STUDY ON
THE PREVALENCE OF SUBCUTANEOUS MYCOSES IN NORTH KERALA

Thesis submitted
in partial fulfilment of the requirements
for the award of the degree of

DOCTOR OF PHILOSOPHY

IN MICROBIOLOGY (MEDICAL FACULTY)

BY

ASHOKAN K KUTTIYIL

DEPARTMENT OF MICROBIOLOGY
MEDICAL COLLEGE, CALICUT- 673008.

AUGUST- 2006

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DECLARATION

I, Ashokan K.Kuttiyil; do hereby declare that this thesis, **"The prevalence of subcutaneous mycoses in North Kerala"** has not been submitted by me for the award of a Degree, Diploma, Title or Recognition, before.

Calicut

20-08-06

(ASHOKAN K KUTTIYIL)

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CERTIFICATE

This is to certify that this report is an authentic work carried out by Mr.Ashokan K Kuttiyil, under my supervision and guidance and that thereof no part of it has been submitted anywhere for any other degree before

Calicut

20-08-06

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Ashokan K. Kuttiyil

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CONTENTS

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INTRODUCTION & REVIEW OF LITERATURE

Medical Mycology has emerged as an important branch of Microbiology due to increase in the isolation of opportunistic fungal pathogens especially in immunocompromised patients. Organisms once thought to be contaminants are now considered as pathogens in compromised patients. Fungal infections, however, are extremely common and some of them are very serious and even fatal. With the control of most bacterial infections in the developed countries, there is an increased incidence of fungal infections. Modern advances in treatment such as bone marrow and various organ transplantations, newer antibiotics, steroids and immunosuppressive agents have led to an increase in opportunistic fungal infections. Even though a given isolate is not a documented fungal pathogen, its isolation from a normal sterile site and its ability to grow at 37°C points to the fact that it may be considered a possible pathogen. With few exceptions, all the fungi that infect humans share the ability to grow at 37 ° C. The study of pathogenic fungi has received scanty attention in comparison with the study of other pathogens. This is probably due to the relatively benign nature of most of the common mycotic diseases and the techniques employed in mycology are more those of botanists than of bacteriologists.

The word "mycology" in fact, is derived from mykes, the Greek word for mushroom. Fungi were initially classified with the plants and much of the botanical influence is still seen, even though the organisms have been transferred to a separate kingdom on the basis of cell structure. Fungi are ubiquitous in nature. They are eukaryotic and cell wall containing chitin and/or cellulose, chemo- heterotrophic. They function as saprophytes and also as a decomposer in nature. Mycological identification can be frustrating because of the importance placed upon the morphology and certain structures and terms. The morphology and the clinical aspects of the fungi serve as a protocol for their identification. Clinicians, mycologist and

pathologists are essential for the diagnosis. Fungi are extremely successful organisms, as evidenced by their ubiquity in nature. They are an important component in energy, where they function as decomposers cycle. Of the estimated 25,000 species, less than 150 are known to be primary pathogens of humans. The infection of humans seems to be an accident of nature, since it represents a dead end for the fungus. Most fungal infections are not contagious but are acquired through exposure to a point source in nature, where the organism exists as a saprophyte.

Fungi reproduce by the formation of spores, which may be either asexual (involving mitosis only) or sexual (involving meiosis; preceded by the fusion of the protoplasm and the nuclei of two cells). One fungus can produce both sexual and asexual spores. Specialized structures (fruiting bodies) may be associated with either sexual or asexual spores and are helpful for identification. Asexual spores are of two general types: sporangiospores and conidia. Sporangiospores are characteristic of lower fungi, zygomycetes. Conidia are the asexual spores of higher fungi. They are represented by the classes Ascomycetes, Basidiomycetes, and Deuteromycetes. The sexual spores of Ascomycetes is the ascospore, basidiomycetes is the basidiospore. The Deuteromycetes (Fungi imperfecti) have no sexual spores.

One method of classifying mycoses is by geographical distribution.(Emmons C.W *etal,*1970). This has some validity and usefulness. Actinomycosis, cryptococcosis, candidiasis, some dermatophytoses, certain mycetoma, nocardiosis, sporotrichosis, phycomycosis (mucormycosis), aspergillosis and histoplasmosis occur widely over temperate and tropical areas of the world. Chromoblastomycosis occurs all around the world; both north and south of the equator, but the predominant etiologic species vary in geographical areas. Certain mycetoma and agents causing subcutaneous mycosis are geographically limited in distribution. Some of these

mycoses appear to be permanently limited by soil or climatic conditions which are necessary for the growth and survival of the etiologic fungus in soil. These fungi are isolated from soil, many of them from specialized habitats characterized by elevated temperatures, high salinity, or enrichment of the substrates by excreta of birds and bats. In these special habitats the fungi grow as free living saprobes, permanent members of the flora and fauna of soil, apparently able to survive the competition of other micro-organisms without any necessity for reseeding of the substrate by an infected animal. Man, other animals and birds are accidental hosts, and periodic or cyclic parasitism of a host is not essential for the survival of the fungus in a suitable ecologic niche. Under these conditions of saprobic growth the pathogens produce enormous numbers of conidia which are resistant to unfavourable conditions. These conidia enter into the host either by penetrating wound or by splinters which implant them subcutaneously in the patients. The actual geographic distribution in soil may be much wider for the fungi which cause chromomycosis and mycetoma, than the relative frequencies of these mycoses in different parts of the world indicate. In these cases the socio-economic status of a country may influence the frequency of mycoses. Chromoblastomycosis and mycetoma occur most often on the feet and in persons who work in the fields without adequate protection of shoes. A topographic classification may be more important to the physician than geographical distribution. The mycetoma, chromomycosis and sporotrichosis are primarily (but not exclusively) cutaneous, sub cutaneous or lymphatic in distribution. They are often localized (but not exclusively) on the feet, legs and arms.

Infectious diseases are responsible for considerable morbidity and mortality in developing countries like India. Bacterial and viral diseases are largely under check due to skillful use of antibiotics and intensive prophylactic vaccination. Fungal

infections continue to be a cause of concern because of its chronic nature of infection and the fewer number of antifungal agents highly toxic to the ordinary tissues. Differential diagnosis is very difficult in subcutaneous fungal infections because of the clinical similarity with other subcutaneous skin infections like cutaneous tuberculosis, leishmaniasis, tertiary syphilis, and yaws. Diagnosis is often delayed because of the associated bacterial colonization which may be mistaken for the primary infectious agents. Accurate diagnosis is essential for treatment of fungal infections since antifungal agents are highly toxic and act directly on fungal agents. The other similar clinical conditions caused by bacterial, viral or protozoal have their own treatment profile.

The subcutaneous mycoses include a heterogeneous group of infections that involve the skin and sub cutaneous tissue, generally without dissemination to other organs of the body. The agents causing subcutaneous infection may also produce dissimilated infection. The etiological agents are found in several unrelated fungal genera, all which may exist as saprophytes in nature. Humans and animals serve as accidental hosts after traumatic inoculation of the fungal spores into cutaneous and subcutaneous tissue. These mycoses are not opportunistic in the usual sense, since they occur in otherwise healthy people; they are chronic, evolving over a lengthy period, and may tend to be disfiguring. There is evidence of a causal relationship between the manner of acquisition of infection and pathologic process, particularly in view of the facts that in the few instances of primary cutaneous inoculation of some of the so called systemic fungi such as coccidiodes and histoplasma, the ensuing disease processes have had a remarkable resemblance to those of the subcutaneous mycoses rather than to the ordinary course of events in systemic infection. These infections are characterized by the development of a lesion at the site of inoculation. Unlike the

systemic mycoses, whose primary mode of entry is usually pulmonary, these infections are the result of traumatic implantation of the fungus into the skin. In general the ensuing disease remains localized to a particular area or slowly spreads to the surrounding tissue. In some diseases slow extension via lymphatic channels is a frequent occurrence (sporotrichosis), and in others hematogenous and lymphatic dissemination is rarely recorded.

Subcutaneous mycoses can be classified into

CHROMOBLASTOMYCOSIS

PHAEOHYPHOMYCOSES

MYCETOMA

HYALOHYPHOMYCOSIS

SPOROTRICHOSIS

RHINOSPORIDIOSIS

SUB CUTANEOUS PHYCOMYCOSES

LOBOMYCOSIS

CHROMOBLASTOMYCOSIS

Chromoblastomycosis or verrucous dermatitis is caused by any of several morphologically related dematiaceous fungi. It is defined by Carrion A.L (1910) as chronic granulomatous diseases of the skin, confined most frequently to one of the lower extremities, but occurring also on other exposed areas such as the arm, head, neck or trunk and characterized clinically by the formation of warty cutaneous nodules or plaques which develop very slowly, ultimately forming prominent papillomatous vegetations which may or may not ulcerate. The infection is characterized by the development of a papule at the site of the traumatic insult that spread to form warty or tumor like lesions described as 'cauliflower like'. The tissue

form, the sclerotic / muriform bodies are brown or copper colored, septate cells that appear to be dividing and are identical in all case of chromoblastomycosis. The presence of muriform bodies in cutaneous or sub cutaneous tissue is pathognomonic of chromoblastomycosis. The agents of chromoblastomycosis must be cultured because the appearance of the muriform bodies formed by all agents of chromoblastomycosis is similar, the fungus cannot be identified on the basis of tissue morphology.

The groups of fungi known to cause chromoblastomycosis are dematiaceous. All are slow growing and produce heaped up and slightly folded darkly pigmented colonies with a grayish – velvety appearance. The reverse side of the colony is jet black. The taxonomy of the organisms that causes chromoblastomycosis is complex. Their identification is based on distinct microscopic morphologic features. Three genera, Cladosporium, Phialophora, and Fonsecaea are known to cause chromoblastomycosis frequently.

The genus Cladosporium includes those species which produce long chains of conidia (blastoconidia) that have a dark septal scar.

The genus Phialophora includes those species that produce short, flask shaped or tubular phialides, usually with a well-developed collarette. Clusters of conidia are produced by the phialides through an apical pore.

The genus Fonsecaea includes the organisms which exhibits a mixed type of sporulation, that uniquely includes one celled primary conidia that are produced on either side of the conidiophores resembling a series of bent knees. Conidia are produced sympodially. The primary conidia gives rise to secondary conidia that appear to occur in loose heads. This is known as rhinocladiella type sporulation. *F.pedrosoi* and *F.compactum* are example for this type of sporulation.

The characteristic features of the three genera are summarized as follows

1. **Cladosporium**:- Cladosporium type sporulation with long chains of elliptical conidia (2-3 μm x 4-5 μm) are born erect, tall branching conidiophore. The important species are *Cladosporium carrionii* and *C.bantianum*. Differentiation from the morphologically similar *C.carrionii* is made on the following basis

   a) The *C.bantianum* generally grow slowly

   b).There is a difference in spore size, distribution, *C.bantianum* having regular conidia which tend to be more elongate than those of *C.carrionii*.

   c) The maximum temperature for growth of *C.bantianum* is 42 to 43°C where as that of *C.carrionii* is 35 to 36°C

   d) C.carrionii fail to induce brain abscess when injected into mice, whereas most strains of *C.bantianum* will do so.

   e) In *C.carrionii* long chains of conidia are seen. Pathogenic species may be differentiated from saprophytes of this genus on the basis of physiologic characters. The pathogen grow well at 21 to 25°C and 37°C and are slow growng, where as the saprophytic forms are fast growing at 21 to 25°C but do not grow at 37 °C

2. **Phialophora** (*Phialophora verrucosa*)

Tube like or flask like phialides each with a distinct collarette. Conidia are produced endogenously and occurs in clusters at the tip of the phialide. Examples are

P.verrucosa

P.jeanselmei

3. **Fonsecaea** (*F.pedrosoi and F.compactum*) conidial heads with sympodial arrangement of conidia, with primary conidia giving rise to secondary conidia. Cladosporium type of sporulation may occur and phialides with collarettes may also

be present. *F.pedrosoi* is differentiated from *F.compactum* by the production of loose heads in contrast to more compact heads produced by *F.compactum*.

The laboratory diagnosis of chromoblastomycosis is made easily. Biopsy was taken from the lesion for culture, direct mount using 10 % KOH and for histopathologic examination. The presence of copper colored sclerotic/ muriform bodies which appear rounded, brown and 4-10 μm in diameter resembling copper pennies is diagnostic.

PHAEOHYPHOMYCOSIS

Dematiaceous fungi are characterized by the presence of a brown to black color in the cell walls of their vegetative cells, conidia, or both that results in colonies ranging from olive or gray to black. The dark pigmentation of the majority of medically important fungi is caused by the deposition of dihydroxynaphthalene melanin formed via pentaketide metabolism. In chromoblastomycosis disease is characterized by the presence of muriform (sclerotic) bodies in infected tissue. Phaeohyphomycosis is characterized by the presence of dematiaceous yeast like cells, pseudohyphae, or any combination of these forms. The name phaeohyphomycosis is not restricted to hyphomycetes; it encompasses all fungi having dematiaceous cells in infected tissue, regardless of the taxonomic classification of the etiologic agents. The hyphae observed in clinical specimens may be regular and uniform in diameter or irregular in shape with many swollen cells and may be short or very long. Phaeohyphomycosis encompasses a spectrum of opportunistic entities that range from purely cosmetic conditions to fatal cerebral infections. As with chromoblastomycosis, the identities of etiologic agents of phaeohyphomycosis cannot be determined from microscopic examination of clinical specimens. These fungi must be grown in laboratory culture media before they can be identified. Masson-Fontana stain is used

for demonstrating the melanin pigment present in the cell wall of the fungi present in the tissue. The most common phaeohyphomycosis syndrome includes allergic fungal sinusitis, keratitis, and sub cutaneous infections. Subcutaneous infections commonly present are either a cyst or a diffuse lesion and are chronic, and usually remain localized.

Medically important dematiaceous fungi causing Phaeohyphomycosis

Alternaria:- The important species are *A.alternata, A.tenuissima, A.chlamysopsora, A.longipes*

Aureobasidium:- *A.pullulans*

Bipolaris:- *B.spicifera, B.hawaiensis, B. australiensis*

Chaetomium:- *C.globosum, C.strumarium, C.atrobrunnem*

Cladosporium:- *C.cladosporioides*

Cladophialophora:- *C.bantiana, C.emmonsii*

Coniothyrium:- *C.fuckelii*

Curvularia:- *C.lunata, C.senegalensis,C.verruculosa, C.clavata, C.geniculata*

Dactylaria :- *D.constricta, D.gallopova*

Exophiala:- *E.dermatitidis, E.jeanselmei, E.jeanselmei var.lecanii-corn*

Exserohilum:- *E.rostratum, E.meginnisii*

Fonsecaea:- *F.pedrosoi, F.compatum,*

Hormonema

Lasiodiplodia:- *L.theobromae*

Lecythophora:- *L.hoffmannii, L.mutabilis*

Nattrassia:- *N.mangiferae*

Phaeoannellomyces:- *P.werneckii, P.elegans*

Phaeococcomyces:- *P.exophialae*

Phialemonium:- *P.curvatum, P.obovatum*

Phaeosclera:- *P.dermatoides*

Phialophora:- *P.richardsiae*

Phialoacremonium:_ *P.parasiticm,P.inflatipes, P.rubrgenum.*

Phiacremonium:- *P.rugrigenum*

Phoma:- *P.eupyrena, P.minutispora, P.oculo-hominis, P.sorghina.*

Rhinocladiella:- *R.aquaspersa, R.atrovirens, R.*

Ramichloridium :- *R.schulzeri, R.mackenzei, R.subulatum*

Scedosporium:- *S.apiospermum, S.prolificans*

Scytalidium:- *S.dimidiatum. S.lignicola, S.hyalinum*

Sporothrix:- *S.schenckii, S.cyanescens*

Wangiella:- *W.dermatitidis*

Xylohypha:- *X.bantiana, X.emmonsii*

MYCETOMA

Mycetoma (literally fungal tumor) is a chronic granulomatous infection that usually involves the lower extremities but may occur in any other part of the body. The disease was originally reported by Gill (1842) from Madurai, South India, and Carter (1860) established its fungal etiology. The infection is characterized by swelling, purplish discoloration and tumor like deformities of the subcutaneous tissue and multiple sinus tracts that drains pus containing yellow, white, red or black granules. The granules contain micro colonies of the causative agent. The infection gradually progresses to involve the bone muscle or other contagious tissue and ultimately requires amputation in most cases. There may be dissemination to other organs including the brain, however this type of infection is relatively uncommon. Mycetoma is common among persons who live in tropical and sub tropical regions of

the world, where outdoor occupation and failure to wear protective clothing predisposes the infections.

Two types of mycetoma are described – 1) Actinomycotic mycetoma, which is caused by species of aerobic and anaerobic actinomycetes including *Nocardia, Actinomadura*, and *Streptomyces* and 2) eumycotic mycetoma caused by heterogenous group of species having true septate hyphae.

