provides better contrast and stains fungal cells that are refractory to the GF and PAS procedures. The GMS staining time must be varied not only according to the control slide but also according to the aetiologic agent under consideration. Slides must be periodically removed from the silver nitrate bath and examined under the light microscope to determine when optimal staining has been achieved.

The colour should never be so intense as to obscure the morphological detail of a fungus. Staining is prolonged for old and nonviable fungal elements. Precautions must be taken to prevent over staining of tissues with the GMS procedure, since erythrocytes and naked nuclei will stain and can mimic the appearance of yeast cells. Over stained blood vessels may mimic the appearance of the zygomycetes, particularly if the vessel is branched, within the same size range, and empty. Calcific bodies, whose appearance may mimic yeast cells, are dissolved by the chromic acid used in the GMF & GF procedures. These bodies take PAS stain.

Since the special fungal stains mask the natural colour of fungi, they are not useful in determining whether fungal elements are hyaline or dematiaceous. Such determinations are crucial in establishing a diagnosis of Phaeohyphomycosis, Chromoblastomycosis and other diseases caused by dematiaceous fungi. To overcome this H&E is used as the counter stain for GMS procedure.

Mayer's mucicarmine procedure stains the mucopolysaccharide capsular material of *Cryptococcus neoformans* a brilliant red. This stain is not specific *C. neoformans* because *Rhinosporidium seeberi* and some cells of *Blastomyces dermatidis* are variably stained with Mayer's mucicarmine.

Tissue Gram stains such as the Brown & Brenn and the Brown-Hopps procedures are recommended for demonstrating the gram-positive filaments of Actinomyces, Nocardia and Streptomyces species which appears bluish-black on a yellow background. Gram stains is used in the identification of bacteria other than the actinomycetes which may coexist with a mycotic infection.

Acid-fast stains are used in the histological diagnosis of infections caused by the Nocardia sp. Since nocardiae are weakly acid-fast , a weak decolourising agent such as 0.5-1.0% aqueous sulfuric acid must be used in these procedures instead of acid alcohol. The acid fast stains are modified Kinyoun or Fite-Faraco procedures.

Whenever possible, cultural studies should always complement histopathologic procedures. This is important when tissue forms of a fungus cannot be demonstrated. If a mycosis is suspected with H&E stained section, serial sections are treated with the following battery of special stains: GMS, Brown and Brenn and the modified Fite-Faraco acid-fast.

Histological sections contain normal and abnormal tissue components. These when coloured by certain stains, resemble fungi, e.g., Russel bodies, karyorrhectic debris, corpora amylacea, calcific bodies, reticulin and elastic fibres, small blood vessels, and the structures seen in the phenomenon termed myospherulosis.

When special stains are used, quality control must be achieved by using appropriate positive tissue substrates². The Centre for Disease Control has made available for distribution of the control tissues that may be used in staining procedures for identification of certain agents and structures: carminophilic and noncarminophilic fungi, gram-positive and gram-negative bacteria, acid-fast bacteria, spirochetes and

amyloid. The Control Tissue Register is maintained to assist technologists in determining whether the special stains are working properly.

Increasingly, cytologic materials, rather than tissue biopsy, are obtained for the diagnosis of fungal infections¹. Fungi can be adequately detected in cytologic specimens obtained by fine-needle aspiration biopsy, brushing, washing or scraping. The cytomorphology of the organism is identical to that seen in tissue biopsy specimens. A drawback of cytologic specimens is the inability to distinguish invasive fungal infections from fungal colonization.

The utility of histopathology in the diagnosis of infectious disease has been well established. Microscopic identification of a pathogen by its morphological features on staining continues to be the mainstay of diagnostic histopathology but recent developments in immunohistochemistry and molecular diagnostics will definitely be more rapid and also specific. However, the routine histopathological identification of microorganisms cannot replace conventional microbiologic culture techniques. The successful characterization of the infectious disease pathology requires the proper characterization of the inflammatory response, knowledge of associated pathogens, use of special histochemical stains and, in some instances, use of highly specific molecular technologies.

If microbiologists, pathologist and clinicians communicate effectively, timely and often correct diagnosis of many difficult to diagnose diseases can be efficiently made. Tissue biopsies should also be submitted for culture and isolation of pathogens. Before culture all biopsies should be examined for the presence of pathogen or suggestive features leading to infection. Important information is often missed if careful microscopic visualization of the tissue sample is not carried out.

Histological features of the fungal species¹

| Disease | Biological Agents | Typical morphology in tissue | Usual host reaction |
|------------------|---|---|--|
| Aspergillosis | Aspergillus fumigatus group, A.Flavus group, A.niger group | Septate, dichotomously branched hyphae of uniform width (3 – 6 micrometer),conidial heads may be formed in cavitory lesions. | Nodular infarcts, rarely granulomatous or suppurative, tendency for angioinvasion. |
| Actinomycosis | Actinomyces israelii, A.naeslundii,,A.viscosus, A.odontolyticus, A.bovis, Arachnia propianica, Rothia dentocariosa. | Organized aggregates (granules) composed of delicate, branched filaments about 1 micrometer wide: entire granules 30 – 3000 micrometer dia | Suppurative with multiple abscesses.extensive fibrosis, and formation of sinus tracts; splendore – Hoepplimaterial usually borders granules. |
| Rhinosporidiosis | Rhinosporidium seeberi | Large sporngia. 100-350 micrometer diameter, with thin walls (3 – 5 micrometer) that enclose numerous sporangiospores, 6-8 micrometer diameter. | Nonspecific chronic inflammatory or granulomatous. |
| candidiasis | Candida albicans, C.tropicalis, C.parapsilosis, C.krusei, C.guilliermondii, | Oval, budding yeastlike cells, 2 – 6 micrometer diameter, and pseudohyphae; septate hyphae may also be present | Suppurative, less commonly granulomatous or infarcive;minimal inflammation in preterminal infection; tendency for angioinvasion. |

| | | | |
|--------------------------------|--|---|---|
| | C.stellatoidea, and others | | |
| Zygomycosis (Mucor mycosis) | Absidia corymbifera, Apophysomyces elegans, Cunninghamella berthloetiae, Mucor ramosissimus, Rhizomucor pusillus, Rhizopus oryzae, R. rhizopodiformis, Saksenaea vasiformis, and others | Broad, thin – walled, infrequently septate hyphae, 6-25 micrometer wide, with nonparallel sides and randomly spaced branches | Suppurative necrosis, less commonly granulomatous; tendency for angioinvasion and infarction. |
| Mycetoma (antimycotic) | Actinomyces madurae, A. pelletieri, Streptomyces somaliensis, Nocardia spp., and others pseudallescheria boydii, madurella grisea, M. mycetomatis, Curvularia geniculata, Exophiala jeanselmei, Leptosphaeria senegalensis, and others | Granules, 0.1 to several mm dia, composed of delicate filaments (about 1 micrometer wide) that are often branched and beaded Granules, 0.2 to several mm diameter, composed of broad (2-6 micrometer) hyaline (white to yellow granules) or dematiaceous (black granules), septate hyphae that often branch and form chlamydoconidia. | Like Actinomycosis Like Actinomycosis |
| Mycetoma (eumycotic) | | | |
| Cryptococcosis | Cryptococcus neoformans; rarely, other Cryptococcus spp | Pleomorphic yeast – like cells, 2 – 20 micrometer diameter, with gelatinous, carminophilic capsules and single or multiple narrow – based buds; some strains are capsule deficient and may not be carminophilic. | Varies from minimal reaction (‘cystic ‘or’ ‘mucoid’ lesion) to granulomatous. |

CONTAMINANTS AND ARTIFACTS¹⁰⁷

Contamination of tissue sections with organisms, particularly bacteria and fungi, and their subsequent demonstration with routine and special staining methods is a potential cause of false positivity and misdiagnosis. Contamination may occur during several stages of tissue processing, cutting and staining. The most common source of contamination involves the sections floatation bath, which is normally set between 45 degree centigrade and 50 degree centigrade and provides an environment for bacteria, algae and fungi to grow. Tap water, which may contain a variety of organisms, should not be used in floatation bath. Fresh distilled water should be used and changed at the start of the day.

Floatation baths should be cleaned daily and left empty overnight. Bacterial and fungi will also grow in buffers, reagents and stains kept at an ambient temperature for long periods. Contamination of mounted tissue sections from washing or staining solutions will deposit organisms on top of the sections and above the focal plane of the section. Generally, deposition of organisms onto or under tissue section will be randomly distributed and not confined to areas of pathological significance or even to the section itself. If a contaminant is suspected, stains should be repeated after any potential sources of contamination have been discarded and reagents freshly prepared.

Immunohistology^{1,2}

The usefulness of the fluorescent antibody (FA) technique as a diagnostic and research tool in medical mycology has been fully established. It can be used for the rapid detection and identification of both viable and nonviable fungi in cultures and in most types of clinical materials.

Paraffin embedded tissues are adequate for direct fluorescent antibody (DFA) studies of fungi because the polysaccharide antigens in fungal cell walls are not destroyed by formalin fixation. DFA can greatly increase the accuracy of conventional histologic evaluations, especially when only atypical forms of a fungus are present.

A broad battery of sensitive and specific fluorescent antibody (FA) reagents is available for detecting and identifying many of the common pathogenic fungi. Basically, the FA technique is an immunochemical staining procedure. Fluorochrome or fluorescent dye is coupled with antibody so that the antigen - antibody reaction can be observed. The fluorochrome antibody complex, often referred to as labelled antibody or conjugate, fluoresces when examined under a fluorescence microscope.

MATERIAL AND METHODS

Study Design

Descriptive Study

Study Population

Patients of all age groups with fungal lesions attending the out patient and inpatient departments of Coimbatore Medical College and Hospital.

Sample size

40 cases

Study period

2 years (June 2007 – June 2009)

Inclusion Criteria

- Clinically suspected cases
- Incidental cases in biopsy from various sites

Exclusion Criteria

Nil

METHODOLOGY

A series of 6300 specimens received at Coimbatore Medical College and Hospital during the two years period from June' 07 to June' 09 was searched for fungal infections. Fungal infections were diagnosed in 50 cases by tissue examination on the basis of the type of reaction and morphological findings of the organism present. Male and female patients of all groups were included.

The formalin fixed specimens were received with complete clinical history.

A detailed clinical history like age, occupation, history of drugs such as chemotherapeutic drugs/ immunosuppressants, steroids and antibiotics, immunological status, chronic diseases like diabetes mellitus and bronchial asthma were obtained.

Required tissue bits were taken for processing. Using the paraffin embedded tissue blocks, routine hamatoxylin and eosin stain was put for all cases. Achieving a successful histopathological diagnosis begins with the selection of the tissue samples to be examined. Those obvious or suspected cases of fungal infections were subjected to special fungal stains.

The most commonly used special fungal stains were Gomori's methenamine silver stain, Periodic Acid Schiff's stain and Gridley's fungal stain. The fungal cell walls are rich in polysaccharides. In presence of chromic or Periodic acid, adjacent hydroxyl groups of the complex polysaccharites in fungal cell walls are oxidized to aldehydes. Microwave oven was used for GMS staining of tissues.

The other special stains used were Southgate's Mucicarmin stain, Brown and Brenn stain and India ink stain for specific fungal pathogens.

The major advantages of histopathology are speed, low cost and the ability to provide a presumptive identification of the fungus as well as demonstrating the tissue reaction. However, unless special techniques such as immunofluorescence are used, or the infecting fungus possesses unique structure such as spherules, definitive species identification of the aetiologic agent by histopathology is difficult.

The various staining procedures are given below:

I. HAEMATOXYLIN AND EOSIN STAINING

Fixation – 10% Formalin

Technic – Paraffin section cut at 6 microns

SOLUTION PREPARATION

Haematoxylin - 10gram.

Absolute alcohol - 100ml dissolve with light heat

Aluminum Potassium sulphate 200 gram dissolved in warm 2 liters of distilled water. Both are mixed and boiled : 5 gms of mercuric oxide is added while boiling and cooled after two minutes. Prior to use, 3 ml of acetic acid for 100 ml of hematoxylin is added.

1% ACID ALCOHOL

70% alcohol - 990 ml.

Con.HCl - 10 ml.

EOSIN

| | | |
|------------------------------|--|-----------|
| Eosin - 10 gram | | dissolved |
| D.H ₂ O - 100 ml. | | |

| | | |
|----------------------------|--|-----------|
| Phloxine 'B' - 100 mg | | dissolved |
| D.H ₂ O - 20 ml | | |

Both are mixed and 780 ml of 90% alcohol is added. 4 ml of glacial acetic acid and saturated Lithium carbonate are added.

PROCEDURE

1. The slide is kept in xylene for 15 minutes.
2. It is washed in graded alcohol absolute 90% : 80% each 2 dips
3. Slide is washed in water for 5 minutes.
4. Stained in haematoxylin for 5 minutes.
5. It is washed in water for 5 minutes.
6. Differentiated in 1% acid alcohol 2 dips
7. Washed in water for 2 minutes.
8. Twice dipped in Lithium carborate for blueing
9. Washed in water for 10 minutes
10. Dipped in 80% alcohol
11. Stained with eosin for 5 minutes.
12. Dehydrated in graded alcohol 80%, 90% then absolute alcohol
13. Cleared in xylene.
14. Mounted in D.P.X.

Result

Nuclei - Blue

Cytoplasm - Pink

II. GOMORI'S – METHENAMINE - SILVER NITRATE (GMS)**STAIN**

Gomori – Methenamine silver (GMS) method, the best of the special fungal stains for screening a tissue section was carried out in all the suspected cases and found positive in 49 cases.