Geographical distribution:- Maduramycosis occurs most frequently in tropical and sub tropical zones where few people wear shoes and the feet come in direct contact with the soil. Instances have been reported from India, mainly Tamil Nadu, Africa, Europe, South America, Mexico, Canada and the United States. The source of infection is exogenous, and more than a half of the patients give a history of an injury, such as a minor scratch or a wound produced by a splinter. Of the organisms isolated from maduramycosis, most are thought to occur either as saprophytes in the soil or on plants.

Table 1. Organisms associated with maduramycosis

Black granules:-

1. *Madurella mycetomii* (Laveraan) Brumpt, (1905)
2. *M.grisea.*(MacKinnon), Ferrada and Montemayer, (1949)
3. *Pyrenochaeta romeroi* Borelli, (1959)
4. *Leptosphaeria senegalensis* Baylet, Camain, and Segretain (1959)
5. *Phialophora jeanselmei* (Langeran) Emmons.(1945)
6. *Curvularia lunata* (Wakker) Boedijn.(1933)
7. *Corynespora lassicola*
8. *Curvularia geniculata*
9. *Exophiala.jeanselmei*

10. *Plenodomus avramii*

11.*Pseudochaetosphaeronema larense*

12.*Pyrenochaeta mackinnonii*

13. *Pyrenochaeta romeroi*

White to Yellow Granules:-

1. *Allescheria boydii.* Shear (1921)
2. *Monosporium apiospermum* Saccardo, (1911)
3. *Cephalosporium falciforme* Carrion (1951)
4. *Neotestudina rosatii*, Segretain and Destombes,(1961)
5. *Cephalosporium recifei*, Leao and Lobo.(1934)
6. *Acremonium falciforme*
7. *Acremonium.recifei*
8. *Aspergillus nidulans*
9. *Cylindrocarpon destructans*
10. *Fusarium moniliforme*
11. *Fusarium.solani*
12. *Polycytella hominis*

Symptomatology:-

The mode of onset is neither characteristic nor uniform with or without a history of previous injury. The first detectable lesion may be (1) a small papule, (2) a small nodule which is deep seated and fixed, (3) and indurated area surrounded by a vesicle (4) and an abscess which ruptures with subsequent formation of a fistula. The disease progresses slowly and is at first characterized by periods of remissions and relapses. As many as 6 or 8 papules may form in succession, or as many as 12 abscesses may develop and disappear over a periods of months or years before the

entire foot becomes involved and presents the characteristic clinical picture. The classical picture of swellings and deformities may develop with in a period of months, but it usually takes much longer, sometimes as many as 10-15 years. As the infection extends deeper into the tissues, the muscles, bones, fascia and tendons may become involved and the foot or hand becomes club shaped or may develop into globose mass two or three times the normal size. The skin is discolored, with pitted scars, nodules and multiple sinuses develop. There is no loss of sensation in the skin. Nodules frequently develop around the openings of fistulas from which serosanguineous or oily fluid containing the diagnostic granules drains. The color of the granules varies depending upon the infecting organism. There is little systemic reaction unless the lesions are secondarily infected. Pain is present rarely even when the affected part is manipulated. The patient generally can walk until the disease has progressed to the stage where marked wastage of the leg muscles. The numerous fungi that have been isolated from cases of maduramycosis fall into two classes: Ascomycetes and Deuteromycetes (Fungi Imperfcti).

HYALOHYPHOMYCOSIS

Hyalohyphomycosis refers to mycotic infections of man or animals caused by a number of hyaline (Non-dematiaceous) hyphomycetes, where the causative fungi appear as hyaline (non pigmented) hyphae in the infected tissue. Etiological agents include species of *Scedosporium (S.prolificans) Penicillium spp, Paecilomyces (P.lilacinus, P.varioti) Acremonium (A.falciforme, A.kiliense and A.recifei, A.alabamensis, A.potroni, A.roseo-griseum, and A.stricitum). Beauveria, (B.bassiana). Fusarium, (F.solani, F.oxysporum, F.vericilloides, F.napiforme), Scopulariopsis (S.brevicaulis, B.brumptii), Scytalidium (S.hyalinum), Trichoderma, T.viridae, T.koningii, T.koningii, T.longibrachiatum, Colletotrichum*

(C.gleosporioides), Onychola, (O.canadendsis) and Cheatomium, (C.globosum, C.atrtrobrunneum). Recently species of many other genera, viz *Cylindrocarpon, Lecythophora and Philaemonium* have been found to be responsible for severe infection in immunocompromised patients.

SPOROTRICHOSIS

Sporotrichosis is a chronic infection of world- wide distribution caused by the dimorphic fungus, *Sporothrix schenckii*, where natural habitat is living or dead vegetation. Sporotrichosis is typically a cutaneous to sub cutaneous chronic infection that may undergo lymphatic spread. Musculoskeletal involment and disseminated infection may occur rarely, and pulmonary infection following inhalation of the conidia has been documented. Human beings aquired infection through trauma (thorn prick or splinters) usually on the hand, arm or leg. More than one dozen species of sporothrix have been described. *Sporothrix schenckii* is the only documented pathogen. Howard A and Orr (1963) isolated sporothrix from soil and considered their isolation from nature to be a variants of *S.schenckii* and they were non pathogenic to human beings. Mackinnon (1969) and his associates found their isolates to be indistinguishable from *S.schenckii* in all respects. *Sporothrix cyanescens*, a recently described species that is distinctive for its purple blue diffusible pigment, can be recovered from clinical material, but animal studies have shown that it is not pathogenic by de Hoog and G.A.deVries (1973). Isolates of Sporothrix species that have been recovered from environmental sources lacks the dematiaceous thick walled conidia and are non virulent in mice. In order to identify a mold recovered from clinical material as *Sporothrix schenckii*, one must demonstrate its dimorphic capability, i.e. its ability to grow as both mold and stable yeast. To do so, isolates must be sub cultured on an enriched medium such as brain heart infusion broth

containing 0.1% agar and incubated under 5% CO_2 at 37 ^{o} C, *S.schenckii* forms yeast phase and the color of *S.schenckii* transforms to a soft, cream colored to white, yeast like colony. The Primary lesion begins as a small non healing ulcer, commonly of the index finger or the back of the hand. The infection is characterized by the lymph nodes, skin or subcutaneous tissues of nodular lesions which soften and break down to form indolent ulcers. The disease becomes rarely disseminated and pulmonary infection may also be seen. The infection is an occupational hazard for farmers, gardeners, nursery workers and miners. So it is commonly known as *"Rose Gardner's Disease."*

Sporothrix species are basidiomycetes. It is a dimorphic fungus which may appear in human tissues as cigar-shaped to oval, budding cells (3-5 μm in length), usually within the polymorphonuclear leukocytes, or in the form of an asteroid body (amorphous, radiating eosinphilic mass surrounding a cryptococcus- like yeast cell) However, it is extremely difficult, or usually impossible, to demonstrate the organism in pus or tissue sections from human lesions (an exception is pulmonary sporotrichosis, where lesions generally reveal large number of the yeast cells). Microscopically, the hyphae are delicate (1-2 μm thick) septate, exhibit branching and bear one celled conidia, 2-5 μm in diameter. These are borne bouquet like in clusters from the tips of single conidiophores by an individual delicate, thread like structure (denticle). As the culture ages, single celled, thick walled, black pigmented conidia, borne along the sides of the hyphae.

RHINOSPORIDIOSIS

Rhinosporidiosis is a chronic granulomatous disease characterized by the production of polyps as hyperplasia on mucus membrane. The etiologic agent is *Rhinosporidium seeberi*. Most of the early studies of rhinosporidiosis were made in

India and Ceylon where the disease occurs frequently. It is also reported from other parts of the world. The systematic position of *R.seeberi* is still uncertain. Most investigators consider it to be a fungus, though it has not been isolated in culture. Although rhinosporidiosis is most often seen in children and young adults, it occurs at any age group. Infections are seen most often in laborers and in those with frequent exposure to water in streams and pools. The disease is not contagious, and sources of infection are exogenous. Rhinosporidiosis was observed in workmen who lived under water to bring up sand in bucket, but not in their associates who carried the sand from the water's edge. It has been suggested that water insects or fish may be the hosts of the fungus.

Laboratory diagnosis :- Direct examination of the surface of the polyp may reveal the subsurface position of sporangia which are white and large (up to 350 μm in diameter) that they can be seen with the naked eye. The mature sporangiums contain numerous endospores.

SUBCUTANEOUS PHYCOMYCOSIS

(Subcutaneous zygomycosis)

CONIDIOBOLOMYCOSIS:- Is a chronic mycosis affecting the subcutaneous tissue. It originates in the nasal sinuses and spreads to the adjacent subcutaneous tissues of the face, causing disfigurement. The disease occurs mainly in the tropical rain forests of Africa, South and Central America and South and East Asia. *Conidiobolus coronatus* (*Entomophthora coronata*) lives as a saprophyte in soil humus and on decomposing materials in moist warm climates. It can parasite certain insects. The disease is most common among adult males particularly those working in tropical rain forests. Infection is acquired through inhalation of spores or through their introduction into the nasal cavities by soiled hands. Clinical manifestation of

conidiobolous infection generally begins with unilateral involvement of the nasal mucosa. The most common symptoms is obstruction along with frequent nosebleed. Subcutaneous nodules then develop in the nasal and para nasal regions. The spread of infection is slow but relentless. The infection is usually confined to the face with the development of gross facial swelling involving the forehead, periorbital region, and upper lip is very distinctive. The lesions are firmly attached to the underlying tissue. Although the bone is spared, the skin remains intact. Spread to the lymph nodes has been reported

Diagnosis:- Microscopic examination of smear or tissue from the nasal mucosa will reveal broad, non septate thin walled mycelial filaments.

Culture:- Cultures are difficult to obtain, and the specimen must be inoculated onto the largest possible number of media. The media should be incubated between 25 and 35 $^{\circ}$ C to enhance the growth of *Conidiobolus coronatus*. The colonies which grow rapidly are flat, cream colored, glabrous and become radially folded and covered by a fine powdery white surface mycelium and conidiphores. The colony becomes tan to brown with age.

Microscopic morphology:- Conidiophores are simple, forming solitary, terminal conidia that are spherical, 10 to 25 μm in diameter, single celled and have a prominent papilla. Conidia may also produce hair like appendages called villae. The histopathological examination shows fibroblastic proliferation and an inflammatory reaction with lymphocytes, plasma cells, histiocytes, eosinophils and giant cells. Broad, thin walled hyphae with occasional septa branched at right angles are seen.

BASIDIOBOLOMYCOSIS

Is a chronic subcutaneous infection of the trunk and limbs. This disease is mainly seen in the tropical regions of East and West Africa, Indonesia and India. The

causative agent *Basidiobolus ranarum* is the sole agent causing the disease and that *Basidiobolus meristosporus* and *B.haptosporus* are only synonyms of the former. *B.ranarum* has been isolated from the guts of frogs, toads and lizards, ants and decaying plant matter. Inoculation through a thorn prick or an insect bite has been suggested. The disease is usually localized to the back of the shoulders and to the arms, but it may be found on the buttock and thigh. The initial swelling may be rapid or slow in onset and is hard and painless. The spread is slow and relentless, and a large mass that is attached to the skin but not the underlying tissue (unlike conidiobolous infection). This is a disfiguring infection, but the skin covering the lesion does not ulcerate. Lymphatic obstruction may occur and can result massive lymphoedema. The basidiobolomycosis closely resembles soft tissue sarcoma, mycetoma, bacterial cellulitis. All these conditions can be reliably distinguished from zygomycosis by biopsy and fungal culture of the lesion.

LOBOMYCOSIS

Lobomycosis is a chronic, localized, sub epidermal infection characterized by the presence of keloidal, verrucoid, nodular lesion or sometimes by vegetating crusty plaques and tumors. The lesions contain masses of the spheroidal yeast like organism tentatively referred to as *Lobo loboi*. There is no systemic spread. The disease has been found in man and dolphins. The disease was first described in 1931 by Jorge Lobo. The etiologic agent of lobo mycosis has not been isolated in culture. Its inability to be cultured in vitro and its clinical restriction to the cooler part of the body explain the organism is an obligate parasite of some lower animal forms.

Clinical disease:- The initial infection occurs at the site of some trauma to the skin. The lesion begins as small, hard nodules resembling keloids that are sharply defined, freely movable, and have a smooth surface. The color of the nodules may be slightly

brownish. The surrounding cutaneous area is normal and erythema is absent. The lesion are painless or slightly pruritic. The disease may be transferred to other area of the skin by subsequent abrasions and auto inoculation, in which case, groups of nodules may be formed on several different areas of the body. Lobomycosis causes little discomfort to the patients, and there are no generalized symptoms. The most successful treatment for the condition is wide surgical excision of the affected areas.

MYCOLOGY HISTORICAL BACKGROUND

The study of mycology started as early as 1677 when Hook constructed a magnifying lens. With this finding people became aware of microscopic organisms especially fungi. First human fungal infection was described by Mayer & Emmert, (1815) in a jay. Augustino Bassi (1773-1865) in 1815 observed that muscardine disease of the silk worms (Bombyx mori) was caused by a fungus called *Beauveria bassiana*. But the name aspergillus dates back to Michaeli (1729) who described the swollen vesicle of aspergillus. Fungal etiology of skin infection was first noticed by Remak (1937), Schoenlein (1839) established the pathogenic nature of a dermatomycosis (favus) after whom the pathogenic agent was named later as *Trichophyton schoenleinii*. After this scientists concentrated on various aspects of dermatomycosis .

The discipline of Medical Mycology attained recognition in the world sciences in 1910 when French dermatologist Raymond Jacques Sabouraud (1864-1936) published his monumental work on dematophytes, "*Les Teignes*". He has rightly been called the "Father of Medical Mycology". Similarly, P.A. Saccardo has played a significant role in the establishment in the field of Medical Mycology in earlier days. The other scientists who played an active role in the development of mycology are Schoenle, Norman Conant, Chester Emmons, David Gruby, Rippon J.W, Ajello L

and Kwon-Chung K.J. The research in the field of mycology was renewed in the later days of 19 th century and beginning of 20 th century Renon, (1897); Sabouraud, (1910). This may be due to the increasing incidence of various fungal infections, both superficial and deep, due to the increased use of antibiotics against bacterial infections. In 1918, Ernst cultured *Mucor* from sputum of a patient having pneumonitis. In 1952 Kligman demonstrated the development of *Candida albicans* infection after antibiotic treatment. It was noted by many researchers that debilitating conditions such as leukemia, diabetes and cancer predispose fungal infections Hausmann, (1958). Prolonged exposure to corticosteroids, antibiotics and immunosuppressive drugs also contribute to the predisposing factors to make one susceptible to fungal infection. Emmons etal (1970).

Now nearly 24 well defined mycoses of man are recognized and about 150 saprobic fungi can adapt to parasitism in man. With a few exceptions the systemic, lymphatic, and sub cutaneous mycoses are caused by fungi which are essentially free living saprobes in nature. These mycoses are not contagious, and infection in man and animals follows inhalations or traumatic implantation of the fungi or its spore. In India diseases of mycotic origin were known from vedic period. Mycetoma was first described from India by Van Dyke Carter in 1860. Kanthaack in 1892 and Vincent in 1984 further defined the disease. Sporotrichosis was first reported from United States. In 1898 Schenck described a case and isolated the fungus *Sporotrix schenckii.* Pioneer workers in the field of mycology in India are Powel, Panja G, Gosh L.M and Day N.C. Most of the early studies of rhinosporidiosis were made in India and Ceylon where the disease occur frequently.

Contrary to what appears in most textbooks, chromoblastomycosis was first described by Max Rudolph in 1914 and not by Lane or Medlar in 1915. In 1987,

Castro and Castro reported that Max Rudolph, a German physician living in Brazil, published a preliminary communication where the first 6 cases of the disease were described. Rudolph was also able to isolate a dark-colored fungus from 4 of 6 patients; this fungus grew in culture as a dark grey-to-black–colored furlike colony. Rudolph believed this fungus to be a type of blastomycete, and he successfully inoculated the disease in 4 white rats and 2 monkeys. Surprisingly, he did not describe the histologic aspects of the disease or the pathognomonic sclerotic cells, which both Lane and Medlar described 1 year later. In 1908 Guiteras had observed 10 cases of a disease known as chapa in which the clinical aspects resembled those of chromoblastomycosis. Unfortunately, those cases were not published. *Torula bantianum* was first reported by Saccaro (1910).