PRINCIPLE

In the presence of chromic acid, adjacent hydroxyl groups of the complex polysaccharides in fungal cell walls are oxidized to aldehydes. The aldehyde reduces the methenamine silver nitrate complex, resulting in the brown – black staining of fungal cell walls due to the deposition of reduced silver wherever aldehyde is located. The depth of the colour produced depends on the amount of aldehyde present.

TECHNIQUE:

Fixation - formaline 10 percent

Technique - paraffin, celloidin or frozen sections

Solutions

1. 5 percent chromic acid

Chromic acid – 5 gm

Distilled water – 100 cc

2. 5% silver nitrate solution

Silver nitrate – 5 gm

Distilled water -100cc

3. 3% Methenamine solution

Hexamethylenetetramine - 3 gm

Distilled water - 100cc

4. 5% Borax

Borax - 5 gm

Distilled water - 100cc

5. Stock Methenamine - Silver nitrate solution
 - Silver nitrate 5% - 5 cc
 - Methenamine 3% - 100 cc

6. Stock Methenamine - Silver nitrate solution
 - Silver nitrate 5% - 5 cc
 - Methenamine 3% - 100 cc

7. Working methenamine silver nitrate Solution
 - Borax 5% solution - 2 cc
 - Distilled water - 25 cc
 - Methenamine silver Nitrate stock solution - 25 cc

8. 1% sodium bisulfite
 - Sodium bisulfite - 1 gm
 - Distilled water - 100 cc

9. 0.1% gold chloride
 - Gold chloride 1% Solution - 10 cc
 - Distilled water - 90 cc

10. 2% sodium thiosulfate
 - Sodium thiosulfate - 2 gm
 - Distilled water - 100 cc

11. Light green - stock

Light green SF (yellow) - 0.2 gm

Distilled water - 100 cc

Glacial acetic acid - 0.2 cc

12. Working light green solution

Light green stock - 10.0 cc

Distilled water - 90.00 cc

PROCEDURE:

1. Sections are deparaffinised through 2 changes of xylene, solute and 95 percent alcohol to distilled water.
2. Oxidised in 5 percent chromic acid solution for 1 hr.
3. Washed in running tap water for a few seconds
4. Rinsed in 1 percent sodium bisulfite for 1 min to remove any residual chromic acid.
5. Washed with 3 – 4 changes of distilled water.
6. Placed in working methenamine silver nitrate solution in microwave oven at 58 degree to 60 degrees for 30 – 60 min, until section turns yellowish brown. Paraffin coated forceps is used to remove slide from this solution; slide is dipped in distilled water and checked for adequate silver impregnation with microscope. Fungi should be dark brown at this stage. Rinsed in 6 changes of distilled water
7. Toned in 0.1 percent gold chloride solution of 2 – 5 min
8. Rinsed in distilled water

9. Unreduced silver is removed with 2 percent sodium thiosulfate solution for 2 – 5 min
10. Washed thoroughly in tap water
11. Counterstained with working light green solution for 30 – 45 sec.
12. Dehydrated with 2 changes of 95 percent alcohol, absolute alcohol, clear with 2 – 3 changes of xylene and mount in DPX.

RESULTS:

- Fungi - Sharply delineated in black
- Mucin - Dark grey
- Inner parts of mycelia and hyphae – old rose
- Background - Pale green

III. PERIODIC ACID SCHIFFS REAGENT STAIN (PAS)

- Fixation - 10% Formalin, Alcohol, Buffered Formalin.
- Technique - Paraffin Section cut at 6 microns

SOLUTION PREPARATION

1% Periodic Acid

Periodic Acid - 1 gram

D.H₂O - 100 cc

SCHIFFS REAGENT

In 100ml of warm distilled water 5 gram of Basic fuchsin is added and allowed to boil. It is then cooled and 10 gram of potassium metabisulphite and 50ml of 1 Normal HCL 50ml are added and kept in dark place over night (24 hours) Then 25 gram of charcoal powder is added, shaken and kept in dark place for 2 hours. It is filtered and stored in fridge.

1. NORMAL HCL

HCL - 8.35ml

D.H₂O - 91.65 ml

PROCEDURE

1. The slide is deparaffinised in 15 ml of xylene.
2. Washed in Graded alcohol ab.90%,80% each 2 dips
3. Washed on H₂O for 5
4. Placed in 1% periodic acid 5 minutes
5. Washed in H₂O for 5
6. Placed in Schiffs reagent 15 minutes
7. Washed in H₂O for 10
8. Stained in Haematoxylin for 3 minutes
9. Then washed in H₂O for 2
10. Differentiated in 1% acid alcohol 3 dips
11. Washed in H₂O for 2
12. Lithium carbonate 1 dip
13. Washed in H₂O
14. Dehydrated (80%, 90%, Alcohol) cleared and mounted

Result

Glycogen, mucin, reticulin, Basement membranes, amyloid and other elements may show a positive reaction – rose to purplish red

Nuclei - Blue

Fungi - Red.

PAS WITH DIASTASE

Before step 4 saliva is put or 0.5% diastase for 45minutes. Then the PAS reaction is continued.

RESULT

Glycogen - Negative

PRINCIPLE

The Principle of the reaction is that periodic acid will bring about oxidative cleavage of the carbon bond in 1-2 Glycols or their amino or alkylamino derivative, to form dialdehydes. These aldehydes will react with fuchsin – Sulfurous acid which combines with Basic fuchsin to form a magenta colour compound.

IV. BROWN&BRENN GRAM'S STAINING METHOD

FIXATION

Formalin, 10% Buffer Neutral.

SECTION

Paraffin sections, 6 microns.

STAINING PROCEDURE

1. Deparaffinized and hydrated to distilled water.
2. 1.0ml Crystal Violet, 1% Aq., with 5 drops Sodium Bicarbonate, 5%, Aq., is mixed and pour onto slides held in a staining rack. Agitated gently to cover section. Slides are stained for 1 min. Rinsed in distilled water.
3. Flooded with Gram's Iodine, 1 min., rinsed with water and carefully blotted with filter paper to dryness.

4. Decolorized with Acetone – Alcohol, 1: 1 by dropping onto the slide until no more color run off.
5. Stained in the basic fuchsin working, or (dilute one vol. Basic fuchsin stock, 0.25%, Aq., with 10 vol. distilled water), 1min; washed in water, blotted carefully but not to complete dryness as in setp
6. Differentiated in Acetone, i.e. one quick dip, then transferred immediately to the picric acid – Acetone solution, 0.1%, to complete. Differentiated until sections show yellowish – pink.
7. Rinsed quickly in Acetone, then Acetone – Xylene, Cleared in 3 – 4 changes Xylene, alone.
8. Mounted

Results

| | |
|---|--------|
| Gram + ve, Nocardia and Actinomyces Filaments | Blue |
| Gram – Ve, Nuclei | Red |
| Additional tissue elements | Yellow |

V. SOUTHGATE’S MUCICARMINE STAIN

PURPOSE:

The Mucicarmine is useful in detecting the mucin secreted by intestinal epithelial cells in inflammation and intestinal carcinomas. It is also useful in staining encapsulated fungi such as Cryptococcus and Rhinosporidiosis.

PRINCIPLE:

Aluminium is believed to form a chelation complex with the carmine, changing the molecule to a positive charge allowing it to bind with the acid substrates of low density such as mucins.

CONTROL: small intestine

FIXATIVE: 10% buffered formalin.

TECHNIQUE: Cut paraffin sections 4 – 5 microns.

EQUIPMENT:

Glassware is rinsed in distilled water. Stirring hot plate, magnetic stir bars, 500ml beaker, coplin jars, microwave oven.

REAGENTS:

Southgate's Mucicarmine Solution

| | |
|--------------------|---------|
| Carmine, alum lake | 1.0 gm |
| Aluminum hydroxide | 1.0 gm |
| 50% alcohol | 100.0ml |
| Mix well, add: | |
| Aluminum chloride, | |
| Anhydrous | 0.5 gm |

Boiled gently for 21/2 minutes. Cooled, filtered, refrigerated. Good for 6 months.

Metanil Yellow Solution:

| | |
|---------------------|---------|
| Metanil yellow | 0.25gm |
| Distilled water | 100.0ml |
| Glacial acetic acid | 0.25 ml |

Mixed well.

Aluminum chloride is added gently because it is water reactive.

PROCEDURE:

1. Deparaffinized and hydrated to distilled water
2. Mayer's hematoxylin for 10 minutes
3. Washed in running water for 5 minutes
4. Mucicarmine solution, microwave HI power, 45 seconds.
5. Rinsed quickly in distilled water.
6. Metanil yellow, 30 seconds to 1 minute.
7. Dehydrated quickly in three changes of absolute alcohol, clear and coverslip in permount.

Conventional method: Mucicarmine solution at room temperature for 1 hour.

RESULTS:

| | |
|-----------------------|-----------|
| Mucin | deep rose |
| Nuclei | black |
| Other tissue elements | yellow |

OBSERVATION AND RESULTS

Of the 6,300 materials received in the department of pathology, Coimbatore Medical College and Hospital during the study period from June'07 to June'09, nearly 300 histopathologically suspicious slides (on routine H & E staining) were subjected to special fungal stains. Of them 50 positive cases were taken up for the study.

Most common age group for fungal infection in this study was above 40 years. The mean age of presentation was 38.2 years. Men predominated (54%) over women who accounted for only 23% of cases. Of all the cases included in this study, the incidence of *Aspergillus* (46%) was more than that of the other fungal species. Next in our series were *Actinomycosis*, *Rhinosporidiosis*, *Candida*, *Mucormycosis*, *Mycetoma*, *Cryptococcus neoformans* and *Streptomyces somaliensis* in the descending order.

The common site of infection was Head & Neck region (72%) which included the nasal cavity, paranasal sinus, ear and oral cavity among which nasal cavity was the commonest site (30%). Extremities accounted for the next higher incidence of involvement (10%). The other sites involved were GIT, Lung, CNS, Ovary, Spine, Kidney, Scalp and Skin which totally accounted for 20% of cases.

Three unusual sites were affected; peculiar modes of presentation seen were cutaneous *Rhinosporidiosis*, Ovarian *Actinomycosis* and GIT *Mucormycosis*.

All the subjects in this study group except one (*Crptococcus* identified in CSF) were subjected to GMS staining. According to the type of fungus identified in GMS stain, PAS stain was carried out. Brown and Brenn stain was used for *Actinomycosis* and *Mycetoma*. Mucicarmine stain was used for *Rhinosporidiosis* and *Cryptococcus neoformans*.

TABLE – 1
AGEWISE DISTRIBUTION

| Age group in yrs | No.of Patients (n = 50) | Percent |
|-------------------------|------------------------------------|----------------|
| 0-20 Yrs | 7 | 14.0 |
| 21-30 Yrs | 13 | 26.0 |
| 31-40 Yrs | 12 | 24.0 |
| > 40 Yrs | 18 | 36.0 |

The age of presentation ranged from 1st to 7th decade of life. Most common age group was above 40 years. The mean age of presentation was 38.2 years.

TABLE - 2
GENDERWISE DISTRIBUTION

| Sex | Number of Patients (n = 50) | Percentage |
|------------|--|-------------------|
| Male | 28 | 56 |
| Female | 22 | 44 |

The sex distribution of fungal infections in this study showed a higher predilection for men (56%) as compared to women (44%) with the male to female ratio being 1.3 : 1 .

TABLE – 3
DISTRIBUTION OF ORGANS INVOLVED

| S.No | Site | Number of Patients (n = 50) | Percentage |
|-------------|--------------|--|-------------------|
| 1 | Extremities | 3 | 6 |
| 2 | Oral cavity | 8 | 16 |
| 3 | Nasal cavity | 15 | 30 |
| 4 | Sinus | 7 | 14 |
| 5 | Lung | 1 | 2 |
| 6 | Ear | 7 | 14 |
| 7 | GIT | 2 | 4 |
| 8 | CNS | 1 | 2 |
| 9 | Ovary | 1 | 2 |
| 10 | Eye | 1 | 2 |
| 11 | Spine | 1 | 2 |
| 12 | Kidney | 1 | 2 |
| 13 | Scalp | 1 | 2 |
| 14 | Skin | 1 | 2 |

Most common organ affected with fungal infection was nasal cavity (30%), followed by oral cavity, sinus, ear, extremities, GIT, lung, CNS, ovary, eye, spine, kidney, scalp, skin.

TABLE – 4
SITewise DISTRIBUTION

| S. No | Site | Number of Patients (n = 50) | Percentage |
|--------------|--|-------------------------------------|-------------------|
| 1 | Nasal / maxillary sinus / lung | 23 | 46.0 |
| 2 | Ear | 7 | 14.0 |
| 3 | Tonsil / Tongue / Cheek / Vocal cord / Oesophagus /GIT | 10 | 20.0 |
| 4 | Ovary / Kidney | 2 | 4.0 |
| 5 | Scalp / Forearm / Thigh / Foot | 5 | 10.0 |
| 6 | Spine / CNS / Eye ball | 3 | 6.0 |

The respiratory tract and paranasal sinuses comprising the nasal cavity, lung and maxillary sinus were predominantly involved by the fungal lesions (46%).

TABLE – 5
INCIDENCE OF FUNGUS

| S.No | Type of Fungal Lesion | Number of patients (n = 50) | Percentage |
|-------------|------------------------------|--|-------------------|
| 1 | Aspergillus | 22 | 44 |
| 2 | Rhinosporidiosis | 8 | 16 |
| 3 | Actinomycosis | 8 | 16 |
| 4 | Candida | 3 | 6 |
| 5 | Mucormycosis | 3 | 6 |
| 6 | Mycetoma | 2 | 4 |
| 7 | Streptomyces | 1 | 2 |
| 8 | Cryptococcus | 1 | 2 |
| 9 | Mixed infections | 2 | 4 |

The most common pathogen was Aspergillus (44%), followed by Actinomycosis, Rhinosporidiosis, Candida, Mucormycosis, Mycetoma, Streptomyces somaliensis, Cryptococcus neoformans.