In 1920, two Brazilian physicians, Pedroso and Gomes, published 4 cases that had been under observation for many years, the first one since 1911. According to them, all 4 cases were caused by *P verrucosa.* Two years later, in 1922, Brumpt concluded that the agents isolated by Pedroso and Gomes could not be classified as *Phialophora* species, and he coined the denomination *Hormodendrum pedrosoi,* later renamed *F pedrosoi* by Negroni in 1936. By 1930, new cases had been described outside the American continents in France, Sumatra, and Poland (Rippon, 1982). Four different genera are now widely accepted to cause chromoblastomycosis: *F pedrosoi, P verrucosa, C carrionii,* and *F compacta* (Lacaz, 1991). Rare cases of chromoblastomycosis caused by *Rhinocladiella aquaspersa* and *Exophiala* species have also been reported, allowing the inclusion of these species among those that cause the disease (South, 1981; Naka, 1986; Barba-Gómez, 1992; Queiroz-Telles, 1996; Padhye, 1996). Infection in animal as well as man due to dematiaceous fungi was reported first time by Kano K (1934). Emmons in Binford *etal*, (1944).

Phialophora dermatitidis by Kano (1937). Carrion identified one of his case and named it *Hormodendrum compactum*. Simmon, (1946) studied Chromoblastomycosis in Australia, described *Cladosporium carrionii* Trejos.(1954). *Phialophora gougerotii* was first reported by Borelli (1955). One case of phaeosporotrichosis caused by *P.richardsiae* was reported by Schwartz and Emmons (1968).

Since its identification of chromoblastomycosis in the early 1910s, the name of the disease has been frequently misused to encompass other infections caused by dematiaceous fungi. More recently, the advent of immunosuppressive therapies and diseases brought more confusion because of the identification of new agents and clinical settings. With the introduction of the concept of phaeohyphomycosis by Ajello and colleague in 1975 and McGinnis in 1983, differentiation among these diseases became more obvious. The features of chromoblastomycosis are distinctive enough to be considered as an independent clinical entity. The infection should not be confused with mycoses, such as mycetoma or phaeohyphomycosis, caused by other dematiaceous fungi. Nowadays, the term chromoblastomycosis is restricted to the cases in which sclerotic cells are present in tissue. Sclerotic cells, also known as Medlar bodies, are globe-shaped, brown-colored, thick-walled structures that are 4-12 mm in diameter. Medlar first described them in 1915. These structures multiply by septation, and they induce a purulent and granulomatous inflammatory reaction in tissue. In 1935 chromoblastomycosis was contracted to chromomycosis as it was considered that the former term implied that the causal agents occurred as yeasts in tissues.

Chromoblastomycosis (CM) is a chronic granulomatous mycotic infection of the skin and subcutaneous tissue caused by pigmented fungi, the most being *F.pedrosoi*, Cathy P.M (1989). Other causative fungi are *Phialophora verrucosa*

Medlar E.M *etal* (1915) Lane C.U (1915), *Fonsecaea compactum* Carrion A.L (1935) , *F.dermatitidis*, *Cladosporium carrionii* Trejos (1954) and *Rhinocladiella aquaspersa*,Borelli D (1972) It typically occurs on the exposed surfaces of the lower leg following traumatic implantation of the organisms, Greer K.E *etal* (1979), Vollum D.I (1977), Carrion A.L,(1975). The lesions can involve other sites either by direct spread or autoinoculation, Rippon *etal* (1974) or by lymphatic extension, Debes V.J *etal* (1964) or haematogenous spread Azulay R.D *etal* (1967).

Chromoblastomycosis caused by several species of dematiaceous fungi is usually confined to one of the lower extremities and affects only the skin and subcutaneous tissue, though the lymph glands draining the diseased focus, may participate in the pathological process Carrion *etal* (1933). Rajam *etal* (1958) studied a case which showed both yeast and mycelial phase in tissue and in culture. Rare cases have been reported affecting the hand, arm, face and buttocks, Bhaktaviziam C *etal* (1970). Mucous membrane is usually not involved, but invasion of the conjunctiva and nasal septum have been reported by Jakamitzu *etal* (1972) and Nagarkatti P.S *etal* (1972). The Chromoblastomycotic lesion may be verrucous with central scarring (tuberculoid), extremely scarred with a serpigenous border (syphiloid), scaly (psoriasifiorm) or indurated with fistula (mycetomatoid), Vollum D.I (1977). The diagnosis of chromoblastomycosis should be confirmed either by direct microscopy of the scarpings from the lesion in 20 % KOH when thick walled dark brown tissue form of the fungus (sclerotic bodies/muriform bodies/copper pennie body) are seen; or by histological examination of a biopsy specimen when the granulomatous reaction and spore are diagnostic; or by culture of scarpings or biopsy material, Vollum D.I *etal* (1977). Kotrajaras R and Chongsathien S, (1979) reported subcutaneous chromomycotic abscesses caused by *Phialophora gougerotii* in 50 year

old woman characterized by subcutaneous abscesses adhering through fistulous tracts, rupturing and leaving black crusts over multiple sinuses mimicking mycetoma. Greer K.E *etal* (1979) reported cystic form of chromoblastomycosis due to *Wangiella dermatitidis* that developed following a nonpenetrating injury to the thumb.

Radhakrishnan K (1981) in his article Chromomycosis due to *Phialophora pedrosoi* (with dimorphism in tissue) reported three case of chromomycosis proved histopathologically. In one case *Phialophora pedrosoi* was grown in culture. The saprobic form of the fungus could also be seen in tissue. Dematiacous fungi characteristically exhibit dimorphism *ie* parasitic tissue form and saprobic mycelial form. In all cases there was hyperplasia of epidermis. Dermis showed chronic inflammatory and giant cells. Characteristic sclerotic and septate dematiaceous mycelial forms were seen in H& E, PAS and Giemsa stains. In one case mycelia were also seen in PAS section. Morales L.A *etal* (1985) reported chromoblastomycosis in a renal transplant recepient with an asymptomatic mass in the right forearm. The infection was cured by aggressive diagnosis and surgical treatment. Jayalakshmi *etal*, (1990) reported nine cases of histologically diagnosed chromoblastomycosis. It was reported from Malaysia. All the patients were males and ranged in age from 56 to 65 years and duration of symptoms varies from 5 months to 13 years and all the lesions were noted in lower extremities.

Rubin H.A *etal* (1991) in his article pointed that the exact pathogenesis of chromoblastomycosis is unknown but direct percutaneous inoculation, inhalation and hematogenous dissemination have been implicated. They reported a case of chromoblastomycosis that followed a well-defined episode of penetrating trauma. The causative organism, *Fonsecaea pedrosoi*, was cultured from the patient's lesions and from the tree branch responsible for trauma. This natural experiment supports the

contention that one cause of chromoblastomycosis is traumatic cutaneous implantation of the fungus. Sanjay Agarwal N *etal* (1991) presented a cystic type of subcutaneous fungal infection and the fungus isolated was *Phialophora.* This was the first reported case of cystic type of chromomycosis from India. Histopathology revealed clusters of pigmented sclerotic bodies (muriform bodies) surrounded by marked granulomatous inflammatory reaction and the presence of foreign body type giant cells. Woodgyer A.J *etal* 1992 reported four non-endemic New Zealand cases of chromoblastomycosis. All these cases were caused by *Fonsecaea pedrosoi.* Deshpande S *etal* (1993) reported two cases of chromomycosis during the period from 1980-1989. The isolates were identified as *Fonsecaea compactum.* Attapattu-M.C (1997) presented a study of the clinical and mycological features of 71 SriLankan patients suffering from chromoblastomycosis for the 16 year period from 1978 to 1993. Out of this 64 *Fonsecaea pedrosoi* (64), *Phialophora verrucosa* (3) and a fungus morphologically compatible to *F.compata* (2)

Tuffanelli-L *etal* (1990), reported the treatment of chromoblastomycosis was frequently difficult and unsatisfactory. Itraconazole, a triazole compound, is considered as the possible drug of choice. Queiroz-Telles-F *etal* (1992), in his article, itraconazole in the treatment of chromoblastomycosis due to *Fonsecaea pedrosoi* evaluated the efficacy and tolerability of itraconazole in chromoblastomycosis due to *Fonsecaea pedrosoi* in a non-comparative open clinical trial in 19 Brazilian patients with histopathologically and mycologically proven active chromoblastomycosis. Result of the study suggesting that itraconazole is an effective compound against chromoblastomycosis due to *Fonsecaea pedrosoi.* Successful treatment of chromoblastomycosis due to *Fonsecaea pedrosoi* by the combination of itraconazole and cryotherapy by Kullavanijaya-P and Rojanavanich (1995). In this

article they had tried itraconazole and cryotherapy. Itraconazole alone showing good response for chromoblastomycosis but it took as long as 18-30 months for lesion to heal. Itraconazole 200 to 400 mg/dl along with monthly liquid nitrogen therapy was tried in 10 cases. Result of the study showed that itraconazole along with cryotherapy was highly effective against chromoblastomycosis.

In a study conducted by De Bedout C, *etal* (1997) with different human isolates of *F.pedrosoi* in Brazil, it was found that 33 % were resistant to amphotericin B, 58 % to 5 fluorocystosine and 66 % to fluconazole. But none of the isolates proved resistant to itraconazole. Saperconazole, a newer drug showed best invitro activity against *F.pedrosoi* in experimental murine chromoblastomycosis. Chromoblastomycosis Combination therapy effective, in one case, presented at the 1998, Atlantic Dermatology Meeting combined azole and triazole antifungal with CO_2 laser therapy, by Robert Hayman and Zoila Flasher, to reduce lesions and manage fungal growth on the leg and foot of a man. Sevignv G.M and Ramos F.A (2000) reported chromoblastomycosis due to *Fonsecaea pedrosoi*, who was treated with 8 months of terbinafine 250 mg by orally daily with histologic and mycologic cure. Cutaneous chromomycosis, three cases reported by Mohan N *etal* (2002) from Bangalore and the culture yielded Cladosporium. Histologically all cases showed chronic granulomatous infiltration with microabscess formation; however no fungal elements were demonstrable. One case was successfully treated with itraconazole with no relapse. The second case failed to respond to itraconazole in spite of 600 mgs daily for 3 months. Two unusual case of chromo mycosis reported by Patra S *etal* (SIHAM 2002), one patient was from Tripura and the fungus isolated was *Fonsecaea pedrosoi*, the patient was treated by ketaconazole and diathermy cautery and the other patient from Mizoram, the isolated fungus was identified as *Cladosporium carrionii*.

The patient was non responsive to treatment with ketaconazole, itracoazle, amphotericin B, potassium iodide and antituberculous drug.

Luiz G.M *etal* during the American Academy of Dermatology's 56th Annual meeting, said that in recent years they have successfully treated a group of chromomycosis with cryosurgery with liquid nitrogen. The clinicians said that the surgical technique is a cost effective method that can be employed in low income population in developing countries, where chromomycosis is most prevalent and they also indicated that follow up itraconazole therapy is at times useful against these diseases. Agarwalla *etal* (2002) reported two cases of chromoblastomycosis from Nepal. The first case, a 67 year old male farmer, presented with itchy hyperkeratotic, scaly plaques with scarring and black dots on the lateral aspects of his left arm and dorsum of his left hand of 28 years duration. The second case, a 75 year old farmer presented with erythematous, crusted, scaly plaques on the dorsum of the left foot of 30 years duration. These two cases were the first reported case of chromoblastomycosis from Nepal. Brandt M.E and Warnock (2003) analyzed the epidemiology, clinical manifestation, and therapy of infections caused by dematiaceous fungi. Among the more important human pathogens are *Alternaria spp, Bipolaris spp, Cladophialophora bantiana, Scedosporium prolificans, Scytalidium dimidiatum,* and *Wangiella dermatitidis.* These infections are more common in tropical and subtropical climates. Disseminated infections are uncommon, but its incidence is increasing, particularly among immunocompromised individuals. *Scedosporium prolificans* is the most frequent cause. A number of dematiaceous fungi are neurotropic, including *Cladophialophora bantiana, Ramichloridium mackenziei* and *Wangiella dermatitidis.* Most forms of diseases caused by dematiaceous fungi require surgical and medical treatment. Itraconazole is the drug of choice for

chromoblastomycosis and phaeohyphomycosis, while ketaconazole is the drug of choice for mycetoma. Koga T *etal* (2003) discussed various therapeutic approaches of subcutaneous mycosis. Results of antifungal susceptibility test may provide valuable information for deciding the appropriate method of treatment. Development of new antifungal agents and combination therapies may result in improvement in the management of subcutaneous mycoses in the future.

Pang K.R *etal* (2004) discussed various sub cutaneous mycosis and their mode of entry to the body. Sporotrichosis, mycetoma and chromoblastomycosis are the common subcutaneous mycoses than rhinosporidosis, zygomycosis, phaeohyphomycosis, and lobomycosis. Bonifaz A *etal* l (2004) Dermatology Service and Mycology Department Mexico described the treatment of chromoblastomycosis with systemic antifungal agents. At present there is no treatment of choice for chromoblastomycosis but rather, several treatment options, with low cure rates and many relapses. The choice of treatment should consider several conditions, such as the causal agent, extension of lesions, clinical topography and health status of the patient. Most oral and systemic antifungals have been used; the best results have been obtained with itraconazole and terbinafine at high doses, for a mean of 6-12 months. In extensive and refractory cases, chemotherapy with oral antifungals may be associated with thermotherapy (local heat/or cryosurgery). Limited or early cases may be managed with surgical methods with oral antifungal agents. It is also important to test the antifungal susceptibility to major casual agents.

Ajello L (1986) in his article hyalohyphomycosis and phaeohyphomycosis: two global disease entities of public health importance clearly defined both diseases. The term chromomycosis was unfortunately used (Mcginnis M.R, *etal* 1983) inappropriately for infections caused by a growing number of diverse dematiaceous

fungi from a number of different genera and species. This prompted Ajello L (1986) to introduce phaeohyphomycosis for those infections, which on the basis of clinical, pathological and mycological grounds, could be distinguished from chromoblastomycosis. In 1992, the International Society for Human and Animal Mycology (ISHAM) recommended that the best name to define the disease was chromoblastomycosis, which Terra *etal* coined in 1922. Therefore, widely or locally used terms, such as chromomycosis, verrucous dermatitis, dermatitis verrucosa chromoparasitaria, black blastomycosis, figueira, chapa, susna, sundam, and mossy foot, should be avoided whenever possible. In contrast to chromoblastomycosis, phaeohyphomycosis is characterized by the presence of dematiaceous yeast like cells, pseudohyphae, or any combination of these forms in the tissue.

Mariat *etal* (1967) proposed the term "phaeosporotrichose" for infections caused by fungi with dematiaceous mycelial tissue forms when they were dealing with infections caused by *Phialophora gougerotii*. Emmons *etal* (1970) changed the term to "phaeosporotrichosis" and included the infection caused by *P.richardsiae*. The suffix 'sporotrichosis' was later considered inappropriate since the Phialophora infections did not resemble sporotrichosis clinically or histopathologically. The term phaeohyphomycosis was proposed by Ajello L (1974), for cutaneous, subcutaneous and systemic disease of man and animals caused by pathogenic fungi which develop dark mycelial elements in the tissue of their hosts. Bennett J.E *etal* (1973) presented an excellent review of cerebral infections by dematiaceous fungi. The *Cladosporium bantianum* which cause cerebral infections was first reported by Banti G (1911) and the second case by Binford C.H, *etal* (1952).

Hironaga M and Watanabe S (1980) reported a rare mycotic infection in a 17 year old Japanese female who had cutaneous alternariosis of the face of nine years

duration but she died of a cerebral infection caused by *Cladosporium bantianum*. This is the first case reported in which two unusual and different mycoses have occurred successively. Peter E.Hohl, *etal* (1983), reported *Wangiella dermatitdis* in a subcutaneous knee infection in a diabetic patient with impared T cell function and cutaneous anergy. It was the first documented case of infection due to this fungus in North America and ninth case documented world wide. McGinnis M.R; (1983). Kirlovic, S.M and Rhodes J.C of the Cincinnati VA Medical Center in Cincinnati, Ohio reported isolation of *Fonsecaea pedrosoi* from an orthopic Liver transplant patient causing Phaeohyphomycotic Osteomyelitis.

Michael R Mcginnis *etal* (1986) reclassified *Cladosporium bantianum* in the genus xylohypha. In this article they proposed dematiaceous hyphomycets *Cladosporium bantianum* into a new genus *Xylohypha bantiana*. This combination is necessary because *X.bantiana* produce conidiophores that are indistinguishable from its vegetative hypha and one celled smooth walled conidia which are borne in long infrequently branched chains. The blastoconidia do not possess darkly pigmented hila. In contrast, members of the genus Cladosporium produce erect, distinct conidiophores and one to four celled smooth to rough walled conidia that occur in short, frequently branched, fragile chains. The blastoconidia have darkly pigmented hila. Another distinguishing characteristic feature is *X.bantiana* can grow at temperature up to approximately 42 to 43°C, where as *Cladosporium carrionii* can grow to a maximum of 35 to 37°C. *X.bantiana* is an extremely important etiologic agent of cerebral phaeohyphomycosis. Appropriate management and early diagnosis of an infection caused by *X.bantiana* is vital. Even with anitfungal chemotherapy and surgical management, mortality owing to this fungus is extremely high.