TABLE – 6
DISTRIBUTION OF FUNGAL INFECTION IN THE HEAD AND NECK
REGION

| S. No | Fungal Infections | Site | Number of Patients (n = 40) | Percentage |
|-------|-------------------------|---|--------------------------------|------------|
| 1 | Aspergillus | Nasal (8) Ear (5) Oral (1) Sinus (7) | 21 | 52.5 |
| 2 | Rhinosporidiosis | Nasal | 7 | 17.5 |
| 3 | Actinomycosis | Oral (6) Scalp (1) | 7 | 17.5 |
| 4 | Candida | Oral | 1 | 2.5 |
| 5 | Mucormycosis | Eye | 1 | 2.5 |
| 6 | Cryptococcus neoformans | CNS | 1 | 2.5 |
| 7 | Mixed infections | Ear | 2 | 5.0 |

The most common site involved by fungal infection in the head & neck region was the nasal cavity followed by sinus, ear, oral cavity, eye and CNS. The commonly detected pathogen was found to be Aspergillus (52.5%), followed by Actinomycosis, Rhinosporidiosis, Candida, Mucormycosis, Cryptococcus neoformans.

TABLE – 7
OCCUPATIONWISE DISTRIBUTION

| S. No | Occupation | Number of Patients (n = 50) | Percentage |
|--------------|--------------------------|--|-------------------|
| 1 | Manual Labourer / Farmer | 11 | 22.0 |
| 2 | Industrial Worker | 28 | 56.0 |
| 3 | Student | 11 | 22.0 |

The most common population in the affected were Industrial workers (56%) followed by farmers (22%) and students (22%).

TABLE - 8
IMMUNOLOGICAL STATUS

| S. No | Immunological status | Number of Patients (n = 50) | Percentage |
|-------|----------------------|--------------------------------|------------|
| 1 | Immunosuppressed | 19 | 38.0 |
| 2 | Immunocompetent | 31 | 62.0 |

TABLE - 9
IMMUNOLOGICAL STATUS

| S. No | Immunological status | Number of Patients | Percentage |
|-------|----------------------------|--------------------|------------|
| 1 | Malignancy | 5 | 10 |
| 2 | HIV | 3 | 6 |
| 3 | Diabetes mellitus | 8 | 16 |
| 4 | Steroid therapy | 2 | 4 |
| 5 | Organ transplant recipient | 1 | 2 |
| 6 | Immunocompetant | 31 | 62 |

The fungal infection was more commonly seen in immunocompetant patients (62%), compared with the immunocompromised patients. Among the immunocompromised patients fungal infections were common in diabetes mellitus, followed by malignancy, HIV disease, Steroid therapy and organ transplant recipient.

TABLE - 10
SPECTRUM OF FUNGAL INFECTIONS AMONG SUBJECTS IN WHOM
CULTURE WAS DONE

| S. No | Histological Diagnosis / Clinical Diagnosis | Number of Patients (n =23) | Culture Done | Name of Organism Cultured |
|--------------|--|-----------------------------------|---------------------|----------------------------------|
| 1 | Otomycosis | 6 | 3 | Aspergillus niger |
| 2 | Allergic fungal rhinosinusitis (AFRS) | 10 | 3 | Aspergillus fumigatus |
| 3 | Fungal ball / Sinus mycetoma | 3 | 2 | Aspergillus fumigatus |
| 4 | Invasive fungal sinusitis (Nasal cavity) | 1 | 1 | Aspergillus flavus |
| 5 | Soft tissue mass (Thigh), Tumour (GIT) | 2 | 1 | Rhizopus |
| 6 | Meningitis (CNS) | 1 | 1 | Cryptococcus neoformans. |

Among the organisms cultured, *Aspergillus niger* was most commonly seen in otomycosis. *Aspergillus fumigatus* was predominantly seen in nasal cavity and paranasal sinuses and *Cryptococcus neoformans* in meningitis.

TABLE - 11
INVASIVE FUNGAL INFECTIONS

| S. No | Site | Type of Fungal Infection | Number of Patients (n = 4) |
|--------------|------------------------------|---|--|
| 1 | Maxillary sinus | Invasive fungal rhinosinusitis-Aspergillus | 1 |
| 2 | Soft tissue mass in thigh | Mucormycosis | 1 |
| 3 | Gastrointestinal tract | Mucormycosis | 1 |
| 4 | Eye | Mucormycosis | 1 |

In the present study 8% of patients were found to have invasive fungal infections.

Mucormycosis was found to be the predominant fungal infection (i.e) 3 out of 4 cases.

TABLE – 12
HISTOLOGICAL REACTIONS
(n = 49 Cases)

| S.No | Type of Fungus | Severe Necrosis | Chronic non Suppurative NonTuberculoid Inflammation | Pyogenic | Mixed Pyogenic & Granulomatous | Histiocytic Granuloma | Granuloma with Necrosis | Granuloma 'SARCOID' Type | Foreign Body Giant Cell Reaction |
|------|------------------|-----------------|---|----------|--------------------------------|-----------------------|-------------------------|--------------------------|----------------------------------|
| 1 | Aspergillus | 13 | 3 | - | - | - | 3 | 1 | 2 |
| 2 | Candida | 2 | - | - | 1 | - | - | - | - |
| 3 | Rhinosporidium | - | 5 | 1 | - | - | - | 1 | 1 |
| 4 | Actinomycosis | 2 | - | 2 | 4 | - | - | - | - |
| 5 | Mycetoma | - | - | - | 2 | - | - | - | - |
| 6 | Streptomyces | - | - | - | - | - | - | - | 1 |
| 7 | Mucormycosis | 2 | - | - | - | - | - | - | 1 |
| 8 | Mixed infections | - | 2 | - | - | - | - | - | - |

The Fungal lesions predominantly showed necrosis. The various reactions showed by the fungal lesions were non specific.

TABLE – 13
SPECIAL STAIN STUDY

| S.No | Type of Fungus | GMS | PAS | BB | Mucicarmine | India Ink |
|------|------------------|-----|-----|----|-------------|-----------|
| 1 | Aspergillus | + | + | -- | -- | -- |
| 2 | Rhinosporidiosis | + | + | -- | + | -- |
| 3 | Actinomycosis | + | -- | + | -- | -- |
| 4 | Candida | + | + | -- | -- | -- |
| 5 | Mucormycosis | + | + | -- | -- | -- |
| 6 | Mycetoma | + | -- | + | -- | -- |
| 7 | Streptomyces | + | -- | -- | -- | -- |
| 8 | Cryptococcus | -- | -- | -- | + | + |

The Aspergillus, Mucormycosis and Candida showed positive staining with GMS and PAS.

1

The Streptomyces somaliensis showed positive staining with GMS.

The Actinomycosis and Mycetoma showed positive Gram staining with Brown and Brenn (B & B) apart from positive staining with GMS.

The Rhinosporidiosis showed positive staining with GMS, PAS and Mucicarmine

The Cryptococcus showed positive staining with Mucicarmine and India ink.

TABLE - 14
ASSOCIATION BETWEEN SITE OF FUNGAL LESIONS AND AGE

| S.No | Site | Age | | | | | | | | TOTAL | |
|------|--|----------|-----|-----------|------|-----------|------|----------|------|-------|-----|
| | | 0-20 YRS | | 21-30 YRS | | 31-40 YRS | | > 40 YRS | | No. | % |
| | | No. | % | No. | % | No. | % | No. | % | | |
| 1 | Nasal / maxillary sinus / lung | - | - | 5 | 38.5 | 8 | 66.7 | 10 | 55.6 | 23 | 46 |
| 2 | Ear | - | - | 4 | 30.8 | - | - | 3 | 16.7 | 7 | 14 |
| 3 | Tonsil / Tongue / Cheek / Vocal cord / Oesophagus /GIT | 4 | 57 | 2 | 15.4 | 2 | 16.7 | 2 | 11.1 | 10 | 20 |
| 4 | Ovary / Kidney | - | - | - | - | 1 | 8.3 | 1 | 5.6 | 2 | 4 |
| 5 | Scalp / Forearm / Thigh / Foot | 2 | 29 | 2 | 15.4 | - | - | 1 | 5.6 | 5 | 10 |
| 6 | Spine / CNS / Eye ball | 1 | 14 | - | - | 1 | 8.3 | 1 | 5.6 | 3 | 6 |
| | TOTAL | 7 | 100 | 13 | 100 | 12 | 100 | 18 | 100 | 50 | 100 |

Fungal infections involving nose, Para nasal sinuses and lung were observed in 66.7% of patients of age group 31 – 40 years, 55.6% of patients of age group of above 40 years and 38.5% of 21 – 40 years age group. However this was not found to be statistically significant (P value > 0.05).

TABLE - 15
ASSOCIATION BETWEEN FUNGAL LESIONS AND VARIOUS AGE
GROUPS

| S.No | Diagnosis | Age | | | | | | | | TOTAL | |
|------|------------------|----------|------|-----------|------|-----------|------|----------|------|-------|-----|
| | | 0-20 YRS | | 21-30 YRS | | 31-40 YRS | | > 40 YRS | | No. | % |
| | | No. | % | No. | % | No. | % | No. | % | | |
| 1 | Actinomycosis | 5 | 71.4 | 1 | 7.7 | 1 | 8.3 | 1 | 5.6 | 8 | 16 |
| 2 | Aspergillus | - | - | 5 | 38.4 | 5 | 41.7 | 12 | 66.7 | 22 | 44 |
| 3 | Candidiasis | - | - | - | - | 2 | 16.7 | 1 | 5.6 | 3 | 6 |
| 4 | Cryptococcus | - | - | - | - | 1 | 8.3 | - | - | 1 | 2 |
| 5 | Mucormycosis | 1 | 14.3 | - | - | - | - | 2 | 11.1 | 3 | 6 |
| 6 | Mycetoma | 1 | 14.3 | 1 | 7.7 | - | - | - | - | 2 | 4 |
| 7 | Rhinosporidiosis | - | - | 3 | 23.1 | 3 | 25 | 2 | 11.1 | 8 | 16 |
| 8 | Streptomyces | - | - | 1 | 7.7 | - | - | - | - | 1 | 2 |
| 9 | Mixed infection | - | - | 2 | 15.3 | - | - | - | - | 2 | 4 |
| | TOTAL | 7 | 100 | 13 | 100 | 12 | 100 | 18 | 100 | 50 | 100 |

Actinomycosis was observed in 71.4% of study subjects belonging to the age group < 20 years, whereas Aspergillosis was observed in 38.4% of patients of 21 – 30 years age group, 41.7% of 31 – 40 years age group and 66.7% of above 40 years age group ($P < 0.01$).

TABLE - 16
ASSOCIATION BETWEEN THE TYPE OF FUNGAL LESIONS AND
GENDER

| S.No | Diagnosis | Gender | | | | TOTAL | |
|------|------------------|--------|------|--------|------|-------|-----|
| | | Male | | Female | | No. | % |
| | | No. | % | No. | % | | |
| 1 | Actinomycosis | 3 | 10.7 | 5 | 22.7 | 8 | 16 |
| 2 | Aspergillus | 11 | 39.2 | 11 | 50 | 22 | 44 |
| 3 | Candidiasis | 2 | 7.1 | 1 | 4.5 | 3 | 6 |
| 4 | Cryptococcus | 1 | 3.6 | - | - | 1 | 2 |
| 5 | Mucormycosis | 3 | 10.7 | - | - | 3 | 6 |
| 6 | Mycetoma | 2 | 7.1 | - | - | 2 | 4 |
| 7 | Rhinosporidiosis | 5 | 17.9 | 3 | 13.6 | 8 | 16 |
| 8 | Streptomyces | - | - | 1 | 4.5 | 1 | 2 |
| 9 | Mixed infection | 1 | 3.5 | 1 | 4.5 | 2 | 4 |
| | TOTAL | 28 | 100 | 22 | 100 | 50 | 100 |

There was no significant association between the type of fungal lesion and gender ($P > 0.05$).

TABLE - 17
ASSOCIATION BETWEEN SITE OF FUNGAL LESION AND GENDER

| S. No | Site | Gender | | | | TOTAL | |
|-------|--|--------|------|--------|------|-------|-----|
| | | Male | | Female | | No. | % |
| | | No. | % | No. | % | | |
| 1 | Nasal / maxillary sinus / lung | 14 | 50 | 9 | 40.9 | 23 | 46 |
| 2 | Ear | 3 | 10.7 | 4 | 18.2 | 7 | 14 |
| 3 | Tonsil / Tongue / Cheek / Vocal cord / Oesophagus /GIT | 5 | 17.9 | 5 | 22.7 | 10 | 20 |
| 4 | Ovary / Kidney | 1 | 3.6 | 1 | 4.5 | 2 | 4 |
| 5 | Scalp / Forearm / Thigh / Foot | 2 | 7.1 | 3 | 13.6 | 5 | 10 |
| 6 | Spine / CNS / Eye ball | 3 | 10.7 | - | - | 3 | 6 |
| | TOTAL | 28 | 100 | 22 | 100 | 50 | 100 |

The site of fungal lesions had no significant association with gender (P value > 0.05).