Padhye A.A *etal* (1988) reported first human phaeohyphomycotic infection

caused by *Xylohypha emmonsii* in an 83 year old woman. The biopsy tissue consists of thin or thick walled, oval to spherical, yeast like cells and septate hyphae. In culture, *X.emmonsii* grew moderately fast at 25°C, showed minimal growth at 37°C and failed to grow at 40°C. It produced acropetal chains of one celled (rarely two celled) conidia laterally and terminally directly from vegetative hyphal cells. However, recent work by de Hoog *etal* (1995) has demonstrated that this organism is nonspecific with another species that had been identified as *xylohypha bantiana* on the basis of rRNA sequencing studies and DNA/DNA reassociation. Furthermore, these authors addressed the cultural differences between these two presumably different species, pointing out that certain strains of *Xylohypha emmonsii*, like *X.bantiana*, could grow at 40°C, and with apparent differences in branching patterns, conidial shape, and pigmentation which could be altered by culture conditions. For these and other reasons, de Hoog *etal* (1995) established the species *C. bantiana* to include both organisms as well as those that had previously been designated *Torula bantiana, Cladosporium bantianum, Cladosporium trichoides*, and *Cladosporium trichoides var.chlamydosporum.* This organism known primarily for the intracerebral involment can rarely produce cutaneous and subcutaneous infection. Immune suppression should be suspected but it is not always clinically apparent. David L *etal* (1988) reported *P.richardsiae* as a rare cause of disease in humans

James W.Patterson and *etal*, (1999) presented a case of cutaneous infection due to *Cladophialophora bantiana*, an agent of phaeohyphomycosis. This fungus was initially designated as *Xylohypha emmonsii* because of certain unique cultural characteristics, including its ablity to grow at 40°C and the formation of short chains of curved blastoconidia. Romano C *etal* (1999) reported a case of cutaneous phaeohyphomycosis caused by *Cladosporium oxysporum*. This is the first reported

case of cutaneous phaeohyphomycosis due to *Cladosporium oxysporum.*. During the past decades, phaeohyphomycoses has been attributed to more than 100 species and 60 genera of fungi in a variety of clinical syndromes, ranging from keratitis and solitary subcutaneous nodules to fulminant, rapidly fatal diseases. Most of the species are considered to be opportunistic pathogens, although some may be true pathogens. Matsumoto T *etal* (1994) and Renu Mathew *etal* (1999), reported a case of subcutaneous phaeohyphomycosis in the form of prepatellar bursitis due to *Fonsecaea pedrosoi* in a renal transplant recipient. *F.pedrosoi* usually recovered from chromoblastomycosis. An unusual cause of Phaeohyphomycosis in a liver transplant patient was reported at 98th general meeting of the American Academy of microbiology by S.M.Kralovic and J.C.Rhodes. A 29 year old orthopic liver transplant recipient who had presented with progressive mid thoracic back pain. MRI showed a tumorous mass occupying the majority of T-7 vertebral body. Biopsy from the mass showed the presence of brown, toruloid hyphae with sclerotic bodies, with a mild granulomatous inflammatory reaction of the surrounding tissue. Culture yielded *F.pedrosoi* and the clinician diagnosed the condition as deep seated phaeohyphomycosis.

Gugnani HC *etal* (2000) reported subcutaneous phaeohyphomycosis caused by *Cladosporium cladosporoides* in a 25 year old male. The clinical presentation was an elevated scaly suppurating lesion with sinuses on the right leg. The lesion healed completely with oral fluconazole therapy. This is the first record of subcutaneous infection due to *C.cladosporoides* from India. Chua JD *etal* (2001) reported *Exophiala jeanselmei*, the most common cause of pheomycotic cyst/subcutaneous phaeohyphomycosis in the United States. A lung-transplant patient with relapsing and invasive Jeanselmei phaeohyphomycosis, who previously had a pheomycotic cyst

excised and treated with oral fluconazole. It was re-excised and the patient was subsequently treated with an eight month course of oral itraconazole. There was no relapse. Liou JM *etal* (2002) reported phaeohyphomycosis caused by exophilala species in three immunocompromised patients. Two of these were due to *E.jeanselmei* and the one was *Exophiala (Wangiella) dermatitidis.*

Sharma NL *etal* (2002) reviewed subcutaneous phaeohyphomycosis in India and reported a case of subcutaneous phaeohyphomycosis in the lumbar region. In India 23 patients with subcutaneous phaeohyphomycosis have been reported, distributed throughout the country in a belt from north to south, sparing the eastern and western regions. The age of the patients ranged from 3 to 60 years, with a male to female ratio 1.3:1. A relatively early age of onset was observed. A history of prior injury was recalled by five patients. The lower extremities were involved in eight cases, upper extremities in five, gluteal region in two, lumbar area and submandibular area in one, face in two and disseminated disease was seen in four cases. Three of these cases died during follow up. Osteomyelitis was observed in two cases, hepatosplenomegaly in one, and lymph node involment in two carcinomatous change developed in a long standing lesion of 33 years. Thirteen species from seven genera of dematiaceous fungi were isolated. *Phialophora dermatitidis* was the most common isolate. *Exophilala dermatitidis* seems to be associated with more fatalities. Treatment with newer azoles seems promising and excision alone or combined with azoles is a good therapeutic modality. Central Venous catheter as a risk factor for disseminated phaeohyphomycosis case reported by Anthony La Rocco Jr, *etal* (2002). *Wangiella dermatitidis* was isolated from the blood of a 61 year old woman receiving chemotherapy through a totally implanted venous device for metastatic breast cancer. The Central Venous Catheter was removed. The patient was treated with Amphotericin B and the patient recovered. There was no metastatic fungal involment.

Severo L.C *etal* (1997) reviewed the literature and presented two cases of cutaneous scedosporiosis caused by *S. apiospermum.* Both patients had lesion localized in the forearm, a solitary ulceration in one and a sporotrichoid- like lesion in the other. Ingo K. Mellinghoff, (2002) presented a case of 41 year old man with acute lymphoblastic leukemia, developed multiple *Scedosporium apiospermum* brain abscess. The infection progressed despite neurological drainage and treatment with itraconazole, amphotericin B, and ketaconazole, but the brain abscesses completely resolved after treatment with posaconazole alone. *S.apiospermum* is the asexual form of *Pseudallescheria boydii*; a saprophytic fungus frequently isolated from soil and water and is usually associated with soft-tissue infection (madura foot). This report highlights the importance of performing a definitive fungal culture and standardized antifungal susceptibility testing. On the basis of their experiences, posaconazole would be a useful agent for treatment of central nervous system infections due to *S.apiospermum, P.boydii* and other posaconazole susceptible molds, especially when options for surgical interventions are limited

Sanjay G Revankar (2004) reviewed 72 cases of disseminated phaeohyphomycosis. *Scedosporium prolificans* is far the most common cause. The presence of melanin in their cell wall may be a virulence factor for these fungi. The outcome of antifungal therapy remains poor with overall mortality rate of 79 % male patients accounted for 41 (57%) of 72 cases and female patients accounted for 31 (43%) of 72 cases. The mean patient age was 42 years (range 0-92). 75 % of all cases were reported during the years of 1992-2001. Of the 28 species isolated, *Scedosporium prolificans* accounted for 30 (42%) of 72 cases. The next common species was *Bipolaris spicifera* (six cases 8 %) followed by *Wangiella dermatitidis* five of 72 patients (7%).

Sub cutaneous phaeohyphomycosis due to *Exophiala jeanselmei* in renal transplant recipient, was reported by A.Chakrabarthi *etal* (2002) in a 53 year old renal transplant recipient. Central Venous catheter as a risk factor for disseminated phaeohyphomycosis. One case reported by Anthony La Rocco Jr; etal (2002). *Wangiella dermatitidis* was isolated from the blood of a 61 year old woman receiving chemotherapy through a totally implanted venous device for metastatic breast cancer. The Central Venous Catheter was removed and the patient was given amphotericin B and the patient recovered well. There was no metastatic fungal involment. M.A. Barron *etal* (2003) reported an invasive mycotic infections caused by *Chaetomium perlucidum*, a new agents of cerebral hyphomycosis. This was the first case of cerebral hyphyomycosis caused by *Chaetomium perlucidum* and this fungus has the ability to disseminate beyond the central nervous system. The other Chaetomium species known to produce neurotropic diseases are *C.atrobrunneum* and *C.strumarium*

Jain SK *etal* (2003) from Gwalior, India, reported a patient suffering from subcutaneous phaeohyphomycosis caused by *Cladophialophora bantiana*. The face and upper site was involved with small, stellate, pyogranulomatous foci and low inflammation. The patient showed good response after amphotericin B and systemic corticosteroid. Kimura M *etal* (2003) reported an 85 year old woman with multifocal purulent subcutaneous nodules on the dorsal side of the right forearm and hand. Histopathologic examination revealed phaeomycotic cyst with faint brown septate hyphae and moniliform fungal elements were found in the granuloma. Culture yielded *Phialophora verrucosa*. This fungus is rarely reported in phaeohyphomycosis. Subcutaneous phaeohyphomycosis usually presents as a single lesion. This is the first report of multifocal subcutaneous phaeohyphomycosis caused by *P.verrucosa* and has responded well with oral itraconazole.

de Monbrison F *etal* (2004) reported two cases of phaeohyphomycosis due to *Exophilala jeansemei*, in cardiac transplant and renal transplant patients. Yehia M *etal* (2004) reviewed the literature of phaeohyphomycosis and described three cases of subcutaneous phaeohyphomycosis developing in the lower limb of renal transplant recipients shortly after transplantation. Each case presented with dark-colored nodules that subsequently ulcerated. Histopathologic examination revealed dematiaceous fungal hyphae with a surrounding granulomatous reaction. The fungi were subsequently identified as *Altetrnaria alternatum* in two cases and *Phialophora richardisiae* in one case. Prolonged course of itraconazole resolved the lesion in one case and combined medical and surgical treatment resulted in cure of the other two cases. Pandhye A.A *etal* (2004) presented subcutaneous phaeohyphomycotic abscess caused by *Pleurophomopsis lignicola.* A 41 year old man with past history of diabetes and AIDS complained with a painful swelling on his right arm. The pus was aspirated and culture was made on SDA agar. The growth was identified as *Pleurophomopsis lignicola.* Sutton D.A *etal* (2004) reported first US report of subcutaneous phaeohyphomycosis caused by *Veronaea botryose* which is a rare agent of human phaeohyphomycosis in heart transplant recipient and reviewed the literature.

Alan Woodgyer, Microbiological Diagnostic Unit, University of Melbourne, in his leading article described Chromoblastomycosis and phaeohyphomycosis as two separate entities and clearly defined the chromoblastomycosis and phaeohyphomycosis. The fungi, which cause both this diseases, are described as dematiaceous. The term dematiaceous is taken to mean that the fungi in culture produce melanin-like pigment in the walls of the hyphae and/or conidia. Fontana Masson Silver stains which specifically stain hyphae containing melanin and this can be used to confirm that the hyphae are dematiaceous.

Infection with the soil fungus *Sporothrix schenckii* is uncommon in human beings and results usually in a localized lymphocutaneous disease after direct inoculation of the fungus into the skin, reported by Werner A.H *etal* (1994). Epidemic cutaneous sporotrichosis by Campos-P etal (1994), reported first cases of epidemic sporotrichosis. They studied four members of two families who contracted sporotrichosis after sleeping in an old and rust stained camping tent. Result of the study showed all cases were presented with polymorphic lesions, three of them with multiple sites inoculation. The camping tent was shown to be the source of infection. In another work by Athol.J.Ware *etal* (1995) presented a disseminated sporotrichosis in a patient with AIDS. Usually sporotrichosis presents as a localized, lymphocutaneous infection that follows trauma, such as injury from a rose thorn. But in a patient having HIV, it may be widespread and disseminated and is a rare opportunistic infection that may affect these patients. Localised sporotrichosis is well respons to therapy but in immunocompromised patients it is life threatening. It is important that clinician be aware of the presentation of this unusual opportunistic infection and that they maintain close communication with pathology and clinical microbiology laboratory to ensure that proper stains and cultures are performed to avoid potential misdiagnosis.

A case of cutaneous sporotrichosis reported by Sumangala Bai *etal* (1998) from Pathanamthitta (Kerala), a 52 year old male working in a rubber plantation, presented with a case of chronic non healing plantar ulcer which failed to respond to anti tuberculous treatment. The culture yielded *Sporothrix schenckii* and was subsequently, successfully treated with itraconazole. Espinosa-Texis A *etal* (2001) studied 50 cases of sporotrichosis in Mexico and evaluated indirect immunoflurescence, hypersensitivity skin reaction, culture and histopathology

laboratory techniques. Metabolic antigen was used to elicit delayed hypersensitivity skin reaction in all patients. Result of the study showed that there was an increased frequency sporotrichosis in women (62%) followed by children and adolescents under 20 years of age (34%) and adults older than 50 years of age (28%). Disease was predominant in farmers (44%) followed by housewives (30%). Lymphagitic form accounted for 82% of cases and these were localized in upper limbs (54%). In 66% of cases, histopathology showed *S.schenckii* yeasts. Hypersensitivity skin reaction was positive in 76 % cases and culture was positive in 94 % cases. By indirect immunoflurescence, parasitic elements were demonstrated in all patients corresponding to both sensitivity and specificity 100 %. In this work indirect immunofluorence was the most efficient diagnostic method followed by culture, hypersensitivity skin reaction and histopathology study.

Koc A..N *etal* (2001) presented a 48 year old man who had subcutaneous sporotrichosis, which is a rare disease in Turkey, and was successfully treated with short term itraconazole and potassium iodide. Ponnighaus M *etal* (2003) reported from Germany, a young man presented an ulcer on his lower leg which had developed over the past 9 weeks. Diagnosis confirmed by histopathologically as sporotrichosis. The patient was then treated with potassium iodide (KI). Bayles-M.A (1992), describes the common tropical subcutaneous and deep mycoses and the drug of choice for sporotrichosis infection as itraconazole. An enhanced efficacy by combining flucytosine and itraconazole was noticed in 3 out of 41 patients. In the present study itraconazole has an impressive safety profile, no side effects were noticed, no adverse reaction occurred and serum chemistry levels remained within the normal limits. Sandhuk and Gupta S (2003) reported a case of lymphocutaneous sporotrichosis that failed to respond to an adequate course of itraconazole yet responded dramatically to treatment with saturated solution of potassium iodide.

Alves S.H *etal* in (2004) reported that sporotrichosis is the most common mycosis observed in Brazil. A rare presentation of sporotrichosis noticed in a Caucasian male agricultural worker whose lesion occurred bilaterally and simultaneously on the upper limb. Coskun B *etal* (2004) reported that sporotrichosis was rare disease in Turkey. They have reviewed the literature and reported a 40 year old woman who had subcutaneous sporotrichosis caused by *S.schenckii* that was successfully treated with terbinafine (250 mg, twice a day) for a period of 6 months. She was also given saturated solution of potassium iodide (KI) orally for two months. So terbinafine and KI are suggested to be the choice treatment for sporotrichosis. Barros M.B *etal* (2004) analysed cat-transmitted sportrichosis epidemic in Rio de Janeiro in Brazil, South America. The usual mode of infection is associated with traumatic implantation of the fungus. 178 cases of culture proven sporotrichosis had been diagnosed during 1998 to 2001. Female patients predominated, and the median age was 39 years. The most frequent clinical presentation was lymphocutaneous disease. Of the 178 patients, 156 reported domiciliary or professional contact with cats with sporotrichosis, and 97 of these patients had a history of receipt of a cat scratch or bite. This study suggests that feline transmission of sporotrichosis was associated with a large and long lasting outbreak of the disease in Brazil.

Geographical variation is very important for the occurrence of subcutaneous mycoses In India, mycetoma is prevalent in Tamilnadu where as in Kerala, it is rare. In Kerala dematiaceous fungi are reported as an important agent in subcutaneous mycosis. Pushpa Talwar *etal* (1979) described 60 cases of clinically suspected mycetoma by which 70% were due to actinomycetoma and 20 % due to eumycetoma and another 20% were due to nocardia. The foot was the common site of infection. Patients of all age groups are susceptible for infection. The common age group

affected was 20-40. Actinomycetoma was the common type in South India. Dasgupta *etal* (1974), Taralakshmi *etal* (1977) and Murthi and Padmavathy (1963) have reported eumycetoma as the common type. Desai *etal* (1970) found actinomycetoma more prevalent in Bombay while Mankodi & Kanavinde (1970) reported eumycetoma as the common infecting form. These studies indicated that the concept of preponderance in the incidence of certain species in different geographical regions is slowly fading. The etiological agents appear to be present ubiquitously as they await suitable opportunities to manifest themselves. There has been a uniform opinion concerning *Madurella mycetomi* as the most common etiological agent in eumycetoma in India.