TABLE - 18
ASSOCIATION BETWEEN OCCUPATION AND TYPE OF FUNGAL
LESION

| S. No | Diagnosis | Occupation | | | | | | TOTAL | |
|-------|------------------|---------------|------|-------------|------|---------|------|-------|-----|
| | | Coolie/Farmer | | Ind. Worker | | Student | | No. | % |
| | | No. | % | No. | % | No. | % | | |
| 1 | Actinomycosis | 2 | 18.2 | 1 | 3.6 | 5 | 45.5 | 8 | 16 |
| 2 | Aspergillus | 7 | 63.6 | 13 | 46.4 | 2 | 18.2 | 24 | 48 |
| 3 | Candidiasis | 1 | 9.1 | 2 | 7.1 | - | - | 3 | 6 |
| 4 | Cryptococcus | - | - | 1 | 3.6 | - | - | 1 | 2 |
| 5 | Mucormycosis | 1 | 9.1 | 1 | 3.6 | 1 | 9.1 | 3 | 6 |
| 6 | Mycetoma | - | - | 1 | 3.6 | 1 | 9.1 | 2 | 4 |
| 7 | Rhinosporidiosis | - | - | 8 | 28.6 | - | - | 8 | 16 |
| 8 | Streptomyces | - | - | 1 | 3.6 | - | - | 1 | 2 |
| 9 | Mixed infections | - | - | - | - | 2 | 18.2 | - | - |
| | TOTAL | 11 | 100 | 28 | 100 | 11 | 100 | 50 | 100 |

Aspergillosis was observed in 63.6% of farmers, 46.4% of Industrial workers and 18.2% of students. Actinomycosis was observed predominantly in students. However, this association was not statistically significant. ($P > 0.05$).

TABLE - 19
ASSOCIATION BETWEEN SITE OF FUNGAL LESIONS AND
OCCUPATION

| S. No | Site | Occupation | | | | | | TOTAL | |
|-------|--|-----------------------|------|-------------|------|---------|------|-------|-----|
| | | Manual Labour /Farmer | | Ind. Worker | | Student | | No | % |
| | | No | % | No | % | No | % | | |
| 1 | Nasal / maxillary sinus / lung | 5 | 45.5 | 18 | 64.3 | - | - | 23 | 46 |
| 2 | Ear | 2 | 18.2 | 1 | 3.6 | 4 | 36.4 | 7 | 14 |
| 3 | Tonsil / Tongue / Cheek / Vocal cord / Oesophagus /GIT | 2 | 18.2 | 4 | 14.3 | 4 | 36.4 | 10 | 20 |
| 4 | Ovary / Kidney | 2 | 18.2 | - | - | - | - | 2 | 4 |
| 5 | Scalp / Forearm / Thigh / Foot | - | - | 3 | 10.7 | 2 | 18.2 | 5 | 10 |
| 6 | Spine / CNS / Eye ball | - | - | 2 | 7.1 | 1 | 9.1 | 3 | 6 |
| | | 11 | 100 | 28 | 100 | 11 | 100 | 50 | 100 |

Sino nasal fungal lesions were observed in 64.3% of Industrial worker and 45.5% of farmers. This was found to be statistically significant ($P < 0.01$).

TABLE - 20
ASSOCIATION BETWEEN TYPE OF FUNGAL LESION AND
IMMUNOLOGICAL STATUS

| S.No | Diagnosis | Immunological status | | | | TOTAL | |
|------|------------------|----------------------|------|------------------|------|-------|-----|
| | | Immuno suppressed | | Immuno competant | | No. | % |
| | | No. | % | No. | % | | |
| 1 | Actinomycosis | 2 | 10.5 | 6 | 19.4 | 8 | 16 |
| 2 | Aspergillus | 7 | 36.8 | 15 | 48.3 | 22 | 44 |
| 3 | Candidiasis | 3 | 15.8 | - | - | 3 | 6 |
| 4 | Cryptococcus | 1 | 5.3 | - | - | 1 | 2 |
| 5 | Mucormycosis | 3 | 15.8 | - | - | 3 | 6 |
| 6 | Mycetoma | - | - | 2 | 6.5 | 2 | 4 |
| 7 | Rhinosporidiosis | 3 | 15.8 | 5 | 16.1 | 8 | 16 |
| 8 | Streptomyces | - | - | 1 | 3.2 | 1 | 2 |
| 9 | Mixed infection | - | - | 2 | 6.4 | 2 | 4 |
| | TOTAL | 19 | 100 | 31 | 100 | 50 | 100 |

Aspergillosis was observed in 48.3% of immunocompetant and 36.8% of immunosuppressed individuals. This was found to be statistically significant ($P < 0.05$).

DISCUSSION

The increased incidence of systemic fungal infections in the past two decades has been overwhelming. However, starting from the 1960's, opportunistic fungi started causing more number of infections, especially in the immunocompromised host. The world health organization and the various ministries of the health have yet to designate a single mycosis as a reportable disease. Hence data on their incidence and prevalence is unavailable. However the present study revealed an incidence of 0.79% of fungal lesions in surgical pathology specimens received in the department of pathology, Coimbatore medical college hospital during the study period from June'07 to June'09

The present study shows that the most common age group affected by fungal lesions was above 40 years. The mean age of presentation was 38.2 years. In a similar study on fungal infections of para nasal sinuses by Jorge.A.Ferrera et al⁶⁵ the mean age was 64 years i.e in the elderly population. However, in a study by U.Zafer et al⁶⁴ on fungal infections of nasal cavity and paranasal sinuses, the mean age of presentation was 22.5 years, thus indicating that the majority of patients were young adults. Fungal infections involving the nose, paranasal sinuses and lung were observed in 66.7% of patients of age group 31 – 40 years, 55.6% of patients of age group of above 40 years and 38.5% of 21 – 40 years age group. However this was not found to be statistically significant. (P value > 0.05).

Actinomycosis was observed in 71.4% of study subjects belonging to the age group < 20 years, whereas aspergillosis was observed in 53.8% of patients of 21 – 30 years age group, 41.7% of 31 – 40 years age group and 66.7% of above 40 years age

group (P value < 0.01). The higher incidence of fungal infections in the elderly is probably due to the normal defence mechanisms being abrogated. Aging is associated with the decline in immune function, termed immune senescence and this is likely to contribute significantly to the increase in susceptibility to fungal infections in the elderly.

The present study shows that the fungal infections were predominantly seen in men (56%) as compared to women (44%) with a male to female ratio being 1.3 : 1. In a similar study on mycotic lesion by Salwa.S.Shikh et al⁶³ the fungal lesions were seen predominantly in men over women in a ratio of 2 : 1. In a study on fungal infections of nasal cavity and paranasal sinuses by U.Zafar et al⁶⁴ fungal infections had a stronger predilection for men as compared to women with a male to female ratio being 1.7 : 1. The high incidence of fungal infections in men is attributed to the fact that a large proportion of the population is made up of outdoor labourers. However, in a study on paranasal sinus fungal ball by Jorge.A.Ferrerio et al⁶⁵, the infections occurred predominantly in elderly women. As regards the association between the type of fungal lesions and gender, there was no significant association (P value > 0.05). Also, the site of fungal lesions had no significant association with gender (P value > 0.05).

The present study shows that the fungal infections were predominantly seen in industrial workers (56%), followed by farmers (22%) and students (22%). In contrast, in a study by J.H.Makannavar et al⁶⁶ on Rhinosporidiosis, the patients affected by fungal infections were predominantly farmers (68%). Since the study population predominantly included patients from urban areas, industrial workers were probably found to be more affected by fungal lesions than farmers. As regards, the association

between occupation and type of fungal lesions, aspergillosis was observed in 63.6% of farmers, 46.4% of industrial workers and 36.4% of students. Actinomycosis was observed predominantly in students. However, this association was not statistically significant (P value > 0.05). Sino nasal fungal lesions were observed in 64.3% of industrial workers and 45.5% of farmers. This was found to be statistically significant (P value < 0.01).

In the present study the predominant site of fungal infections was the nasal cavity (30%). The respiratory tract and the paranasal sinuses comprising of the nasal cavity, lung and maxillary sinus were predominantly involved by the fungal lesions (46%). The most common site involved by fungal infection in the head & neck region was the nasal cavity followed by sinus, ear, oral cavity, eye and CNS. The commonly detected pathogen was found to be *aspergillus* (57.5%), followed by Actinomycosis, Rhinosporidiosis, *Candida*, Mucormycosis, *Cryptococcus neoformans*.

The present study shows that the fungal infections were predominantly seen in immunocompetent patients (62%) compared with the immunocompromised patients. Among the immunocompromised patients fungal infections was common in diabetes mellitus (16%) followed by malignancy, HIV infections, steroid therapy and organ transplantation. Aspergillosis was observed in 54.8% of immunocompetent and 36.8% of immunosuppressed individuals. This was found to be statistically significant (P value < 0.05). No significant association was observed between the site of fungal lesion and immunological status (P value > 0.05). Invasive fungal lesions are prone to occur in immunocompromised individuals whereas superficial mycoses tend to occur in the immunocompetent. The present study predominantly showed superficial

mycoses. Hence it is not surprising to note that immunocompetant individuals formed the predominant group.

In the present study the various fungal lesions identified in different sites were Aspergillosis, Rhinosporidiosis, Actinomycosis, Candida, Mucormycosis, Mycetoma, Streptomyces and Cryptococcus. The most common fungal infections were **Aspergillosis** (24%). The common sites involved by the Aspergillus species are the nasal cavity (30%) and para nasal sinuses (14%). The other sites involved by the aspergillosis are the ear and lung. **Nasal cavity and paranasal aspergillosis** presented as nasal polyp in 10 subjects and as invasive aspergillosis in one subject. In a study by A.Ravikumar et al⁷⁰, allergic fungal sinusitis is to be considered as an important differential diagnosis in patients with sinonasal polyposis. Immunocompetant subjects were predominantly affected. Among the four immunocompromised patients, two patients had diabetes mellitus and two had HIV infection.

Otomycosis is a subacute or chronic superficial fungal infection of the external auditory canal. In the present study all the subjects with otomycosis i.e seven subjects (14%) were found to be infected with aspergillosis. Among the seven subjects with otomycosis six were immunocompetant and one was immunocompromised (steroid therapy). In a study by James Fasulna et al⁷¹ on 1389 subjects with otitis externa it was found that 6.54% of patients had otomycosis. Immunocompromised patients were found to be more prone for otomycosis, the common predisposing factors being diabetes mellitus, malignancy and HIV infections. **Pulmonary Aspergillosis** was found in one subject in the present study. The patient had squamous cell carcinoma of the lung and was on chemotherapy.

The most common histological reaction shown by *Aspergillus* in the study was severe granular necrosis (15 subjects). The septate hyphae with dichotomous branches at acute angles and uniform width were seen (fig.no.2). In three subjects with chronic inflammatory lesions, short globose and distorted hyphae were seen (fig.no.1). *Aspergillus niger* was detected in three subjects with otomycosis, *Aspergillus fumigatus* in three subjects with allergic fungal rhinosinusitis and two subjects with nasal fungal ball.

In the present study **Actinomycosis** was found in eight patients (16%). The predominant site involved was the tonsil (5 subjects). Among the five subjects with **tonsillar actinomycosis**, two were in the paediatric age group and three were adults. In a similar study by A.Aydin et al⁷⁴ in 1820 tonsillectomies, Actinomycosis was seen in 122 (6.7%) subjects. The rate of actinomycosis was significantly higher in adults than in children. In a study by Al-Tatari et al⁷⁷, Actinomycosis was more prevalent in the tissues of patients with obstructive tonsillar hypertrophy. All the five patients in the study with tonsillar Actinomycosis were immunocompetent.

In the present study one patient presented with **Pelvic actinomycosis**. The patient gave a history of intrauterine device (IUD) insertion for four years. The patient was a known diabetic admitted with abdominal mass and severe abdominal pain. The intra operative finding was a solid ovarian mass which mimicked carcinoma. This is comparable with Masahiro Iwasaki et al⁷⁸, who found that 85% of ovarian Actinomycosis occurred in women who have an IUD in place for three or more years. The aberration of the ovarian surface during ovulation makes it vulnerable to infections. In a study by Atay.Y.et al⁷⁹, three cases of ovarian Actinomycosis mimicked ovarian carcinoma. One patient with Actinomycosis in the cheek had squamous cell carcinoma and was on chemotherapy. Another patient with Actinomycosis presented as a growth in the scalp and was immunocompetent.

The most common histological picture seen was mixed pyogenic and granulomatous reaction (4 subjects). The inflammatory reaction in Actinomycosis is suppuration with formation of abscesses that contain one or more granules bordered by eosinophilic club like Splendore – Hoespli material, creating a sun burst appearance (fig. 16,17,18).

In the present study two subjects were identified with **Mycetoma** infections. One subject was a 13 year old male, a student with infection in the spine – D2 vertebra. He had history of injury in the spine with an open wound. In a study by Mohammed Abdul et al ⁸² on intraspinal Mycetoma, two cases were identified with Mycetoma infection which was confirmed by histopathological examination. The other subject was a 27 year old man, farmer by occupation. He had a chronic non healing ulcer in the foot with blackish discoloration. Both the above patients were immunocompetent. Patients with Mycetoma infection usually have a history of thorn prick or minor trauma which facilitate the entry of causative organism from soil into the subcutaneous tissue. The present study also showed comparable results. Both the subjects were found to have eumycotic Mycetoma.