Taralakshmi V. Venugopal *etal* (1977) analysed 150 cases of clinically diagnosied mycetoma in Madras during 1964-1975, and found that the disease was predominantly seen in 21-40 age group. Men were more frequently affected than females and the commonest site of the lesion was foot. Actinomycotic mycetoma (68.9%) was more often found than the maduramycetoma type. *Madurella mycetomi* (27.8%) and *Actinomadura madurae* (26.7%) were the common casual agents. *Nocardia spp.* were the next most common (21.1%) followed by *A.pelletieri* (15.5%), *S.somaliensis*(5.6%) and *Allescheria / Cephalosporium species* from only 3 case of white grain mycetoma. Men were involved 5 times as often as women. This is probably because of their greater out door activities increasing the opportunities for trauma to the exposed surface of the body. Possibly the females may in addition have some protective mechanism against the entry or the proliferation of the fungus into the body. No site is exempt for mycetoma, though the foot is the main site (72%). Stierstorfer M.B *etal* (1998) reported a case of mycetoma affecting the foot of a 38 year old mentally retarded man from northern New England. The casual agent was

identified as *Pseudallescheria boydii*. The patient showed partial response to 8 month ketaconazole therapy.

Venugopal P.V and Venugopal TV (1990) reported Actinomycosis caused by *Actinomadura madurae* from Madras, India. Turiansky GW *etal* (1995) described *Phialophora verrucosa* a new agent causing mycetoma in a 29 year old Thai woman who had draining sinus tracts, tumefaction and granules on the plantar aspects of the foot. *P.verrucosa* is a major agent of chromoblastomycosis. This dematiaceous fungus has not been previously reported to cause mycetoma. McGinnis M.R (1996) in his article described the clinical details of mycetoma and *Madurella mycetomatis* as the important agent causing mycetoma. Combined medical and surgical management with ketaconazole resulted in the best outcome. Peizer K *etal* (2000) isolated both *Sporothrix schenckii* and *Nocardia asteroides* from a mycetoma of the forefoot. A combination of causative agents in mycetoma is rare, and the combination of *S.schenckii* and *N.asteroides* have not been reported earlier from one lesion. Kano R *etal* (2002) reported the first isolation of *Nocadia veterana* from a human mycetoma. It was identified by nucleotide sequence analysis of the16 S ribosomal DNA to reference strain of *N.veterana*.

Dieng M.T *etal* (2003) reported 130 cases of mycetoma in Senagal from 1983 to 2000. Clinical diagnosis of mycetoma was based on open tract sinuses, tumefaction or discharge of grain. Diagnosis was confirmed based on fungal culture and histology. They observed 76 actiniomycetoma and 54 eumycetoma (sex ratio M/F =6.6; mean age =34.7+/-14.8 years). Actinomycetoma was due to A*ctinomadura pelletieri* (54 cases), *Actinomadura madurae* (17 cases) and *Streptomyces somaliensis* (5cases). Eumycetoma was due to *Madurella mycetomatis* (38 cases), *Leptospahria senegalensis* (9cases), *Pseudoallescheria boydii* (6 cases) and *Rhinocladiella*

atrovirens (1case). Clinical inflammatory features significantly associated with actinomyces ($p < 0.001$ or= 2.64) were predominant (85 cases). The geographical distribution of pathogenic mycetoma agents was determined by the annual rainfall. Distinction between eumycetoma and actinomycetoma is very important

Fahal A.H (2004) rievewed various aspects of mycetoma, its geographical distribution, diagnosis and treatment of the eumycetomas. Foltz K.D and Fallat (2004) reviewed mycetoma and a case report of actinomycoses that was successfully treated with surgical resection and long term antibiotic therapy. Mycetoma are primarily found in tropical and subtropical areas of the world and are rare in United States. This case is unique because of the rarity of contracting this type infection in the United States. Ahmed A.O *etal* (2004) presented mycetoma caused by *Madurella mycetomatis* and review on developments in the clinical, epidemiological and diagnostic management of *M.mycetomatis* eumycetoma. They described newly developed molecular diagnostic and gene typing procedures and their application for management of patients and environment research. A mouse model fungal susceptibility test was also developed.

Gordon M.A *etal* (1975) first reported a rare instance of infection of man and other aninmals by *Phoma* or Phoma like mold. The important species of phoma are *P.cava, P.glomerata, P.hominis*. Zaitz C etal (1997) reported a case of subcuataneous phaeohyphomycosis caused by Phoma cava and reviewed the literature. Oh C.K *etal* (1999) reported subcutaneous phaeohyphomycosis caused by Phoma species. Gordon M.Dickinson, *etal* (1983) reported the first case of subcutaneous phaeohyphomycosis caused by *Scytalidium lignicola* in a human. In this article he presented *Scytalidium lignicola* a dematiaceous hypomycetes associated with wood and soil as previously unknown agent of either human or animal disease. Many fungi once thought to be

laboratory contaminants are being documented as etiological agents of phaeohyphomycosis and other opportunistic mycotic infections. Subramanyam V.R *etal* (1993) reported the isolation of *Curvularia species* from a 21 year old man with chronic ulcer on the lower limb. This case was reported from Bhubaneswar, India.

Lobomycosis was first described by Jorge Lobo in 1931 from a patient from Amazon Vally and is charaterised by the appearance of slowly developing keloid like ulcerated, or verrucous nodular or plaque like lesions, usually at the site of local trauma such as from a cut, insect bite, animal bite, or ray sting. The ulcerated lesions in which there are large numbers of spherical to lemon shaped fungus cells. Ciferri R *etal* (1956) described the fungal etiology of Lobos disease. Burns R.A *etal* (2000) described the first case of lobomycosis in United States. Sameer Elsayed *etal* (2004) reported a 42 year old woman with histologically confirmed lobomycosis as a chronic granulomatous infection of the skin and subcutaneous tissues caused by the fungus *L.loboi.*

Subcutaneous phycomycosis or subcutaneous zygomycosis caused by lower fungi was first reported by Drechsler in 1947. The fungus was identified as *Basidiobolus ranarum* by Emmons (Lie-Kian-joe *etal* 1956). Greer and Friedman (1966) identified two more species, *B.haptosporus, B.meristosporus.* In 1967 Srinivasan and Thirumalachar concluded that the correct name was *B.haptosporus*, and that *B.meristosporus* is only a variety of *B.haptosporus.* Infection with *Conidiobolus coronatus* (*Entomophthora coronata*) was reported by Bras,G *etal* in (1967). Dasgupta L.R. *etal* (1976) reported four cases of subcutaneous phycomycosis diagnosed by isolating *Basidiobolus meristosorus* from the affected subcutaneous tissue. These were reported from Pondicherry. The first case of sub cutaneous phycomycosis was reported by Mukerjee S etal (1962) as *B.ranaram.* Shah M.B. etal

(1970) reported 3 cases of *B.meristosporus*. Koshi *etal* (1972) reported three cases caused by *B.haptosporus*. Gugnani HC (1999) reviewed zygomycosis due to *Basidiobolus ranarum*. The laboratory diagnosis is based on histopathology and culture. The typical histopathology feature is the presence of thin-walled, broad often aseptate hyphae or hyphal fragments with an eosinophilic sheath, frequently phagocytised within giant cells.

Sub cutaneous phycomycosis caused by *Basidiobolous species* a case report by Sujatha S *etal* from JIPMER Pondicherry, a 58 old male, agricultural labourer from south India presented with painless subcutaneous swellings on the left thigh of 4 year duration. The isolated fungus was *Basidiobolous species* and the patient was given potassium iodide over a 2 month period which resulted in subsidence of swelling rapidly. Ribes J.A *etal* (2000) reviewed zygomycetes in human disease. There are two order of zygomycetes that cause human diseases, the mucorales and entomophthorales. The important mucorales causing human infections are Mucor, Rhizopus, Rhizomucor, Absidia, Apophysomyces, Saksenaea, Cunninghamella, Cokeromyces, and Syncephalastrum species. Kalpadia S and Polenakovik H (2004) reported subcutaneous zygomycosis following attempted radial artery caused by rhizopus in a 70 year old man with cellulites of the left arm. A large bulla was found on the lateral aspect of the left wrist and aspirated fluid was cultured on SDA. Rhizopus was grown and the patient was started with amphotericin B. The patient died due to various other complications.

Choonhakaran C and Inthraburan K, (2004) reported concurrent subcutaneous and visceral basidiobolomycosis. A 55 year old female renal transplant recipient, who developed chronic hard nonpitting oedema of the right lower extremity and abdominal wall concurrent with infection from the same organism involving uterus, urinary

bladder and intra-abdominal lymph nodes. The patient responded successfully, both clinically and radiographically, to medical treatment without surgical resection. Bigliazzi C *etal* (2004), a young immunocompetent woman who had presented with eosinophilia and lung infiltrates. She died subsequently, and diagnosis of basidiobolomycosis was made on the basis of histological features at autopsy. Chiewchanvit S *etal* (2002) reviewed entomophthoromycosis in Maharaj Nakorn Chiang Mai Hospital, Thialand. Eight cases of entomophthoromycosis were found between 1988 and 1993, with five patients diagnosed as subcutaneous zygomycosis. Five patients were female with age group 7-77 years. They presented with painless subcutaneous mass, which was solitary or multiple and most commonly found on the extremities. The duration of the disease was 3 months to 5 years. Culture yielded the growth of *Basidiobilous ranaram.*

Gutierrea-Rodero F *etal* (1999) reported cutaneous hyalohyphomycosis caused by *Paecilomyces lilacinus* in an immunocompetent host which was successfully treated with itraconazole. This fungal pathogen is highly resistant to many antifungal agents. This case of sporadic cutaneous infection due to *Paecilomyces lilacinus* is believed to be reported first time in Europe and it was first histopathologically proven case successfully treated with itraconazole. Karam A *etal* (2003) presented a paper on subcutaneous mycosis due to *Scopulariopsis brevicaulis* in an aplastic patient. Annellospore group of hyphomycete class Scopulariopsis genera presently includes 16 species considered as opportunistic pathogens. The mycological and histological examinations are fundamental to confirm the diagnosis.

Kurzai O *etal* (2003) reported *Paecilomyces lilacinus* as an agent of subcutaneous infection in a patient with liver cirrhosis. Surgical treatment in combination with systemic amphotericin B led to complete recovery. Retrospectively

performed microdilution testing revealed dose dependent in vitro susceptibility of the isolate to voriconazole (MIC=2g/ml) and terbinafine (MIC=1 microg/ml). Guarro J *etal* 2003 reported two cases of subcutaneous infection due to *Phaeoacremonium species* from Brazil. The first case was caused by *Phaeoacremonium aleophilum* and the patient presented with a unique fistulized nodule on the left ankle. This is the first reported case of human infection caused by this fungus. The second reported case was caused by *Phaeoacremonium rubrigenum.* This patient presented with multiple nodules around the left ankle and foot. Dermatophytes are common pathogens of skin but rarely cause subcutaneous infections. Ran Nir-Paz, *etal* (2003) reported first time the isolation of *Trichophyton rubrum*, usually a skin pathogen, from a patient with multiple subcutaneous nodules on both legs. This patient was suffering from an auto immune disease with liver, cardiac, and lung involment. Biopsy of the nodules showed a granulomatous inflammatory reaction in the dermis and hypodermis composed of monocytes, macarophages, multinucleated giant cells and rare neutrophils. Septate hyphae were revealed by both PAS and GMS stains

Bosma F *etal* (2003) reported two cases of *Scedosporium apiospermum* infection which successfully treated with voriconazole. This agent was considered as an opportunistic pathogen in an immunocompromised patient. Chaverio MA *etal* (2003) reported cutaneous infection due to *Scedosporium apiospermum* in an immunocompromised patient. It is an anamorphic form of *Pseudallescheria boydii.* The patient having rheumatoid arthritis and diabetes, who was submitted to long term therapy with cyclosporine and corticosteoids. Posteraro P etal 2003 reported *Scedosporium apiospermum* infection in a 52 year old male heart transplant patient with a persistent localized subcutaneous infection with *Scedosporium apiospermum.* This patient showed multiple nodules on the right hand that were surgically removed

and he received oral itraconazole, but the infection persisted for two years. Chade M.E *etal* (2003) reported post traumatic subcutaneous mycosis due to *Fusarium solani*. This case was a subcutaneous hyalohyphomycosis. A 24 year old man presented with ulcerative lesion in the right leg of approximately one year duration. It was caused by traumatic implantation of a yerba mate branch. Subcutaneous fungal infection due to *Eurotium herbariorum* presented by Shivani Vishnoi etal from R.D. University Jabalpur, M.P, the patient was 62 year old male, suffering from lichen planus, presented with patchy scaly lesions on neck, upperarm, anterior aspects of lower limb and waistline for the last 12 years. Diagnosis is based on histopathology and culture yielded *E.herbariorum.* Girard C etal (2004) reported subcutaneous phaeohyphomycosis due to *Pyrenochaeta romeroi* in patient with leprosy

Penicillium species are the most common laboratory contaminants; unless it is accompanied by typical fungal elements in tissue specimens its pathologic role is uncertain. The only true pathogen among members of the genus is *Penicillium marneffei*. It is a dimorphic fungi and is common in Eastern Asia. Lis J.S *etal* (1991) reported three cases of disseminated penicilliosis in China. The clinical features were characterized by multiple organ involvement, multiple subcutaneous abscesses, inflammatory papules, nodules, pustules, enlargement of superficial lymph nodes with chill, anemia and leukocytosis. Infection caused by *P.marneffei* are usually disseminated, with multiple organ involment, manifesting lymphadinitis, subcutaneous abscesses, bone lesions, arthritis, enlarged spleen, or lung, liver, bowel lesions Kwon Chung K.J and.Bennett J.E (1992). The potential risk of laboratory acquired infection was demonstrated by the individual who first described *P.marneffei*, who accidently punctured his right index finger while inoculating laboratory rodent. The fungus could be isolated from a small nodule that developed

at the puncture site, Segretain G (1959). Successful treatment with nystatin was initiated since this fungus has been found to be sensitive to this antifungal.

There are only few cases of subcutaneous mycoses reported from Kerala. The earlier study was by Meenakshy and Ananthanarayanan (1961). Maheswariamma *etal* (1979) reported *Cladosporium bantianum* from a case of phaeohypomycosis in Kerala. In this article, they presented *Cladosporium bantianum* from a case of phaeohyphomycosis. This was the first reported case in India; also this was the first known case of infection involving the foot caused by this fungus. The etiologic agents of phaeohyphomycosis are varied in number. At present 16 fungi belonging to 8 genera are recognized as agent of phaeohyphomycosis, one of which is *Cladosporium bantianum*. K.Pavithran (1991) from Kerala, presented a disseminated chromoblastomycosis and the fungi isolated was *Cladosporium carrionii*. K Pavithran (1992) from Kerala reported, chromoblastomycosis masquerading as tuberculoid leprosy (letter). Maheswariamma *etal* (1990) reported phaeohyphomycosis caused by *Phialophora dermatitidis* in Calicut from a case of subcutaneous lesion.

One of the most characteristic features of fungal infections is its refractoriness to treatment. Over the last decades, several different therapeutic schemes have been used, but most proved unsuccessful or of low efficacy. Surgical excision or electrodesiccation of lesions should be avoided because metastasis might ensue. The use of amphotericin B alone or with 5-flucytosine (5-FC) and ketoconazole is no longer indicated. Some physical therapies have produced noteworthy results. To date, two therapeutic approaches are accepted as the best choices: oral itraconazole (as monotherapy or with oral 5-FC) and cryosurgery with liquid nitrogen. Several authors indicated itraconazole as the best choice of therapy Restrepo, (1988); Graybill, (1992); Queiroz-Telles, (1992). Daily doses range from 200-400 mg, and results vary

greatly. Adverse effects are not common, but efficacy is not as high as one would desire. Severe cases should be treated for several years. The author's experience in treating more than 25 patients with varying degrees of severity for up to 5 years shows that itraconazole produces dramatic improvement after a few months of therapy; however, a complete cure is rarely reached, especially in severe cases. These results might be because of the predominantly fungistatic mechanism of action of the drug. In several cases, drug withdrawal led to relapse. Although few studies have been published, the association of itraconazole and 5-FC is promising, Pradinaud, (1991). As with 5-FC and amphotericin B, itraconazole and 5-FC produce a synergistic effect. Multidrug therapy for chromoblastomycosis seems to be an interesting approach and may also be used with cryosurgery.