In a similar study by Abbasher Hussein et al ⁸¹ on 30 subjects with mycetoma infection, commonest site for infection was the foot, most of the lesions were seen on the dorsal aspect of the forefoot. The hand ranks as the second most common site. The most common histological presentation seen was mixed pyogenic and granulomatous reaction. Histologically, granules of actinomycotic Mycetoma contain delicate, gram positive, branched filaments whereas those of eumycotic Mycetoma contain broad, septate, fungal hyphae (Fig.No.19). The mycelium is distorted and bizarre in form and size.

In the present study a 30 year old woman, farmer by occupation presented with a chronic non healing ulcer in the foot. Histopathological examination revealed **Streptomyces somaliensis**. The fungus causes actinomycotic mycetoma in humans⁵⁸. This is common in Somalia⁸³ where it accounts for 50 % of cases. It accounts for 7% of cases worldwide. In a study by PK Maiti et al⁸⁵ on mycetoma infections of the foot in 54 patients in North India, 4 subjects were found to be positive. for *Streptomyces somaliensis*. It appears that 3-4% of all Mycetoma found in India are due to *Streptomyces somaliensis*. The histological reaction shown by *Streptomyces somaliensis* was foreign body giant cell reaction. Histologically, the *Streptomyces* could be mistaken for blood clot, fibrinoid material or amyloid. It is seen as cystic spaces filled with pale fragmented eosinophilic material surrounded by giant cell reaction (Fig.No.30).

In the present study, 8 patients (16%) were found to have **Rhinosporidiosis**, of whom 7 had nasal Rhinosporidiosis and one had primary cutaneous Rhinosporidiosis. All were in the adult age group. Men (5 patients) outnumbered women (3 patients). All the patients were industrial workers. **Primary cutaneous rhinosporidiosis** is a very rare entity, only eleven cases have been reported in India. In a study by J. H. Makannavar et al⁶⁶, 68 % of patients were found to have Rhinosporidiosis. The mean age of the patients was 30. The nose and nasopharynx were the sites predominantly involved. But majority of them (80%) were farmers. The mode of transport of *Rhinosporidium* is probably dust or water and is common in patients with history of bathing in ponds. This could account for the frequency of nasal infections.

In a study by Manjunath et al⁸⁶, one 65 year old man and another 40 year old man presented with secondary cutaneous Rhinosporidiosis in association with

recurrent nasopharyngeal Rhinosporidiosis. The predominant histological reaction seen is chronic non suppurative inflammation. In the present study foreign body giant cell reaction was observed in one subject, in addition to chronic inflammatory reaction (Fig.No.9).

In the present study, **Candidiasis** infection was found in 3 subjects. All of them were immunosuppressed and were industrial workers. One patient was a 36 year old lady, with squamous cell carcinoma of oesophagus, on chemotherapy and the candidal growth was seen in the oesophagus. The second patient was a 40 year old man, with squamous cell carcinoma of the tongue, on chemotherapy and had candidal growth in the tongue. The third patient was a 50 year old man, had undergone renal transplant and was on immunosuppressants. He presented with renal abscess. Histopathological examination revealed renal candidiasis. In a study by Cho and Choi⁴², Candida was found to be the most frequent offending organism in malignancy (52.2%). These organisms are more likely a manifestation of debility in high risk patients.

In contrast, in a study by Berend Rikken et al⁹⁰, renal candidiasis occurred in neonates and young infants with secondary risk factors and underlying congenital urinary tract abnormalities. In a study by C. Kintanar et al⁹¹ on infections in renal transplant patients, Aspergillus and Candidal infection were most commonly reported. The normal bacterial flora at these mucocutaneous surfaces have an inhibitory influence on the growth of candida. Hence an alteration in PH or use of antibiotics and immunosuppressants may permit the fungus to proliferate. More severe the candidal infection, more frequently is an immunological defect encountered. The most common histological reaction usually seen is severe necrosis.

Microscopically these lesions show non-specific acute and chronic inflammation with microabscesses but in their chronic state, granulomatous reaction may develop. In the present study pyogenic and mixed pyogenic and granulomatous reaction was seen. Pseudohyphae was seen in tissue sections (Fig .No.23, 24.).

In the present study 3 subjects were found to be infected with **Mucormycosis**. All of them were immunocompromised. One patient was in the paediatric age group and the other two patients were elderly men. The patient in the paediatric age group was a seven year old male child, who presented with a recurrent soft tissue mass in the thigh. The child had acute lymphoblastic leukemia and was on chemotherapy. Histopathologically the soft tissue mass revealed Mucormycosis in the subcutaneous tissue. In a study by F.W.Chandler et al⁹³, **cutaneous zygomycosis** was reported in elderly diabetic women. The second patient was a seventy two year old man, a known diabetic on irregular treatment found to have **gastrointestinal mucormycosis**. The Patient presented with abdominal pain and malena. There was a perforation in the colon with a 6cm x 5cm growth in the transverse colon. Histopathological examination with special stains confirmed the presence of Mucormycosis. S.R. Thomson et al⁹⁴ reported twenty subjects of gastrointestinal Mucormycosis. All the patients in the study were immunocompromised. The fatal infection was diagnosed histologically. The third patient in the present study was a sixty year old man, a known diabetic on irregular treatment who presented with **orbital mucormycosis**.

The most common histological reaction seen was severe necrosis. Histologically, the hyphae of the mucoraceous zygomycetes have a characteristic appearance in tissue sections. Typical hyphae are broad (6 to 25 micrometer wide), thin walled and pleomorphic, with irregular, non parallel contours (Fig No.25 & 26

).Branches arise haphazardly, often at right angles to the parent hyphae. Septa can be found in some of the hyphae, though most appear nonseptate (cenocytic). Since the hyphae have a little structural stability, they are often folded, twisted, wrinkled, or collapsed. In one subject with GIT mucormycosis, culture examination detected *Rhizopus*. Pulmonary (Disseminated) mucormycosis was not observed in the present study.

In the present study, one patient was found to be infected with **Cryptococcus neoformans**.The patient was a 35 year old man, who was a HIV positive industrial worker who presented with meningitis. Lumbar puncture was done. *Cryptococcus neoformans* (round to oval unicellular yeast forms) was demonstrated in the CSF (Fig. no.35). A clear spherical zone representing the yeast capsule which may have a diameter up to 5 times that of the yeast cell it surrounds is characteristic.It is best visualized by examining the cells in India ink preparation (negative staining).The mucopolysaccharide capsule is mucicarmine positive (Fig .No.36).

In a study by H.C. Chai et al¹⁰¹, on 50 HIV positive patients who presented with meningitis, 70% of them were found to be positive for *Cryptococcus neoformans* by CSF examination. Latex agglutination test, tube agglutination test and indirect fluorescent antibody test are useful serological tests. Pulmonary and cutaneous cryptococcosis was however not observed in the present study.

In the present study two patients presented with **mixed fungal infections**. Both the patients had superficial otomycosis of the external auditory canal. Immunologically both were immunocompetant. One subject had a mixed infection with *Aspergillus* and *Candida* whereas the other subject had infection with three organisms (i.e) *Aspergillus*, *Candida* and Mucormycosis (Fig.No 32). An

immunocompromised host is more susceptible to otomycosis as compared to immunocompetent host. In the immunocompromised hosts, *Aspergillus* may extend to the adjacent mastoid bone or brain. The common causative agents noted in literature are *Aspergillus niger* and *Candida albicans*³. In a study by Mathur et al on otomycosis two out of seventy three patients with otomycosis had mixed infections with *Aspergillus* and *Candida*. The other patients had single infection with *Aspergillus*. Both the patients with mixed infections were immunocompetent.

In the present study **invasive fungal infection** was found in four patients. Of them, three were Mucormycosis and one was *Aspergillus*. One subject had cutaneous Mucormycosis, the second had gastrointestinal Mucormycosis, the third, orbital Mucormycosis and the fourth patient had invasive Aspergillosis of the paranasal sinuses. All the four patients in the study were immunocompromised. **Cutaneous Mucormycosis** was found in the pediatric age group. The patient had hematological malignancy and was on chemotherapy. There was tissue invasion involving the subcutaneous tissue and muscle (Fig.No 29). Severe necrosis was found in the areas of invasion. In a study by Mehmet et al, four patients in the adult age group were found to have cutaneous Mucormycosis. Three of the four patients were immunocompromised and one was immunocompetent. Tissue invasion and angioinvasion were found histologically.

Orbital Mucormycosis was observed in an elderly man who had uncontrolled diabetes mellitus. There was extensive involvement of the extraocular muscles and soft tissues. Herdzri.M.H. et al reported rhinocerebral Mucormycosis with orbital extension. In a study by Punam Prasad et al invasive rhino orbital Mucormycosis presented in 18 year old immunocompetent girl. Clinico radiologically

it was suspected as malignancy. This presentation with suspicion of malignancy resulted in extensive surgical debridement and removal of the eye which was diagnosed as Mucormycosis on histopathological examination. Her eye could have been saved if the Mucormycosis was suspected initially. William.P.Bargh et al reported a case of invasive orbital Mucormycosis with invasion of nerves, blood vessels, cartilage, bone and meninges. Direct invasion by fungal elements resulted in thrombosis and nerve dysfunction. Advancing infections can result in cavernous sinus thrombosis.

Gastrointestinal Mucormycosis in the present study was seen in a seventy two year old man with poorly controlled diabetes mellitus. The patient presented with malena. Intraoperatively there was a growth in the colon associated with deep ulcers and perforation which mimicked a carcinoma. Histopathologically it was confirmed to be Mucormycosis with deep invasion into the muscle coat and serosal layer. In a study by Al –Rikabi et al invasive Mucormycosis was present in a subject with gastric ulcer and the patient was an elderly diabetic. In a study by Antonio et al invasive Mucormycosis was seen in a patient with severe atheromatous vascular disease.

Invasive Aspergillosis of the paranasal sinus was seen in a thirty year old HIV positive man. There was regional tissue invasion involving the wall of the sinus associated with eosinophils, necrosis, fibrosis and vasculitis. In a study by Lulu Ahmed et al five subjects were detected with invasive fungal sinusitis. Among them three were diabetics and two were immunocompetant. The organism detected in all the cases was *Aspergillus*.

SUMMARY

- In the present study fungal infections were predominantly seen in the nasal cavity and paranasal sinuses.
- The predominant age group affected was above 40 years.
- Men were predominantly affected.
- The commonest fungus identified was *Aspergillus*.
- Immunocompetent patients were affected more than the immunocompromised patients. However, they were all superficial mycosis.
- Among the immunocompromised patients, diabetics were predominantly affected.
- Unusual presentations encountered in the present study were ovarian Actinomycosis, primary cutaneous Rhinosporidiosis, GIT Mucormycosis and spinal *Streptomyces somaliensis*.
- Mixed fungal infections were identified in two subjects with otomycosis. *Aspergillus* and *Candida* in one and *Aspergillus*, *Candida* and Mucormycosis in another.
- Invasive Mucormycosis was found in three patients and invasive *Aspergillus* in one. All the four patients were immunocompromised.

CONCLUSION

In today's scenario, fungi are gradually assuming a noteworthy place in the differential diagnosis of all obscure infections. Superficial fungal infections are commonly seen in the immunocompetent while invasive fungal infections are observed in the immunocompromised patients. However, many instances of invasive fungal infections in immunocompetent individuals have been reported in literature. Therefore a high index of suspicion and timely diagnosis are needed. The morbidity and mortality due to invasive fungal lesions could thus be reduced and vital organs salvaged. Immunocompromised individuals who are highly prone for fungal infections have to be periodically screened and promptly treated. Since fungal infections could not uncommonly present as tumours, prompt diagnosis would prevent unnecessary radical intervention. IUD users for more than three years have to be screened regularly for endometrial Actinomycosis by demonstrating the organism in papanicolaou smear even before surgery. Concomitant ovarian Actinomycosis should be looked for.

In short, fungal lesions in various sites have protean clinical manifestations. A comprehensive definitive diagnosis is mandatory for wholesome treatment. Hence, histopathology plays a pivotal role.