In 1996, Esterre et al presented interesting results when using terbinafine to treat more than 100 patients in Madagascar. Similar to that of itraconazole, the drug presented below optimal results, it is expensive, and treatment lasts several months. To date, no reports on the association of terbinafine and itraconazole or terbinafine and 5-FC have appeared in the literature. Posaconazole, a new azole derivative, has been experimentally used to treat chromoblastomycosis, and the results of isolated cases suggest that outcomes may be slightly superior to those obtained by itraconazole or terbinafine (Unpublished data on file, Dr. Shikanai-Yasuda, Department of Infectious Diseases, Univ. São Paulo). Heat therapy is another treatment. Especially in Japan, the use of pocket warmers has proven successful in the treatment of a limited number of cases. Apparently, an increase in skin temperature somehow impairs fungal development (Kinbara, 1982).

Lidiane Meire Kohler *etal* (2004), studied thirty isolates of the yeast form of *Sporothrix schenckii* and their invitro susceptibility to itraconazole and terbinafine by

the recommended NCCLS modified technique (M27-A2). The MIC s of itraconazole obtained oscillated between 0.062 and 4.0 μg /ml, and those of terbinafine oscillated between 0.007-0.50 μg/ml; therefore, terbinafine showed greater invitro activity. Itraconazole is currently considered the treatment of choice to treat the diverse clinical manifestations of sporotrichosis, Koc A.N *etal* (2001). On the other hand terbinafine by virtue of its excellent invitro and invivo activity is under comparative evaluation for its therapeutic potential for a wide range of fungal infections, Hay R.J (1999). Promising invitro result by the technique of macrodilution in a liquid medium (NCCLS M27-A2) with terbinafine for both the fixed and lymphocutaneous forms of sporotrichosis due to *S.schenckii* (Hull P.R. and.Vismer H.F 1992). Perez A. (1999) discussed the allylamine antifungals, of which terbinafine is most effective against chromoblastomycosis, phaeohyphomycosis, maduromycosis mucormycosis and various other fungal infections. Hay R.J (1999) discussed the therapeutic potential of terbinafine in subcutaneous and systemic mycosis

IMPORTANCE OF THE STUDY

OBJECTIVE OF THE STUDY:

Main Objective: To study the prevalent fungal pathogens responsible for subcutaneous mycosis in North Kerala.

The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to health care professionals. The higher incidence of fungal infections in the past 10 years has been attributed to the increased use of newer and more effective antibacterial agents, the AIDS pandemic, and the rapidly expanding number of chemically induced immunosuppressive patients, bone marrow, solid organ transplantation and oncology patients. As a result of improved management protocols, AIDS, cancer, and transplantation populations now survive longer and become highly

susceptible to life-threatening fungal infections. This increase is directly related to the growing population of immunocompromised individuals, resulting from changes in medical practice such as the use of intensive chemotherapy and immunosuppressive therapy.

After the skilful control of bacterial and viral infections by antibiotics and antiviral agents the incidence of fungal infection seems to be increasing. The increased number of diabetes patients also pose a greater challenge for fungal infections. The etiological agents of subcutaneous mycosis widely varies geographically. Some of them are geographically limited in distribution. Accurate diagnosis is essential for fungal infections because of the clinical similarity with other bacterial and parasitic infections like cutaneous tuberculosis, leishmaniasis, tertiary syphilis and yaws and each group has its own treatment profile. Few of the fungi causing sub cutaneous mycosis are dangerous, so early diagnosis and treatment are very essential and failure to treat in time may be fatal.

Preliminary studies done by some workers (personal data) showed that disease chromoblastomycosis is highly prevalent in North Kerala exceptionally in hilly areas of Wynad, hence any study on the prevalence of subcutaneous mycoses and their sources of infection is supposed to yield fruitful results. The biopsy specimens collected from Dermatology and Surgery departments of Medical College, Calicut gives an accurate picture of the prevalence of subcutaneous mycosis in North Kerala.

At present we have a limited data regarding the prevalent fungal agents causing subcutaneous mycosis in this part of Kerala and more over only limited centre are available for the isolation and identification of fungal pathogens. Studies on the etiological agents of subcutaneous mycoses are rarely done in our area. Identification

of the etiologic agent by culture is essential for prognostic and management considerations, since some fungi are more frequently associated with dissemination and are dangerous and are often fatal. Hence the present study is mainly focused on the various subcutaneous mycoses prevalent in this area, its isolation, identification, along with source of infection. Moreover this study will have an added advantage to the health need of Kerala by bringing awareness on the prevalence of fungal disease among health care professionals.

STUDY ON
THE PREVALENCE OF SUBCUTANEOUS MYCOSES IN NORTH KERALA

Thesis submitted
in partial fulfilment of the requirements
for the award of the degree of

DOCTOR OF PHILOSOPHY

IN MICROBIOLOGY (MEDICAL FACULTY)

BY

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MATERIALS & METHODS

Patients getting treatment from Dermatology and Surgery Departments of Calicut Medical College for chronic sub cutaneous infections, referred patients from in and around Calicut, Wynad, Malapuram and Cannanore districts to the above Departments were taken for the study. Since Calicut Medical College is a referral hospital for the above districts, all suspected fungal infections from the peripheral hospitals are referred to this medical college for diagnosis and treatment. So, any study conducted in the Dermatology and other departments of Calicut Medical College would give a correct picture of subcutaneous fungal disease prevalent in this area. Wynad is a hilly area with high altitude and various types of plantations. The tribals are an important population in Wynad. Patients having subcutaneous skin lesions from all age groups of both sexes of varying economic status were included in this study.

The study group consisted of 161542 patients with various skin lesions who attended the dermatology, surgery and ENT departments of Calicut Medical College, for a period of five years from January 2000 to January 2006.

For each case, history, duration of symptoms, clinical features, physical signs, associated diseases like tuberculosis, syphilis, drug addiction etc were noted in detail. Treatments taken for the present illness and history of drugs taken in the past were noted. History of intake of prolonged antibiotics, antituberculous drugs, corticosteriod, and antimalignancy therapy were also considered. X- ray findings, detailed laboratory investigation like total Leucocyte count, Differential leucocyte count, VDRL etc were noted

COLLECTION, TRANSPORT, PROCESSING, AND EXAMINATION OF SPECIMEN

Specimen collection, transport and storage are extremely important components in the provision of accurate results for the diagnosis and management of subcutaneous mycoses. Specimens must be collected under aseptic conditions or after appropriate hygienic preparation to optimize the significance of mycologic results. The specimens most frequently submitted for the recovery of fungi causing subcutaneous mycoses includes aspirate, biopsy samples, skin scrapings and surgical tissue. Swabs are not an effective means of specimen collection and should be avoided when possible. Portion of suspicious necrotic, purulent, or caseous specimens should be examined microscopically and inoculated onto culture media. Tissue specimens should be minced with scalpels into 0.5 to 10 mm pieces and inoculated directly to culture media. Tissue homogenizers should not be used, because some moulds do not have regularly septate hyphae and thus can be easily killed by homogenization

The specimens included tissue from the infected sites. Biopsies were taken from the lesion for:

- Microscopical examination
- Histopathological examination
- Fungal culture
- Routine microbiological culture
- Anti fungal sensitivity testing of the isolated fungi

MICROSCOPICAL EXAMINATION

Wet-Mount or Tease- Mount Technique. The wet preparations are used for diagnosis of fungal infections from clinical specimens or to study the morphological features of the fungal isolates. Wet mount is a rapid method of preparing fungal colonies for microscopic examination. A bent needle or spade of heavy gauge wire is used to remove colony fragments from the culture. When possible, the surface spore material should be removed by gently scraping the surface of the culture toward the center (oldest portion) of the colony.

Wet mount with Normal Saline: This preparation is used for observing pigmented fungi and its structures.

Hydroxide Mounts: The aqueous potassium hydroxide digests protein debris and dissolves the cement substance which holds the keratinized cells together. It is prepared from the following ingredients:

Potassium Hydroxide	:	10 gm
Glycerol	:	10 ml
Distilled Water	:	80 ml

Place a small part of the biopsy tissue or scrapings on a clean glass slide. Pour a drop of 10% KOH on the specimen and place a cover glass over it. Heat the slide gently over the flame and examine under microscope after a few minutes. If the specimen is not properly dissolved, it may be kept for some more time in a wet Petri dish and examined.

Calcofluor White Stain (CFW): Calcofluor White is a water soluble colorless textile dye and fluorescent whitener. This selectively binds to the cellulose and chitin of the fungal cell wall and visualized when exposed to long wavelength of visible light. It fluoresces light blue when exposed to UV light (346-365 nm). Recently it has become

a very popular staining method because of its more sensitivity than other conventional staining techniques.

CFW M2R	:	100 mg
Evans Blue	:	50 mg
Distilled water	:	100 ml

Mix well and store at room temperature in a dark room. The calcofluor solution is prepared by dissolving calcofluor white powder in distilled water with a final concentration of 0.01 %. Evan's Blue is also added (0.1 %) in order to reduce non specific background fluorescence and reveal the surrounding tissue. Evan's Blue counter stain also produces a contrasting orange to ruby-red background, thereby enhancing detection of fungi. This stain gives apple green fluorescence of the fungi. UV light of wavelength between 300 and 412 nm are used for visualizing the fungal structures under fluorescent microscope. It is difficult to differentiate hyphae from collagen fibers and other artifacts by conventional KOH wet mounts. Therefore CFW staining techniques is far superior to the conventional staining techniques for the detection of fungi in clinical specimens. It is technically simple, quick and highly reliable to identify fungi.

PHOL Stain:

The acronym PHOL is derived from the initials of surnames of four scientists i.e. Pal, Hasegawa, Ono, Lee. This is used similar to the LCB stain for the examination of fungal isolates. It contains formalin instead of phenol and methylene blue in place of cotton blue of LCB stain.

Neutral Red Stain: Is useful and easily an applicable method for the evaluation of the viability of the fungal elements. This stain is used as a vital stain.

DIFFERENTIAL STAINS:

1. **Grams stain**: Grams stain is effective for detection of some of the fungal pathogens. Brown and Brenn modification of grams stain is used for Nocardia and Actinomyces species in tissues. In general, the procedure is more suited to sections than to smears. The yeast cells usually show up well stained morphology but filamentous fungi in smears become desiccated and their morphological characteristics are usually lost. The fungi are usually gram positive and seen as violet colored in the stained smear. Fix the smear by passing over a flame. Place 0.5 % aqueous crystal violet solution on the slide for 20 seconds. Wash the smear gently under tap water. Apply grams iodine solution over he slide for 20 seconds. Wash with water and decolorize quickly with solution of equal parts of acetone and 95 % ethanol and wash immediately in the running tap water. Counter stain with 0.5 % aqueous safranine for 10 seconds and again wash with water, air dry. Then observe under a microscope.
2. **Modified Acid-Fast Stain**: the aerobic bacteria like Nocardia species and aerobic Actinomycetes species are difficult to be differentiated because both are filamentous gram positive bacteria. Therefore the smear should be stained with modified acid fast stain (Kinyoun's method) as nocardia is weakly acid fast giving pink or red color to the bacilli.

Make a smear and fix it by passing over the flame. Flood the slide with Kinyoun carbol fuschin for 3-5 minutes. Then pour off the excessive stain and flood the slide with 50 % alcohol and immediately with water. Decolorize with 1 % aqueous sulfuric acid. Wash with tap water. Counter stain with methylene blue for 1 minute. Rinse with water and examine under oil immersion.

Hematoxylin and Eosin Stain:

Hematoxylin and Eosin (H & E) Staining is one of the basic stains used in many of the diagnostic settings. It is used to stain the nuclei by oxidized hematoxylin (hematin) through mordant (chelate) bonds of metals such as aluminum followed by counterstaining by the xanthene dye- eosin, which colors in varying shades the different tissue fibers and cytoplasm. A general tissue demonstration picture is produced and serves as the main diagnostic technique.

(a) Staining solutions

Hematoxylin	:	5 gm
Ethyl alcohol	:	50 ml
Potassium alum	:	100 gm
Distilled water	:	950 ml
Glacial acetic acid	:	40 ml

(1) Eosin 1 % aqueous ws yellowish

(2) Differentiator 1 % HCL in 70 % alcohol

(3) Bluing agent 2% aq.sodium bicarbonate

(b) Staining technique

- Bring sections to water
- Stain with hematoxylin solution for the requisite time
- Wash briefly in water and differentiate in acid alcohol
- Wash well in water and blue for 10-30 seconds.
- Wash in water and stain with eosin solution for 3 minutes
- Wash quickly in water, differentiate and dehydrate in alcohol. Clear and mount as desired.

Results:

- Keratohyalin, nuclei, cytoplasmic RNA, some calcium salts, bacteria - Blue

- Muscle, keratin coarse elastic fibers, fibrin, fibrinoid - Bright red
- Collagen, reticulin, myelinated nerve fibers, amyloid - Pink
- Red blood cells - orange

GIEMSA STAIN

This is a compound stain formed by interaction of methylene blue and eosin. On exposure to acids, alkali and ultraviolet light, a number of oxidation products (methylene azures) are formed from methylene blue which give contrast staining. The modified method *ie* May-Grunwald Giemsa (MGG) technique is commonly used.

Solutions : Grind 0.3 g of May-Grunwald dye in a little methanol, decant and add more methanol and grind until the dye is in solution and make up to a final volume of 100 ml and filter.

Dilute 20 parts of May- Grunwald solution with 30 parts of pH 6.8 phosphate buffer.

Staining Procedure

Fix the smears in methanol for 5 to 10 minutes.

Stain in dilute M-G solution for 10 minutes

Rinse in pH 6.8 buffer.

Stain in Giemsa solution for 30 minutes.

Wash and differentiate in pH 6.8 buffer for 5-20 minutes until the desired color balance is achieved. Air dry and see under a microscope.

Results

Nuclei	:	purple
Cell cytoplasm	:	blue to mauve
Red blood cells	:	pink

PERIODIC ACID SCHIFF STAIN

This stain is very useful for demonstrating fungi in tissues that are usually stained darker than the surrounding tissues. The disadvantages are that many components of tissues which are carbohydrate in composition, are stained by PAS stain. Moreover, actinomycetes such as Nocardia are not stained by the PAS method but are stained by the methenamine stain. The principle of PAS stain is based on Feulgen reaction is that hydrolysis with HCL liberates aldehydes which recolor Schiff's reagent. The polysaccharides of fungi and bacteria are oxidized by periodic acid to form aldehyde groups that yield red colored compounds with Schiff's fuchsin sulphite. The protein and nucleic acids remain unstained. The nuclei stain blue, fungi magenta or red and background is light green.

Solutions

1% aqueous periodic acid Schiff's reagent:

Basic fuchsin	:	1 gm
Distilled water	:	200 ml
Sodium metabisulphite	:	2gm
Analar conc. hydrochloric acid	:	2ml
Decolorizing charcoal	:	2 gm

Staining procedure:

- Bring the sections to water
- Oxidize with periodic acid solution for 5 minutes
- Rinse well in distilled water
- Treat with Schiff's reagents for 15 minutes
- Wash in running water for 5 to 10 minutes
- Stain nuclei with Harris' hematoxylin solution
- Dehydrate in alcohol, clear in xylene and mount in a synthetic resin medium

Result:

PAS positive substances	:	Magenta or red
Nuclei	:	Red

Gridley's Fungal Stain: In the Gridley's fungal stain, the mycelia, yeasts, elastic tissue, mucin are stained as purple and background as yellow. This is like PAS staining but chromic acid is used as the oxidizing agent. The aldehyde that is produced recolor Schiff's reagent, giving the fungi purple color. The elastic fibers and some connective tissue mucin stain purple, making fungus demonstration more difficult in tissues such as skin.

GOMORI'S METHENAMINE –SILVER STAIN FOR FUNGAL HYPHAE.

This stain works on the principle of liberation of aldehyde groups and their subsequent identification by reduced silver method. It is used for the demonstration of polysaccharide content of the fungus in tissue sections. The aldehydes reduce the methenamine silver nitrate complex, resulting in the brown- black staining fungal cell wall due to deposition of reduced silver wherever aldehydes are located. Grocott's modification of Gomori's methenamine silver stain is usually used. Tendolkar and colleagues have devised simplified Grocott's silver staining by use of running water for washing, avoiding use of alcohol, xylene and expensive gold chloride which do not affect the staining character of fungi.

The fungi and bacteria are stained black, mucopolysaccharide dark grey and cytoplasm old rose and tissue pale green. The GMS stain is better than other fungal stains as:

I. It stains both live and dead fungi as compared to PAS which stain only live fungi
II. It also stains the filamentous higher bacteria of Actinomycetes (Actinomyces, Nocardia, Steptomyces and Actinomadura) which are not stained by other fungal stains

Fixation:- 10% Formalin

Technique:- Paraffin section

Solutions: 1) 5% Chromic acid

2) 5% Silver Nitrate Solution

3) 3 % Methenamine solution

4) 5% Borax solution

5) 1% Sodium Bislfite Solution

6) 0.1% Gold Chloride

7) 2 % Sodium Thiosulfate(Hypo) Solution

8) Stock Methenamine – Silver Nitrate Solution

Silver Nitrate,5 % Solution

Methenamine,3 % Solution

9) Working Methenamin-Silver Nitrate Solution

Borax, 5% solution-----2.0 ml

D.water----------25.0 ml

Mix and add

Methenamine-Silver Nitrate stock solution---25 ml

10) Stock Light Green

Light Green-----0.2 gm

D.Water --------100 ml

Glacial Acetic Acid—0.2 ml

11) Working Light Green

Light Green (Stock)----10 ml

Distilled Water---------- 50 ml

Staining Procedure:

1. De-parafinize sections through 2 changes of xylene, absolute alcohols to distilled water as usual.
2. Oxidize in 5% chromic acid solution for 1 hour.
3. Wash with running tap water for few seconds.
4. Rinse in 1% solution of sodium bisulfite for 1 minute to remove any residual chromic acid.
5. Wash in running tap water for 5 to 10 minutes.
6. Wash with 3-4 changes of distilled water
7. Place in working methenamine-silver nitrate solution in an oven at 58 to 60 ^{o}C for 30 to 60 minutes until sections turns yellowish-brown. Dip slide in distilled water and check for an adequate silver impregnation with a microscope.
8. Rinse 6 times in distilled water.
9. Tone in 0.1 % gold chloride solution for 2 to 5 minutes
10. Rinse in distilled water.
11. Remove unreduced silver with 2 % sodium thiosulfate for 2 to 5 minutes.
12. wash thoroughly in tap water.
13. Counter stain with light green for 30 to 45 seconds.
14. Dehydrate with 2 changes of 95 % alcohol, absolute alcohol,clear with 2 to 3 changes of xylene and mount in permount.

RESULT:

Fungi- sharply delineated in black

Mucin- taupe to dark gray

Inner part of mycelia and hyphae- old rose

Back ground – pale green.

MAYER'S MUCICARMINE STAIN

This is used for staining of Cryptococcus and Rhinosporidium species. Cryptococcus stains deep rose red, nuclei black, tissue yellow. In case of rhinosporidiosis, the sporangium and the endospores are stained by mucicarmine stain.

Staining Procedures

- Bring sections to water
- Stain the nuclei with an alum hematoxyli solution.
- Stain with mucicarmine solution for 20 minutes
- Wash in water, dehydrate, clear and mount as desired

Masson- Fontana Silver Stain

The Masson-Fontana Silver Stain (MFSS) is used to identify phaeoid (dematiaceous) fungi. The histopathological examination of tissue is one of the most accurate means of documenting invasive fungal infection. Despite these advantages, histopathological stains are non specific and they do not provide identification of a fungal pathogen. The phaeoid fungi are now among the emerging fungal pathogens. These organisms are classified as phaeoid because they have melanin in their cell wall. MFSS specifically stain melanin of the phaeoid fungi.

- Bring sections to distilled water
- Treat with ammoniacal silver solution in a dark container
- Wash well with several changes of distilled water
- Treat with 0.5% sodium thiosulphate for 2 minutes
- Wash, counter stain in the neutral red solution for 3-5 minutes
- Wash, dehydrate, clear and mount.

Results:- Melanin, argentaffin, chromaffin, some lipofuscin pigments stain black and nuclei stain red.

TISSUE PROCESSING AND STAINING

The tissue taken from the lesion (biopsy) is collected in 10 % formalin, which acts as a preservative and sent for tissue processing and staining. The following steps are performed for tissue processing. Molten wax is used for Impregnation

DEHYDRATION:- As paraffin wax is immiscible with water, removal of water from the tissue is required, for this ascending grades of alcohol is used.

CLEARING:- Alcohol used in the first step is also immiscible with paraffin wax. So in the next step, removal of alcohol from the tissue is necessary. This is performed by adding a solvent which is miscible with molten wax. For this procedure chloroform is used.

IMPREGNATION WITH WAX:- After clearing, the tissue is infiltrated with molten paraffin wax, for preserving the cellular morphology and integrity.

EMBEDDING OR BLOCKING:- The tissue is finally transferred from the paraffin wax bath to the molten wax containing mold with a pair of warm forceps. Allow to solidify and the block may be removed.

SCHEDULE FOR PARAFFIN WAX PROCESSING

Reagent	Time required	
70 % alcohol----------	1 hour	
80 % „ „ ----------	1 hour	
90 % „ „ ----------	1 hour	
100 % alcohol----------	1 hour	Dehydration
100 % „ „ ----------	1 hour	
100 % „ „ ----------	1 hour	

Chloroform---------- 1 hour

Chloroform---------- 1 hour

Chloroform---------- 1/2 hour

Clearing

Paraffin Wax (molten)---------- 1 ½ hour

Paraffin Wax(molten)---------- 1 ½ hour

Paraffin Wax(molten)---------- 30 minute

in vacuum

Impregnation

Sections are made from the paraffin embedded tissue by an instrument called "MICROTOME". The section thickness should be between 4 to 5 μm.

STAINING AND MOUNTING

Staining and mounting of paraffin section is as follows

1. Removal of wax with xylene
2. Hydration through alchohol
3. Staining
4. Dehydration with alchohol
5. Clearing with xylene
6. mounting under a cover slip

PROCEDURE FOR HEMATOXILIN AND EOSIN STAINING

1. Xylene 1 minute
2. Xylene 1 minute
3. Xylene 1 minute
4. Absolute alchohol 1 minute

5. 90 % alcohol 1 minute
6. 80 % alchohol 1minute
7. 70 % alchohol 1 minute
8. Distilled water 1 minute
9. Harris Hemotoxillin 5 minute
10. Wash in water
11. Differentiation in acid/alcohol for 30 minute
12. Blue in tap water
13. Eosin 1 minute
14. Dehydration, 70 % alcohol 1 minute
15. 80 % alcohol 1 minute
16. 90 % alcohol 1 minute
17. Absolute alcohol 1 minute
18. Xylene 1 minute
19. Xylene 1 minute
20. Mount in DPX

H& E staining is to examine the tissue form of the fungus eg. Sclerotic / muriform bodies. Inorder to see the fungal hyphae in tissue, Gomori's Methenamine Silver Nitrate Stain (Grocott's Application to Fungi) is used.

Simultaneous routine bacterial cultures were put up followed by inoculation into LJ media and RCM to detect other bacterial infections.

CULTURE

Common culture media used for culture were:

1. Sabouraud's Dextrose Agar (SDA):- This is the most commonly used medium in the diagnostic mycology laboratory. The Sabouraud dextrose agar

(SDA) is the name recommended for the present day versions of the medium originally designed by the French dermatologist Raymond Sabouraud. The ingredients of this medium are as follows:

Peptone : 10 gm

Dextrose : 40 gm

Agar : 20 gm

Distilled water: 1000 ml

Autoclave the ingredients at 121 ^{o} C for 15 minutes and adjust the final pH to 5.6. Sometimes, saprobic fungi grow rapidly on this medium and often overgrown obscuring the true pathogen.

2. Neutral Sabouraud's Dextrose Agar (SDA) [Emmons modification]

The Emmons' modification differs from the original Sabouraud's formulation with lower concentration of glucose and a neutral pH. It contain 2 % dextrose and neopeptone with a final pH of 6.8-7.0.

Neopeptone : 10 gm

Dextose : 20 gm

Agar : 20 gm

Distilled water : 1000 ml

3. Sabouraud's Dextrose Agar with antibiotics (mg/ml Chloramphenicol) by dissolving 50 mg of chloramphenicol in 10 ml of 95 % Ethyl Alcohol and the adding to the boilng medium (1 litre) and Cycloheximide (Actidione) 0.5mg/ml by dissolving 500 mg of cycloheximide in 10 ml of acetone then adding to boiling medium (1 litre).

4. New media for fungal isolation and its storage

A novel media was prepared from indigenously available ingredients for the

isolation and maintenances of fungal culture. It has an added advantage over the conventional SDA as it is cheap and the ingredients are easily available. It gives a luxuriant growth of all types fungi. Compared to SDA, it is a good media for the storage of different fungal cultures.

The ingredient of this special media is

Bengal gram : 1%

Green gram : 1%

Sodium Chloride : 0.5%

Glucose : 2%

pH : 6.5

Before adding glucose, mix the media by boiling and filter to make it transparent. Add glucose sterilized at 10 pound pressure and dispense in test tubes similar to SDA.

5. Cornmeal Agar/ Cornmeal Tween agar: It is a nutritionally deficient media hence suppress the vegetative growth and stimulates sporulation in fungi

   Cornmeal : 5 gm

   Agar : 4 gm

   Distilled water : 200 ml

   Tween 80 (1%) : 2 gm

6. A new preparation for keeping permanent Wet mount for long periods

   Gycerol : 2 ml

   Lactic Acid: 1 ml

   Phenol : 0.5 ml

   Glycerol prevents drying , phenol kills the fungus and lactic acid preserve the fungus. Mainly this is useful for keeping the undisturbed fungal structure from a slide culture and is also useful for dematiaceous fungi.

STUDY OF THE FUNGAL CULTURES

GROSS MORPHOLOGY :-

Fungal growth on SDA were observed. The important factors to be noted are

1. Rate of growth:- fungi develop varying characteristics in different media, so it is important to describe the characteristics on standard medium, such as Sabouraud's Dextrose Agar. Virtually all the medically important fungi are normally described by their appearance on SDA, which is one reason this medium continues to be used as the primary isolation medium. A rapidly growing fungus develops characteristic morphology with in 2 to 5 days. Whereas a slow growing colony may take 2 to 3 weeks : intermediate growers mature with in 6 to 10 days. The growth rate will vary with media and temperature changes.
2. General topography, whether flat, hemispherical, or raised ,folded, verrucose, cerebriform and heaped margins regular or irregular.
3. Texture, whether yeast like, glabrous, powdery, granular, velvetty or cottony.
4. Surface pigmentation.
5. Pigmentation on the reverse.

MICROSCOPY

Growth from the culture tubes were examined microscopically after placing a small portion of the growth on a glass slide, teasing it with two sterile needles after adding lactophenol cotton blue/ normal saline in case of pigmented fungi, and then a coverslip.

Under microscope the following features were noted

Mycelium- Whether true or pseudomycelium

Hyphae- Whether septate or non septate

Whether branching or not

Whether pigmentation present or absent

Spore Bearing Structures:- Determine how the spores are attached. Do they develop directly from the hypha, as arthrospore and chlamydospores, or from specialized structures known as conidiophore. Are the spore bearing structures simple, such as a short unbranched stalk, or are they more complex with branching and / or whorls

Conidia:- size, shape, and arrangement of conidia

whether smooth or rough

Also noted the presence of any granule from the lesion, color, shape and size of the granules. Cultures were examined at regular intervals and sub culturing was done if contaminants threatened to overgrow the suspected pathogen. All the cultures were retained 2 months before discarding as negative. The major disadvantage of wet mount is that the characteristic arrangement of spores is disrupted when pressure is applied to the coverslip

There are two commonly used methods for examining the undisturbed microscopic morphology of fungi and they are (1) Adhesive Tape method and Microslide culture method.

LACTOPHENOL COTTON BLUE:

Lactophenol Cotton Blue (LCB) is used to study the morphological features of the fungal isolates.

1. Plain LCB:

Melted Phenol	:	20 ml
Lactic Acid	:	20 ml
Glycerol	:	40 ml

Cotton Blue : 0.05 gm

Distilled Water : 20 ml

The small amount of fungal growth is transferred to a drop of Lactophenol Cotton Blue (LCB) on a clean glass slide and teased apart with dissecting needles. The lactophenol cotton blue kills, preserves, and stains the fungal specimen. A cover slip is applied and the specimen is examined microscopically under low magnifiaction and then to high magnification. The method is limited in usefulness because the spores are often separated from the spore bearing structures, and this makes identification difficult for a number of fungi. This stain is useful for studying the morphology of fungus which are hyaline. The LCB preparation can be permanently preserved if Polyvinyl alcohol is used. It contains the following ingredients:

Polyvinyl alcohol power : 15 gm

Distilled water : 100 ml

Mix the powder at 80^{o} C in a beaker placed in water bath and filter through double-layered cloth.

Staining solution:

PVA stock solution : 56 ml

Melted Pheno : 22 ml

Lactic acid : 22 ml

Cotton Blue : 0.05 gm.

SLIDE CULTURE TECHNIQUE

Adhesive (Scotch) tape preparation:-

The transparent adhesive tape preparation allows one to observe the micro-organism microscopically approximately the way it sporulates in culture. The spores are intact, and the microscopic identification of an organism can be made easily.

1. Touch the adhesive side of a small length of transparent tape to the surface of the colony.
2. Adhere the length of tape to the surface of a microscope slide to which a drop of lactophenol cotton blue has been added.
3. Observe microscopically for the characteristic shape and arrangement of the spore.

Microslide culture

This method might appear to be the most suitable for making the microscopic identification of an organism because it allows one to observe microscopically the fungus growing directly underneath the coverslip. This technique was used to study the undisturbed relationship between reproductive structures and mycelium and also the sporulation characteristics of the organism. Microscopic features should be easily discerned, structures should be intact, and representative areas of growth are available for observation.

1. Cut a small block of suitable agar medium in 4x4 mm thickness.
2. Place the agar block over a sterile glass slide in a Petri dish.
3. With a right ankled wire, inoculate the four quadrants of the agar block with the organism.
4. Apply a sterile coverslip onto the surface of the inoculated agar block.
5. Add small amount of sterile distilled water and incubate at 30 ^{o} C
6. After a suitable incubation period, remove the coverslip and place it on a microscope slide containing a drop of lacto phenol cotton blue/ normal saline in case of dematiaceous fungi.
7. Observe microscopically for the characteristic shape and arrangement of spores.

INVITRO ANTIFUNGAL SUSCEPTIBILITY TESTING:

Antifungal susceptibility tests are designed to provide information that will allow the physician to select the appropriate antifungal agent useful for treating a specific infection. Compared to antibacterial susceptibility test, antifungal susceptibility testing is in a primitive form. Invitro antifungal susceptibility testing is influenced by a number of variables, including Inoculum size and preparation, medium formulation and pH, duration and temperature of incubation, and the criterion used for MIC endpoint determination. In addition, antifungal susceptibility testing is complicated by problems unique to fungi, such as slow growth rates (relative to bacteria) and the ability of certain dimorphic fungi to growth either as a unicellular yeast form that produces blastoconidia or as a hyphal or filamentous fungal form that may produce asexual spores, depending on pH, temperature and medium composition Mc Ginnis, M.R; and M.G.Rinaldi (1986).

The basic properties of antifungal agents themselves, such as solubility, chemical stability, mode of action, and the tendency to produce partial inhibition of growth over a wide range of concentrations, must be taken into account. All variables have been standardizes, and efforts are under way to develop interpretative guidelines for different antifungal agents. Numerous antifungal agents have been developed, and the newer agents are on the horizon.

The antifungal susceptibility testing is performed to provide information that allows the clinician to select an appropriate antifungal agent useful for treating a particular fungal infection. The agents may appear resistant in vitro and still have clinical efficacy. Sometimes, there has been no correlation between clinical response and susceptibility with in a fungal species. Unfortunately, this testing has not progressed as far as tests used for deciding the susceptibility of bacteria to the antimicrobial agents. There is no standard method used by all laboratories and there is

disagreement concerning specific conditions of incubation and other variables necessary for performing the test. The problems associated with antifungal susceptibility, which are given below.

1. Problems in relation to the fungal organism
    a. Slow growth rates in relation to bacteria.
    b. Dimorphism in fungal growth as yeast-mold.
2. Problems in relation to the antifungal agents
    a. Solubility in aqueous media.
    b. Stability of the antifungal agent.
    c. Partial inhibition of growth.
    d. Mechanism of action
3. Test conditions that affect the MIC of antifungal agent
    a. Composition and pH of medium
    b. Inoculum preparation and size.
    c. Incubation temperature and time
    d. Endpoint criteria
4. Lack of correlation between results of antifungal susceptibility testing and the clinical outcome.

Despite of all the difficulties, these tests are important for selection of appropriate antifungal agent and as a method to detect the development of resistance in certain organisms during the antifungal therapy.

Classification of Antifungal Drugs

A. ANTIFUNGAL ANTIBIOTICS

1. **Polyene Antibiotics**

    a) Amphotericin B

!) Conventional amphotericin B.