CHART – 1

AGEWISE DISTRIBUTION

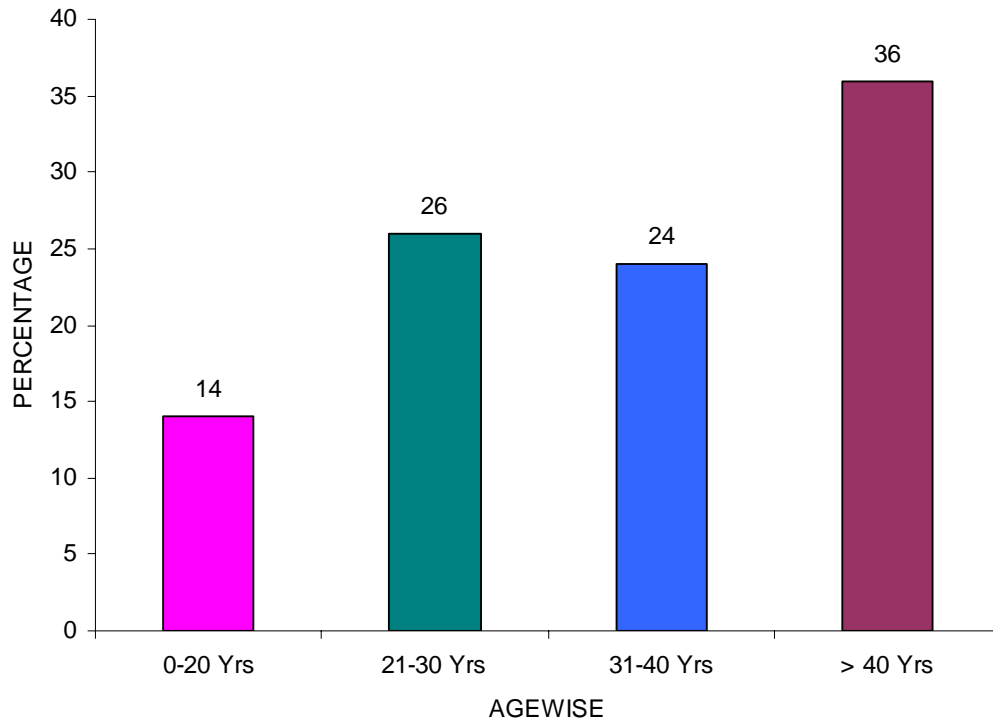


CHART - 2

GENDERWISE DISTRIBUTION

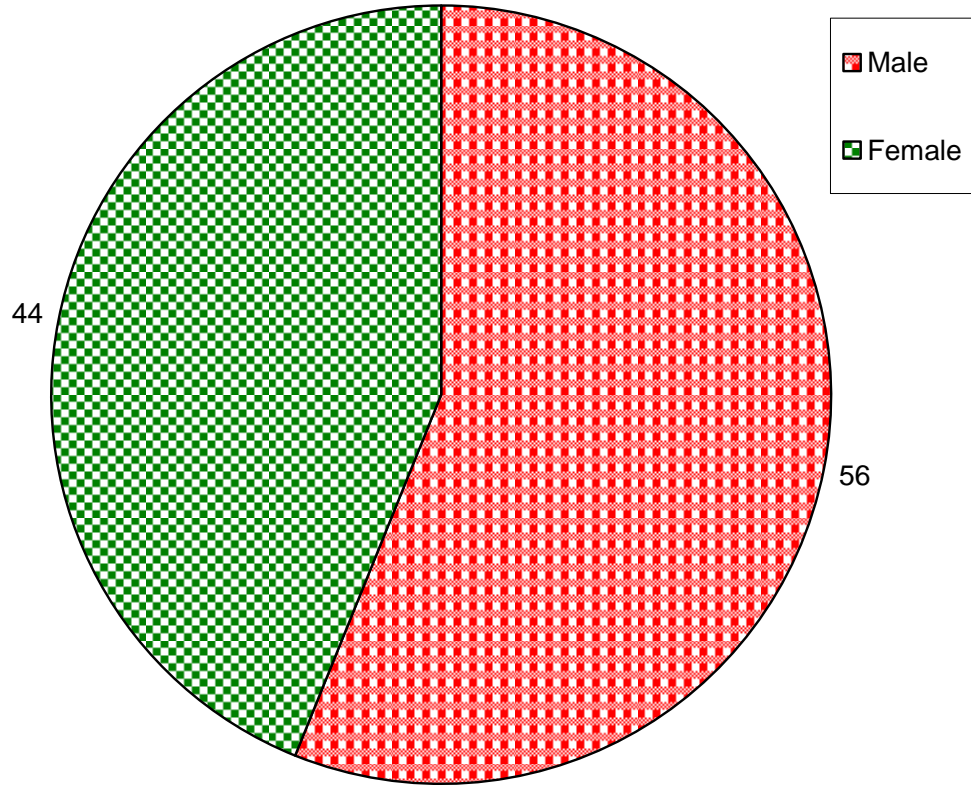


CHART – 3

DISTRIBUTION OF ORGANS INVOLVED

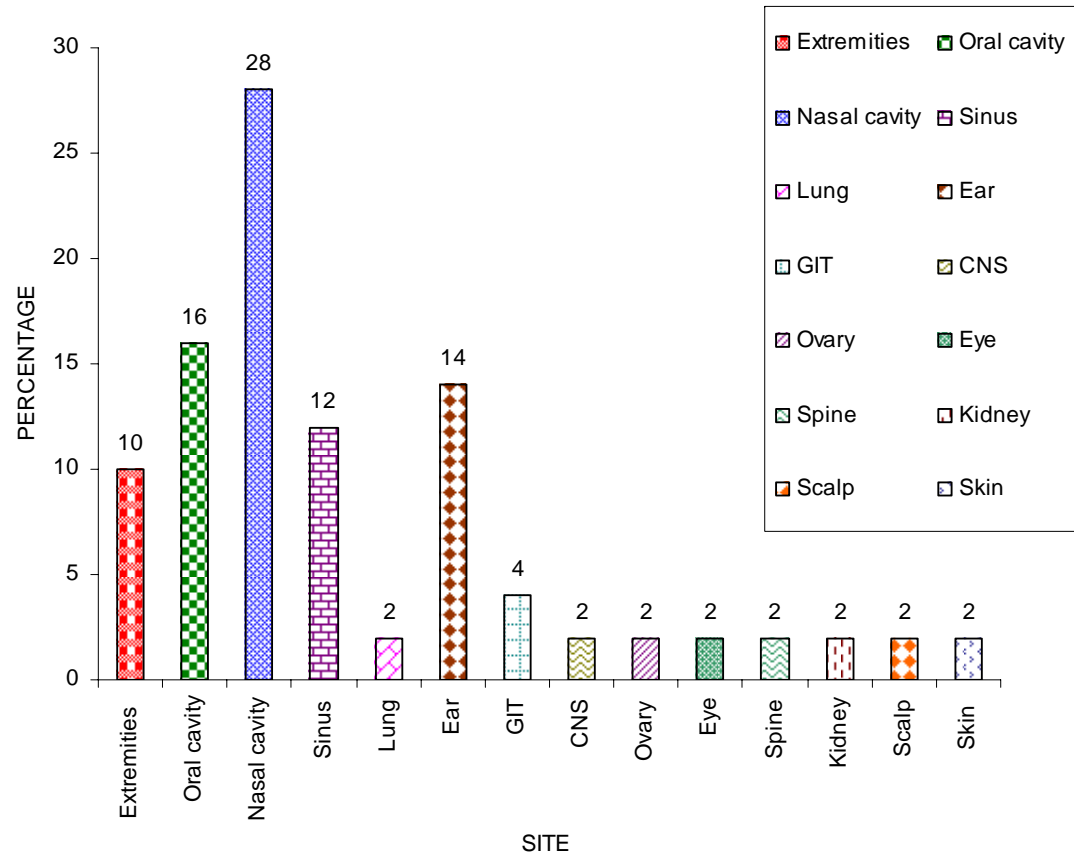


CHART - 4
INCIDENCE OF FUNGUS

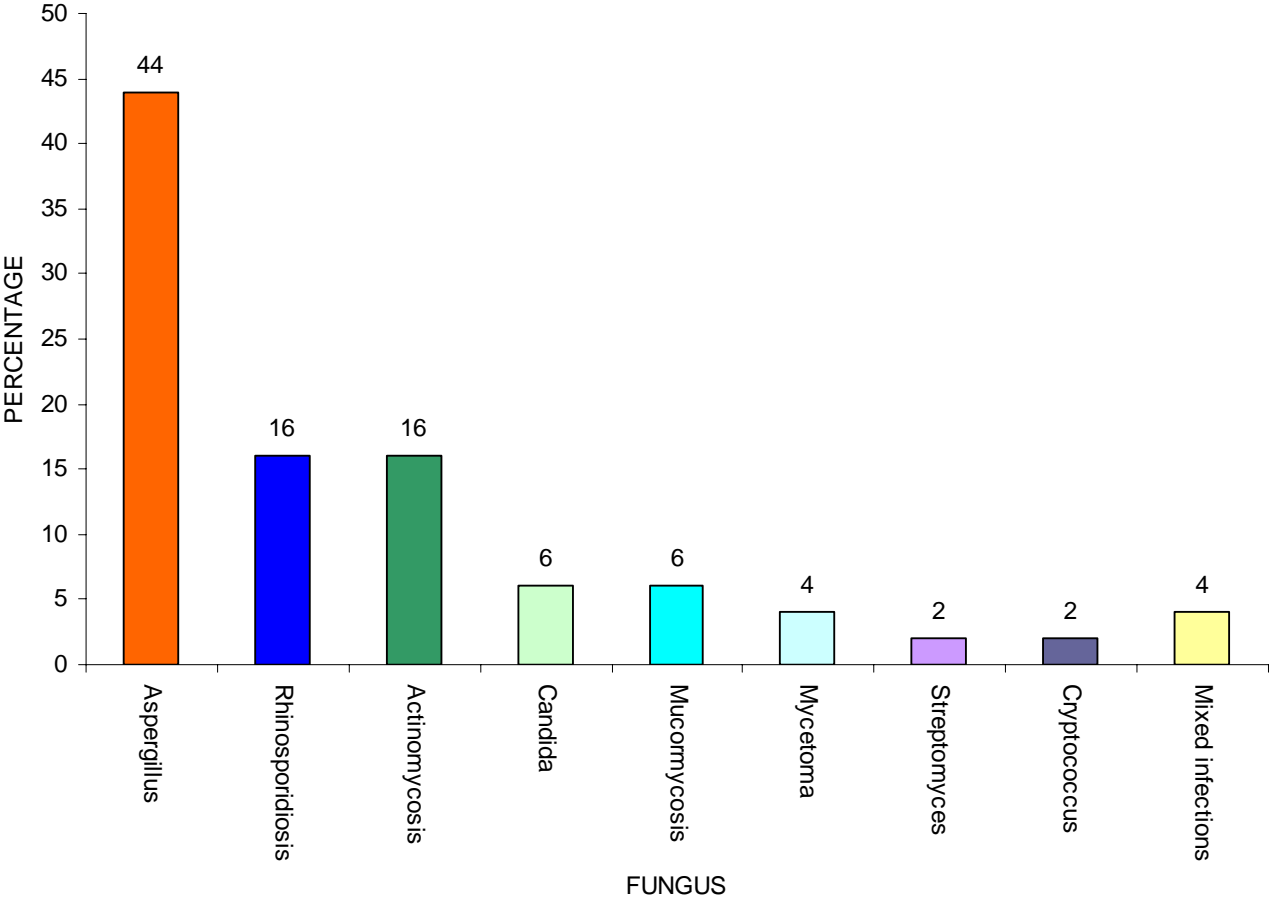


CHART – 5
DISTRIBUTION OF FUNGAL INFECTION IN THE HEAD & NECK REGION

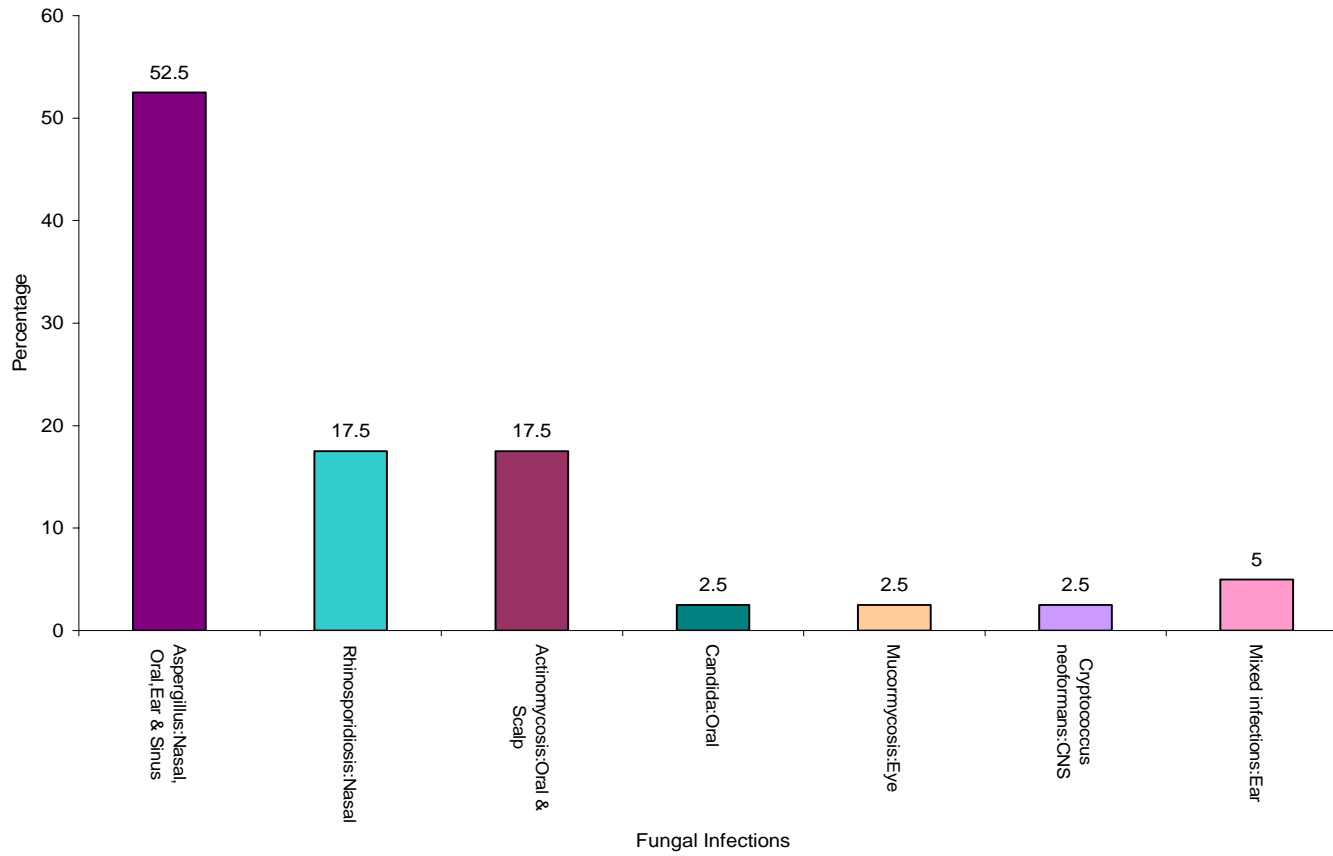


CHART – 6
OCCUPATIONWISE DISTRIBUTION

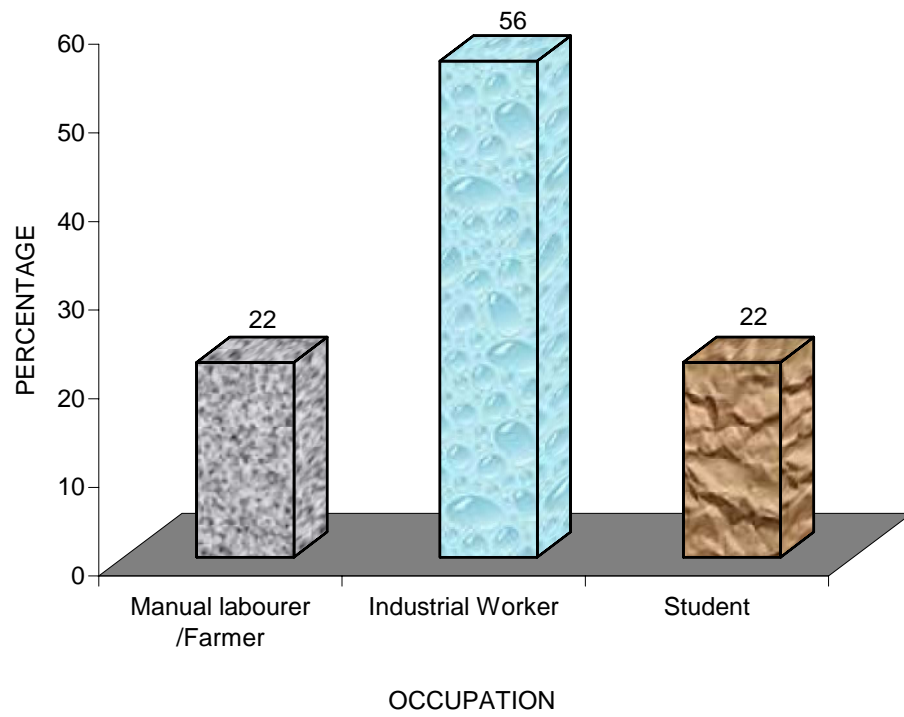


CHART - 7
IMMUNOLOGICAL STATUS

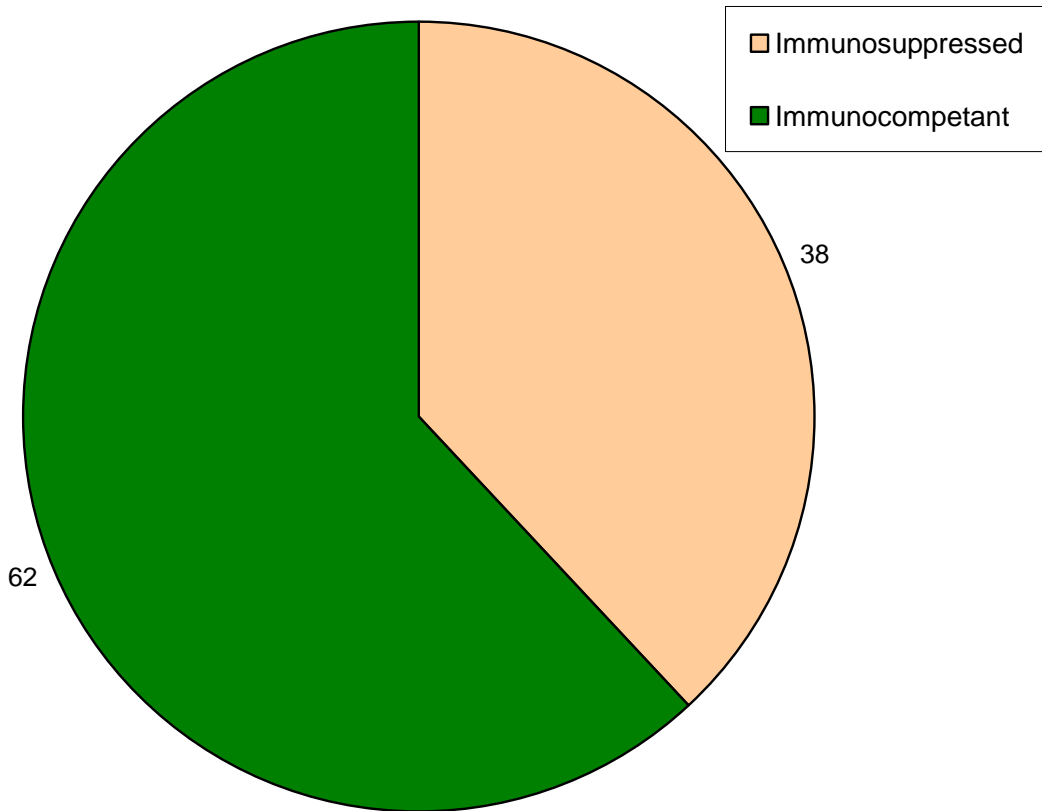
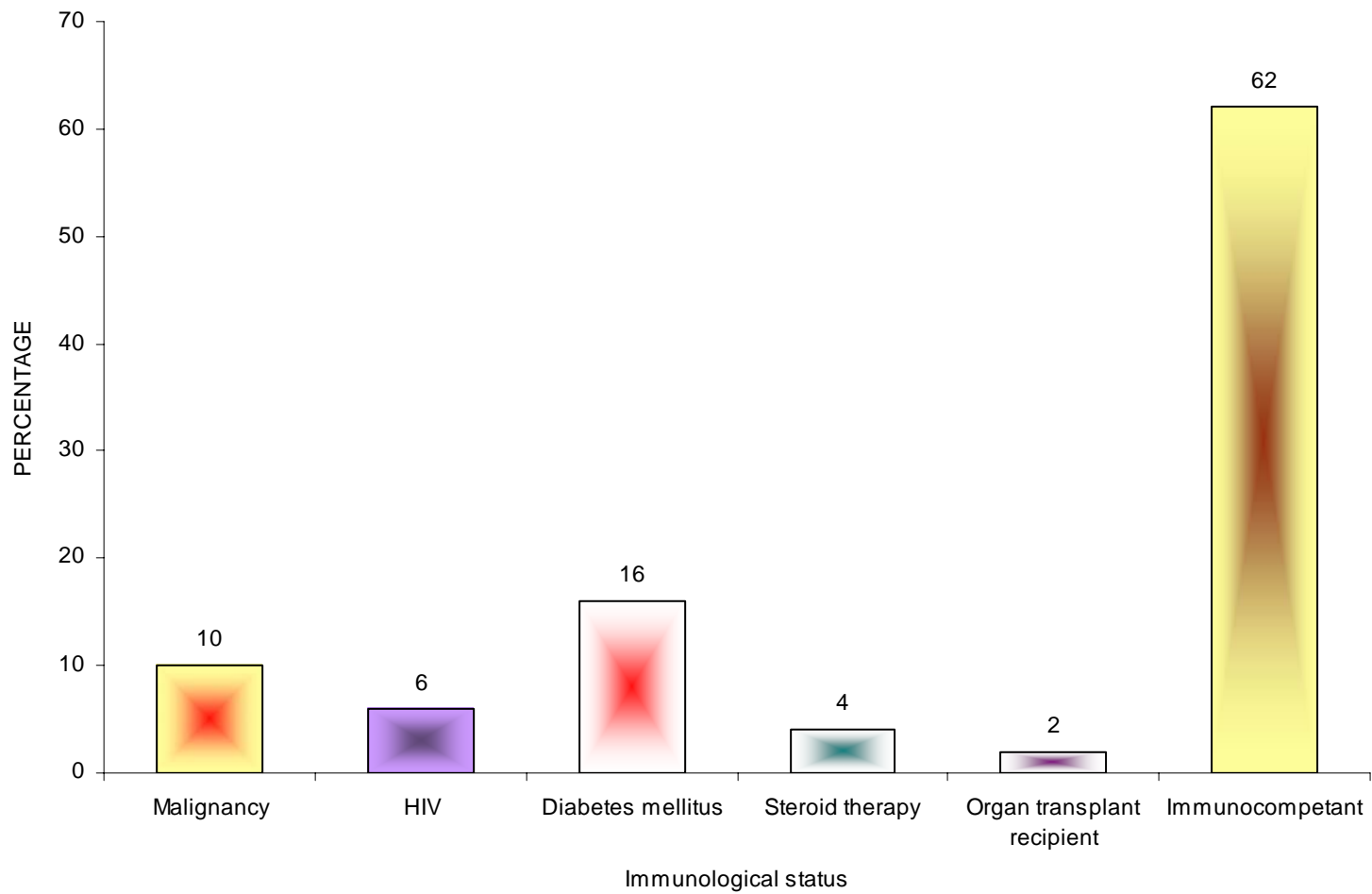


CHART - 8
IMMUNOLOGICAL STATUS



ANNEXURE - I

PROFORMA

1. Name :
2. Age :
3. Sex :
4. Residential Address :
5. Occupation :
6. Symptoms :
7. History of Chronic
Drug intake
Immunosuppressants /
Steroid therapy :
8. HIV status :
9. Comorbid conditions :
 - a. TB
 - b. Diabetes
 - c. Malignancy
 - d. Rheumatoid arthritis/
other connective
tissue disorders
 - e. Bronchial asthma :
10. Site of lesion :
11. Gross Appearance :

12. Microscopic Picture
Haemotoxylin &
Eosin (H & E) :
13. Special stain :
- a. Gomori –
Methenamine silver(GMS) :
 - b. Periodic acid –
Schiff (PAS) :
 - c. Brown & Brenn :
 - d. Mucicarmine :
 - e. India Ink :
14. Inference :

ANNEXURE - II

TAXONOMY²

Basic to an understanding of the mycoses and their aetiologic agents is the use of logically developed and organized classification schemes².

The fungal infections represent the invasion of tissues by one or more species of fungi that range from superficial localized skin condition to deeper tissue infections, serious lung infection, septicemia or systemic diseases. Some fungi are opportunistic while others are pathogenic causing diseases whether the immune system is healthy or not.

Infections involving fungi may occur on the surface of the skin, in skin folds and in other areas kept warm and moist. They may occur at the site of an injury, in mucus membranes the sinuses and lungs. Fungal infections trigger the body's immune systems, can cause inflammation and tissue damage and in some people may trigger and allergic reaction.

Fungal diseases are grouped into four broad categories based on the predominant location of infection within the body².

- Superficial
- Cutaneous
- subcutaneous
- Systemic

I. Superficial Mycoses

Superficial fungal infections may be caused by both yeast and mould forms of fungi. Skin is normally populated with a mixture of microorganisms called normal flora. Most of the time, normal flora do not cause illness and do not stimulate the immune system. If there is a break in the skin or the immune system becomes compromised, then any of the microorganism present can cause a wound or skin infection.

If there is a shift in the balance of the microorganisms, such as a decrease in the bacteria and an increase in the growth of fungi, the person may experience a fungal

infection associated with the imbalance².The invading pathogen is confined to the stratum corneum with little or no tissue reaction.

In the case of hair infections (black and white piedra), growth is generally superficial with minimal damage to the hair and no host reaction.

A.Black Piedra

1. *Piedraia hortai*.

B.Tinea Nigra.

1. *Exophiala werneckii*, *Stenella araguata*.

C. Tinea Versicolor.

1. *Malassezia furfur*.

D.White Piedra.

1. *Trichosporon beigeli*.

II. Cutaneous Mycoses

In the cutaneous mycoses all keratinised tissue, i.e., skin, hair, nail, feathers are attacked. Although the pathogens are generally confined to the nonliving cornified layers of the skin and its appendages, destruction of these tissues is extensive and host immunological reactions may be severe.

A. Cutaneous Candidiasis

1. *Candida albicans*

B. Dermatomycoses

C. Dermatophytoses

1. *Epidermophyton floccosum*
2. *Microsporum* species
3. *Trichophyton* species

D. Cutaneous Zygomycosis

1. *Rhizopus rhizopodiformis*

III. Subcutaneous Mycoses

The subcutaneous mycoses constitute a heterogeneous group of diseases caused by a variety of fungi that invade the cutaneous and subcutaneous tissues after traumatic implantation. Some infections may remain localised, slowly spreading to contiguous tissues.

A. Chromoblastomycosis

1. *Cladosporium carrionii*
2. *Fonsecaea compacta*
3. *F.pedrosoi*
4. *Phialophora verrucosa*
5. *Rhinocladiella cerophilum*

B. Lobomycosis

1. *Loboa loboii*

C. Mycetomas (Eumycotic)

1. *Acremonium falciforme*
2. *A.kilense*
3. *A.recifei*
4. *Aspergillus nidulans*
5. *Corynespora cassicola*
6. *Curvularia geniculata*
7. *Exophiala jeanselmei*
8. *Fusarium moniliforme*
9. *Leptosphaeria senegalensis*
10. *L.tompkinsii*
11. *Madurella grisea*
12. *M.mycetomatis*
13. *Neotestudina rosatii*
14. *Petriellidium boydii*
15. *Pyrenochaeta mackinnonii*
16. *Pyrenochaeta romeroi*

D. Subcutaneous Phaeohyphomycosis

E. Rhinosporidiosis

1. *Rhinosporidium seeberi*

F. Sporotrichosis

1. *Sporothrix schenckii*
 - (1) *S.s.var.schenckii*
 - (2) *S.s.var.luriei*

G. Subcutaneous Zygomycosis

1. *Basidiobolus haptosporus*
2. *Conidiobolus coronatus*

IV Systemic Mycoses

These are basically pulmonary disease in that the primary site of infection is almost invariably the lungs. Systemic mycoses, except in their subclinical, benign forms, may have grave consequences. All of the vital organs may be attacked and lesions may be extensive. Cutaneous and subcutaneous forms of these diseases do occur as the result of dissemination or as a consequence of direct inoculation following an injury.

A. Adiaspiromycosis

1. *Chrysosporium parvum*
 - (a) *C.p.var.parvum*
 - (b) *C.p.var.crescens*

B. Aspergillosis

1. *Aspergillus fumigatus* group
2. *A.flavus* group
3. *A.nidulans* group
4. *A.niger* group
5. *A.oryzae* group
6. *A.terreus* group

C. Blastomycosis

1. *Blastomyces dermatitidis*

D. Systemic Candidiasis

1. *Candida albicans*
2. *C.glabrata*
3. *C.guilliermondii*
4. *C.krusei*
5. *C.parapsilosis*
6. *C.tropicalis*

E. Coccidioidomycosis

1. *Coccidioides immitis*

F. Cryptococcosis

1. *Cryptococcus neoformans*

G. Histoplasmosis Capsulati

1. *Histoplasma capsulatum*.var.*capsulatum*.

H. Histoplasmosis Duboisii

1. *H.capsulatum* var.*duboisii*

I. Histoplasmosis Farciminosi

1. *H. farciminosum*

J. Paracoccidioidomycosis

1. *Paracoccidioides brasiliensis*

K. Systemic Phaeohyphomycosis

L. Systemic Zygomycosis

1. *Absidia corymbifera*
2. *Cunninghamella bertholletiae*
3. *Conidiobolus incongruus*
4. *Mucor ramosissimus*
5. *Mucor rouxianus*
6. *Rhizomucor pusillus*

7. *Rhizopus arrhizus*
8. *R. microsporus*
9. *R. oryzae*
10. *R. rhizopodiformis*
11. *Saksenaea vasiformis*

The diseases caused by the anaerobic and aerobic actinomycetes are treated separately since these organisms are bacteria and not fungi. However, traditionally, the actinomycoses have been considered part of the province of medical mycology. Thus, they are dealt with in this Atlas.

I Actinomycosis

1. *Acinomyces bovis*
2. *A. israelii*
3. *A. naeslundii*
4. *A. odontolyticus*
5. *A. viscocus*
6. *Arachnia propionica*
7. *Rothia dentocariosa*

II Actinomycotic Mycetomas

1. *Actinomadura madurae*
2. *A. pelletiera*
3. *Nocardia asteroides*
4. *N. brasiliensis*
5. *N. caviae*
6. *Streptomyces somaliensis*

III Dermatophilosis

1. *Dermatophilus congolensis*

IV Nocardiosis

1. *Nocardia asteroides*
2. *N. brasiliensis*
3. *N. caviae*

V Streptomyces

1. *Streptomyces griseus*.

ANNEXURE - III

MYCOLOGIC TERMINOLOGY

Arthroconidium

Conidium formed by mycelial disarticulation

Bud (blastoconidium)

Conidium produced by lateral outgrowth from a parent cell: buds may be single or multiple.

Chlamydoconidium

Thick – walled, rounded, resistant conidium formed by direct differentiation of the mycelium.

Conidium

A sexual spore formed on but easily detached from a conidiophore.

Conidiophore

Specialized hypha that produces and bears conidia

Dematiaceous : Naturally pigmented, usually brown or black

Dimorphic

Term applied to fungi that grow as hyphae in vitro at 25 degree calicoes and as budding yeastlike cells or spherules infected tissues or in vitro at 37 degree calicoes on special media

Endospore

A sexual spore formed within a closed structure such as a spherule

Germ tube

Tube like process, produced by a germinating conidium, that eventually develops into a hypha.

Granule (grain): Compact aggregate of organized mycelium that may be embedded in a cementlike substance; formed in actinomycosis, and in actinomycotic and eumycotic mycetomas; also formed by nonfilamentous bacteria in botryomycosis.

Hypha : Filament that forms the thallus or body of most fungi.

Mycelium : Mass of intertwined and branched hyphae

Pseudohypha : Short hyphal – like filament produced by the successive buds of a yeast that elongate and fail to separate.

Septate : Having cross – walls

Spherule : Closed, thick – walled, spherical structure within which asexual endospores are produced by progressive cytoplasmic cleavage.

Splendore-Hoeppli material: Eosinophilic, refractile substance that surrounds some fungi and represents a localised antigen antibody reaction in the hypersensitized host.

Yeast: Spherical to oval unicellular fungus that reproduces by budding.

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MASTER CHART

| S.NO | HPE No | AGE in yrs | SEX | Occupation | Site | Clinical Features | Immuno suppressed | DrugH/o | Spl. Fungal Stains | | | | Diagnosis |
|------|----------|---------------|-----|----------------------------|-------------------|-------------------------|----------------------|---------|--------------------|-----|-----|-----|------------------|
| | | | | | | | | | GMS | PAS | B&B | Muc | |
| 1 | 1796/07 | 38 | M | Industrial worker | Nasal | Nasal Polyp | | | + | + | -- | + | Rhinosporidiosis |
| 2 | G2207/07 | 40 | F | Manual Labourer/ farmer | Ovary | Abdominal mass | DM | | + | -- | + | -- | Actinomycosis |
| 3 | 138/08 | 30 | F | Industrial worker | Nasal | Nasal polyp | | | + | + | -- | + | Rhinosporidiosis |
| 4 | 292/08 | 11 | M | Student | Tonsil | Tonsilar growth | | | + | -- | + | -- | Actinomycosis |
| 5 | 326/08 | 5 | M | Student | Tonsil | Tonsilar growth | | | + | -- | + | -- | Actinomycosis |
| 6 | 1019/08 | 25 | M | Industrial worker | Nasal | Nasal polyp | | | + | + | -- | + | Rhinosporidiosis |
| 7 | 1045/08 | 7 | M | Student | Thigh | soft tissue mass | ALL | CT | + | + | -- | -- | Mucormycosis |
| 8 | 2242/08 | 35 | M | Industrial worker | Maxillary sinus | Nasal Stuffiness | HIV | | + | + | -- | -- | Aspergillus |
| 9 | 2380/08 | 60 | F | Manual Labourer/ farmer | Cheek | Growth in cheek | Scc - Cheek | CT | + | -- | + | -- | Actinomycosis |
| 10 | 2472/08 | 23 | M | Industrial worker | Nasal | Nasal polyp | BA on steroid | Steroid | + | + | -- | + | Rhinosporidiosis |
| 11 | 2482/08 | 40 | F | Industrial worker | Nasal | Nasal polyp | | | + | + | -- | + | Rhinosporidiosis |
| 12 | 2596/08 | 70 | F | Manual Labourer/ farmer | Maxillary sinus | Nasal obstruction | DM | | + | + | -- | -- | Aspergillus |
| 13 | 2627/08 | 60 | M | Manual Labourer/ Farmer | Lung | lung mass | Scc - Lung | CT | + | + | -- | -- | Aspergillus |
| 14 | 2645/08 | 13 | M | Student | Spine-D2 vertebra | H/O Injury Back pain | | | + | -- | + | -- | Mycetoma |

| S.NO | HPE No | AGE in yrs | SEX | Occupation | Site | Clinical Features | Immuno suppressed | DrugH/o | Spl.Fungal Stains | | | | Diagnosis |
|------|----------|---------------|-----|----------------------------|------------------|----------------------|--------------------------|---------|-------------------|-----|-----|-----|------------------|
| | | | | | | | | | GMS | PAS | B&B | Muc | |
| 15 | 2664/08 | 58 | M | Industrial worker | Nasal | Nasal polyp | DM | | + | + | -- | + | Rhinosporidiosis |
| 16 | 2757/08 | 53 | F | Manual Labourer/ Farmer | Maxillary Sinus | Nasal stuffiness | | | + | + | -- | -- | Aspergillus |
| 17 | 2855/08 | 72 | M | Manual Labourer/ Farmer | Ear | Polyp | | | + | + | -- | -- | Aspergillus |
| 18 | 2871/08 | 16 | F | Student | Scalp | Growth | | | + | -- | + | -- | Actinomycosis |
| 19 | 6092/08 | 50 | F | Industrial worker | Forearm | Growth | DM | | + | + | -- | + | Rhinosporidiosis |
| 20 | 7371/08 | 51 | F | Manual Labourer/ farmer | Maxillary Sinus | Mass | | | + | + | -- | -- | Aspergillus |
| 21 | 7432/08 | 30 | F | Industrial worker | Foot | Ulcer | | | + | -- | -- | -- | Streptomyces |
| 22 | 8740/08 | 50 | M | Industrial worker | Renal | Renal Abscess | Organ transplantation | | + | + | -- | -- | Candidiasis |
| 23 | 11053/08 | 60 | F | Manual Labourer/ farmer | Nasal | Nasal mass | | | + | + | -- | -- | Aspergillus |
| 24 | 11472/08 | 34 | F | Industrial worker | Maxillary antrum | Mass | | | + | + | -- | -- | Aspergillus |
| 25 | 11615/08 | 55 | M | Industrial worker | Maxillary antrum | Mass | | | + | + | -- | -- | Aspergillus |
| 26 | 11630/08 | 18 | F | Student | Tonsil | Growth | | | + | -- | + | -- | Actinomycosis |
| 27 | 12524/08 | 28 | M | Industrial worker | Tonsil | Growth | | | + | -- | + | -- | Actinomycosis |
| 28 | P210/09 | 18 | F | Student | Tonsil | Sore throat | | | + | -- | + | -- | Actinomycosis |

| S.NO | HPE No | AGE in yrs | SEX | Occupation | Site | Clinical Features | Immuno suppressed | DrugH/o | Spl.Fungal Stains | | | | Diagonosis |
|------|---------|---------------|-----|-------------------|------------------|----------------------|----------------------|---------|-------------------|-----|-----|-----|------------------|
| | | | | | | | | | GMS | PAS | B&B | Muc | |
| 29 | 317/09 | 35 | M | Industrial worker | Nasal | Nasal Polyp | | | + | + | -- | + | Rhinosporidiosis |
| 30 | 552/09 | 49 | M | Industrial worker | Ear | Ear ache | | | + | + | -- | -- | Aspergillus |
| 31 | 754/09 | 22 | F | Student | Ear | Ear ache | BA on steroid | Steroid | + | + | -- | -- | Aspergillus |
| 32 | 755/09 | 65 | F | Industrial worker | Ear | Ear ache | DM | | + | + | -- | -- | Aspergillus |
| 33 | 856/09 | 30 | F | Industrial worker | Vocal Cord | Papillary growth | | | + | + | -- | -- | Aspergillus |
| 34 | 1060/09 | 27 | M | Industrial worker | Foot | Ulcer | | | + | -- | + | -- | Mycetoma |
| 35 | 1061/09 | 21 | M | Student | Ear | Ear ache | | | + | + | -- | -- | Aspergillus |
| 36 | 1124/09 | 24 | F | Student | Ear | Ear ache | | | + | + | -- | -- | Aspergillus |
| 37 | 1254/09 | 55 | M | Industrial worker | Nasal | Nasal Polyp | DM | | + | + | -- | -- | Aspergillus |
| 38 | 1300/09 | 32 | F | Industrial worker | Nasal | Nasal Polyp | | | + | + | -- | -- | Aspergillus |
| 39 | 1361/09 | 22 | F | Student | Ear | Ear ache | | | + | + | -- | -- | Aspergillus |
| 40 | 1481/09 | 27 | M | Industrial worker | Maxillary antrum | Nasal Obstruction | | | + | + | -- | -- | Aspergillus |
| 41 | 1527/09 | 36 | F | Industrial worker | Oesophagus | Growth | SCC | CT | + | + | -- | -- | Candidiasis |
| 42 | 1537/09 | 30 | M | Industrial worker | Nasal | Nasal Mass | HIV | | + | + | -- | -- | Aspergillus |

| S.NO | HPE No | AGE in yrs | SEX | Occupation | Site | Clinical Features | Immuno suppressed | DrugH/o | Spl.Fungal Stains | | | | Diagnosis |
|------|---------|---------------|-----|------------------------|----------|----------------------|----------------------|---------|-------------------|-----|-----|-----|--------------|
| | | | | | | | | | GMS | PAS | B&B | Muc | |
| 43 | 1556/09 | 36 | M | Industrial worker | Nasal | Nasal Polyp | | | + | + | -- | -- | Aspergillus |
| 44 | 1574/09 | 40 | M | Industrial worker | Tongue | Growth | SCC | CT | + | + | -- | -- | Candidiasis |
| 45 | 1663/09 | 40 | F | Industrial worker | Nasal | Nasal Pplyp | | | + | + | -- | -- | Aspergillus |
| 46 | 1668/09 | 60 | M | Manual Labourer/farmer | Nasal | Nasal Polyp | | | + | + | -- | -- | Aspergillus |
| 47 | 2327/09 | 72 | M | Manual Labourer/farmer | GIT | Colonic growth | DM | | + | + | -- | -- | Mucormycosis |
| 48 | 5483/09 | 53 | M | Industrial worker | Nasal | Nasal Mass | | | + | + | -- | -- | Aspergillus |
| 49 | 5800/09 | 60 | M | Industrial worker | Eye Ball | Growth | DM | | + | + | -- | -- | Mucormycosis |
| 50 | 6002/09 | 35 | M | Industrial worker | CNS | Meningitis | HIV | | -- | -- | -- | + | Cryptococcus |