Amphotericin B deoxycholate

!!) Liposomal formulations of Amphotericin B

Amphotericin B lipid complex

Amphotericin B colloidal dispersion

Liposomal- encapsulated Amphotericin B

Nystatin

Pimaricin

Hamycin

2) **Other Antibiotics**

Griseofulvin

Pradimicin

B.SYNTHETIC ANTIFUNGAL AGENTS

1. Thicarbamates

Tolnaftate

2. Allylamines and Benzylamines

Naftifine

Terbinafine

Butenafine

3. Azoles

!) Imidazoles

Bifonazole	Butoconazole
Clotrimazole	Econazole
Fenticonazole	Ketaconazole
Miconazole	Omoconazole
Oxiconazole	Sulconazole

!!) Triazoles

Fluconazole	Itraconazole
Voriconazole	Terconazole
Posaconazole	Ravuconazole

B. MISCELLANEOUS ANTIFUNGAL AGENTS

Flucytosine	Ciclopiroxolamine
Amorofine	Whitifield's ointment
Potassium iodide	Selenium sulfide
Undecylenic acid	Haloprogin
Triacetin	Echinocandin
Nikkomycin	Gentian violet paint

The commonly used antifungal agents for treating subcutaneous fungal infections are

Polyene Macrolide Antifungals:- The polyenes are water insoluble and are inactivated by heat, light, and acid. The medium should be well buffered (pH 7.0), and the test solutions should be protected from light. These consist of Amphotericin-B, Nystatin, 5- Flurocystosine, and Griseofulvin.

Amphotericin-B. Is produced by actinomycete Streptomyces nodosus. It binds the ergosterol component of the fungal cell membrane and alter the selective permeability of this membrane. However, other sterols, including those present in mammalian cell membranes, are also bound. The most important adverse reaction associated with amphotericin B is renal insufficiency. A newer agent, liposomal amphoptericin B, reportedly diminishes, this adverse reaction. Conventional and liposomal formulations of amphotericin B are recommended for eumycetoma caused by Madurella and Fusarium species. Before introduction of azoles, amphotericin B was the drug of choice for treatment of relapsed lymphocutaneous infection, pulmonary infection and other unifocal deep form of sporotrichosis

Azole Antifungal agents:- The azole group of antifungal agents consists of the imidazoles and triazoles. The important group in imidazoles is ketaconazole and is useful for sporotrichosis. The triazoles group of antifungal agents like itraconazole, posaconazole and voriconazole are used for treating various subcutaneous fungal infections. These agents disrupt the integrity of the fungal cell membrane by interfering with the synthesis of ergosterol. On the other hand, the azoles, except fluconazole, have relatively good chemical stabilities but shows poor solubility in aqueous media.

Fluconazole:- is a triazoles, which is exceptionally soluble in water. This can be used for oral and intravenous administration. Therapeutic levels are easily reached in the central nervous systems. Side effects of fluconazole therapy are usually minimal. This drug is useful for treating histoplasmosis, blastomycosis, coccidiodomycosis, aspergillosis and for cryptococcal meningitis in AIDS patients.

Itraconazole:- It is a triazoles antifungal agent having a broad spectrum antifungal activity against most pathogenic fungi except zygomycetes. It has a good response against disseminated aspergillosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, phaeohyphomycosis, eumycetoma and chromoblastomycosis caused by cladosporium species.

Posaconazole:- It is also a triazoles antifungal agent and is useful for various systemic and subcutaneous mycosis.

Allylamine and Benzylamine:- Allylamine is a newly developed class of synthetic antifungal agents with activity against wide range of fungi. These agents selectively inhibit the key enzyme, squalene epoxidase, which is required for fungal ergosterol biosynthesis. This inhibition is not mediated through cytochrome p-450, consequently, accumulation of squalene weakens the cell membrane leading to fungal cell death. This group consist of Naftifine, Terbinafine and Butenafine

Terbinafine:- It is useful for very useful for various systemic and subcutaneous mycosis

Miscellaneous Antifungal agents:

Flucytosine:- Flucytosine (5-Fluorocytosine) is a synthetic fluoropyrimidine and mainly used in the treatment of infections caused by yeasts and phaeoid fungi. This is a water soluble drug and can be administered orally. It is converted by fungal cytosine deaminase to antimetabolite, 5- fluorouracil, which inhibit thymidylate synthetase and consequently DNA synthesis. Flucytosine and amphotericin B act synergistically and are useful for treating various fungal infections as combination therapy

Potassium Iodide:- Is the therapy of choice for cutaneous/lymphatic sporotrichosis.

Diaminodiphenylsulphone (dapsone –DDS) This drug is widely used for treating leprosy and is also useful for treating rhinosporidiosis

Some of the antifungal susceptibility testing methods are described below.

a) **Macro and Microdilution methods for Yeast NCCLS (M27-A)** The US National Committee for Clinical Laboratory Standards (NCCLS) has released the approved version (M 27-A) of standardized broth macrodilution and microdilution methods for the antifungal susceptibility testing of yeasts in 1997. The M 27 document was proposed in 1992 After two multicentric studies this method has been approved as the standard method for antifungal susceptibility testing for yeasts.. This document describes a broth macrodilution and microdilution modifications, specifies a defined culture medium as the standard medium(RPMI 1640 broth buffered to pH 7.0), as well as an inoculum standardized by spectrophotometric reading approximately 1000 cells/ml and visual determination of MIC endpoint determination after incubation at 35 ^{o} C for 48 hours to 72 hours

Macro and Microdilution methods for Filamentous Fungi NCCLS(M38-P)

Although the number of serious infections caused by the filamentous fungi is lower than the number of yeast infections, antifungal susceptibility testing of these opportunistic pathogens is important in the clinical laboratory. The determination of MIC s for filamentous fungi can be facilitated with a method that overcomes observer's bias and quantifies the hyphal growth of molds. The NCCLS has also proposed the antifungal susceptibility testing of conidia forming filamentous fungi in 1998 which can be performed as per the M38-P document on a similar pattern as that of yeasts. Both these documents, M27-A and M38-P are currently being used worldwide for antifungal susceptibility testing for yeasts and molds, respectively. As turbidity measurements and colony counts are not useful in the case of filamentous fungi, colorimetric methods based on the measurement of metabolic activity may facilitate determination of MIC.

There have been several multicenter studies involving filamentous fungi which have been used in the development of the National Committee for Clinical Laboratory Standards (NCCLS) reference method for broth dilution antifungal susceptibility testing of conidium forming filamentous fungi. The test employs a methodology similar to that for yeasts but requires spectrometric inoculum determination based on conidial size. Interlaboratory agreement is high for the broth dilution, thus making it suitable as a reference standard., Espinel-Ingroff A etal (1997). The inoculum for each isolate is prepared by first growing the fungus on potato dextrose agar slants for 7 days at 35^{0} C.

A conidial suspension is prepared by flooding each slant with approximately 2 ml sterile 0.85 % saline. The resulting mixture is withdrawn, and the heavy particles are allowed to settle for 3 to 5 min. The upper homogenous suspension containing the

conidia is mixed for 15 second with a vortex. The turbidity of the mixed suspension is measured by using a spectrophotometer at 530 nm and adjusted to a specific final transmission range for each species tested. Only conidial suspensions of approximately 0.05 x 10^4 to 5x 10^4 CFU/ml have been evaluated for antifungal susceptibility testing of mold by this method. The QC organism used for yeast testing plus an isolate of *Paecilomyces variotti*. ATCC 22319 may be tested in the same manner as the other isolates and should be included each time an isolate is evaluated with any antifungal agent.

All tubes and microdilution trays are incubated at 35° C and observed each for the presence of growth, when growth is visible in the growth control, each tube is vortexed for 10 seconds. Immediately prior to being scored, this allows the detection of small amounts of growth. The growth in each tube and well is compared with that of the growth control (drug free) and given a numerical score as follows: 0, optically clear or showing no growth; 1, approximately 75 % reduction in growth; 2, approximately 50 % reduction in growth; 3,approximately 25 % reduction in growth; 4, no reduction in growth. The MIC endpoint criterion for molds is the lowest drug concentration that inhibits approximately ≥75 % of the growth of the fungus being tested compared with the control. Espinel-Ingroff A etal (1993). However, it is somewhat cumbersome to perform and not likely to be used in clinical microbiology laboratories.

Thus modifications of the reference method are acceptable and expected. With this in mind, several modifications of the macrobroth reference method of antifungal susceptibility are currently under investigation. They offen promise as alternative approaches that may better serve practical clinical laboratory needs. To improve objectivity and speed of current antifungal susceptibility testing, the yeast Rapid Susceptibility Assay (RSA) was adapted for *Aspergillus fumigatus* Tracy J.Wetter etal (2003).

This method is based on glucose utilization in the presence of an antifungal drug. Aspergillus fumigatus conidia were incubated in 0.2 % glucose RPMI 1640 containing 0.03 to 16 micro gram of amphotericin B or itraconazole/ml. Drug-related inhibition of glucose utilization correlated with suppression of conidial germination. Following incubation of conidia with various concentration of antifungal drug, the percentage of residual glucose in the growth medium was determined colorimetrically and plotted against drug concentration to determine the MIC. National Committee for Clinical Laboratory Standards (NCCLS) M 38-P testing was also performed to obtain NCCLS MIC's for direct comparison. Result of this study showed that the mold RSA provides a more objective and rapid method for aspergillus species susceptibility testing than the NCCLS M 38-P assay.

A simple screening semisolid agar antifungal susceptibility test (SAAS) method was developed by Cigdem etal (2004). They compared MIC results of the NCCLS M38-P broth dilution method with SAAS screening test for four antifungal agents tested against 54 clinical isolates of filamentous fungi. The antifungal agents used for the study were amphotericin B, amphotericin B lipid complex, itrazonazole, posaconazole. The SAAS test supported the growth of all filamentous fungi tested. They found excellent concordance of results for all four drugs tested and found that the SAAS test compared favourably to NCCLS broth micro dilution test for molds and might be useful preliminary screening test for molds. This test for filamentous fungi uses inocula prepared from a colony swab, without the need for special equipment.

Carmen Castro, M etal 2004 compared the susceptibilities of 63 isolates of Aspergillus species to voriconazole by a modified NCCLS M38-A method and Sensititre Yeast One Colorimetric method.The overall agreement was 82.5%, ranging

from 100% for *Aspergillus niger* and *Aspergillus terreus* to 62.5% for *Aspergillus flavus*. This test is a commercial colorimetric panel that consists of a disposable tray which contains dried serial dilutions of five antifungal agents in individual wells. The wells also contain an oxidation-reduction indicator (Alamar blue) to generate clear-cut endpoints based on a visually detectable color change. The MIC obtained by this method was compared to those obtained by the modified reference broth micro-dilution method. Sanchez Sousa A etal (1999).

The interpretative guidelines as defined by NCCLS are as follows

Antifungal agent	Susceptible concentration	Resistant concentration
Fluconazole	≤ 8.0 μg/ml	≥64.9 μg/ml
Itraconazole	≤ 0.125.0 μg/ml	≥0.1 μg/ml
Fluconazole	≤ 4.0 μg/ml	≥32.0 μg/ml

Amphotericin B susceptibility or resistance cannot be distinguished using the NCCLS method. It is suggested that an MIC of at least 1.0 μg/ml be considered as resistant; however, this information is tentative. Ketaconazole susceptibility testing has suggested that isolates with an MIC between 0.313 and 16 μg/ml be considered as susceptible

b) Disk Diffusion Method:

This method has wide-spread use for antibacterial drug testing. The agar diffusion testing has limited application in antifungal drug susceptibility testing. This method is useful for testing the antifungal action of flucytosine. In this method a disk containing the antifungal agent which diffuses in the surrounding medium, inhibit the growth of fungi and measurements of zone of inhibition are taken.

c) Etest:

The Etest is a patented commercial method for determination of MIC. It is set

up in a similar method as the disc diffusion test except the disk is replaced by a calibrated plastic impregnated with a concentration gradient of the antifungal agent.

d) Fungitest:

This is an alternative to the NCCLS reference procedures in which growth of isolates is measured in cultures containing just one or two antimicrobial drug concentrations that distinguish resistant from susceptible strains.

e) Spectrophotometric Methods

This method is used to determine MIC end points more objectively by reading broth micro dilution plates with spectrophotometer. However, the determination of spectrophotometric MIC s requires the selection of a level of inhibition and different studies have employed different endpoint definitions.

f) Flowcytometry

During the last decades, flowcytometry has been developed as a powerful tool in many diagnostic and research laboratories. A rapid assay of antifungal activity has been developed by utilizing flowcytometry to detect accumulation of a vital dye in drug damaged fungal cells. It has been suggested that flowcytometry may provide an improved, rapid method for determining and comparing the antifungal activities of compounds with different mode of action.

ANTIFUNGAL SUSCEPTIBILITY TESTING USING AGAR DILUTION

In the present study agar dilution method is used for invitro antifungal activity of terbinafine.Serial dilutions of drug are prepared in Sabouraud's agar and poured into tubes. Conidial suspension of the test fungus was prepared in BHI broth at a concentration of 10^6 cells/ml. 0.1 ml of the above conidial suspension was inoculated into the sabourud's tube with serial dilutions of antifungal agent. The tubes were incubated at room temperature and observe for growth. Control tubes without antibiotics were also included.

STUDY ON
THE PREVALENCE OF SUBCUTANEOUS MYCOSES IN NORTH KERALA

Thesis submitted
in partial fulfilment of the requirements
for the award of the degree of

DOCTOR OF PHILOSOPHY

IN MICROBIOLOGY (MEDICAL FACULTY)

BY

ASHOKAN K KUTTIYIL

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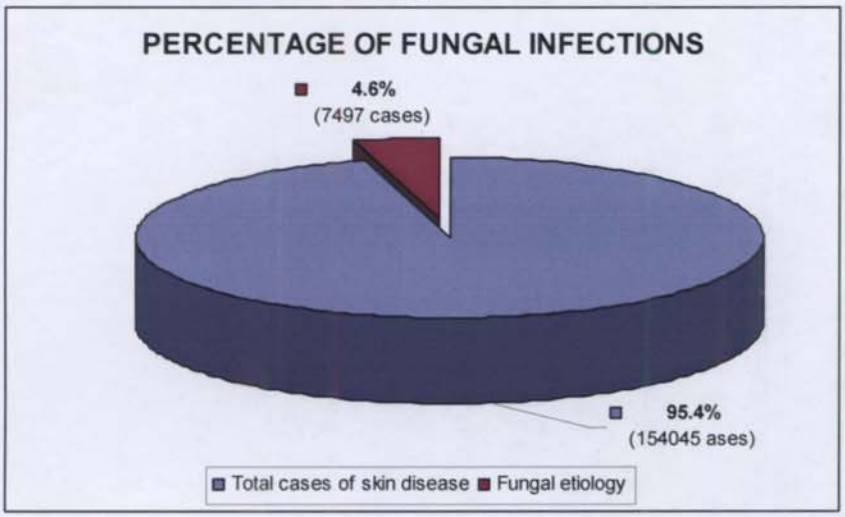
AUGUST- 2006

RESULTS

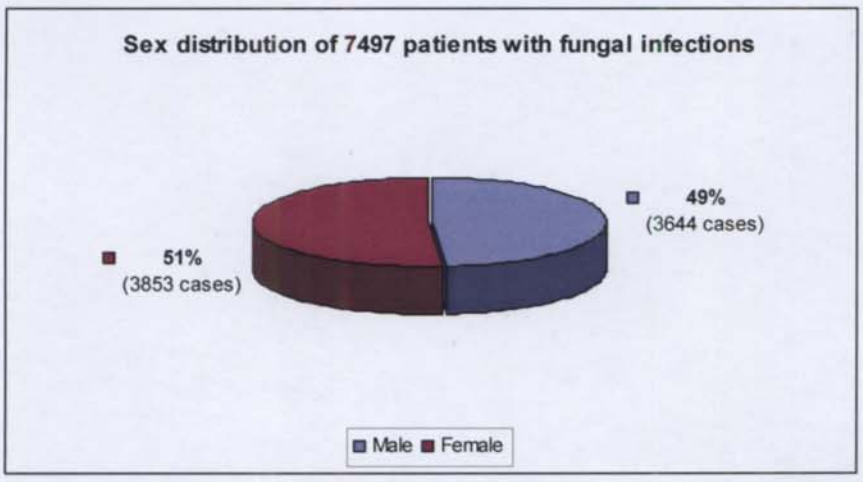
The study group consisted of 161542 patients with various skin lesions who attended the Dermatology, Surgery and ENT departments of Calicut Medical College, for a period of five years from January 2000 to January 2006.

Fig: 1



As shown in Fig 1, of the 161542 patients with various types of skin diseases, 7497 (4.64%) were suspected to be associated with fungal etiology. 154045 cases (95.4%) were associated with other infections.

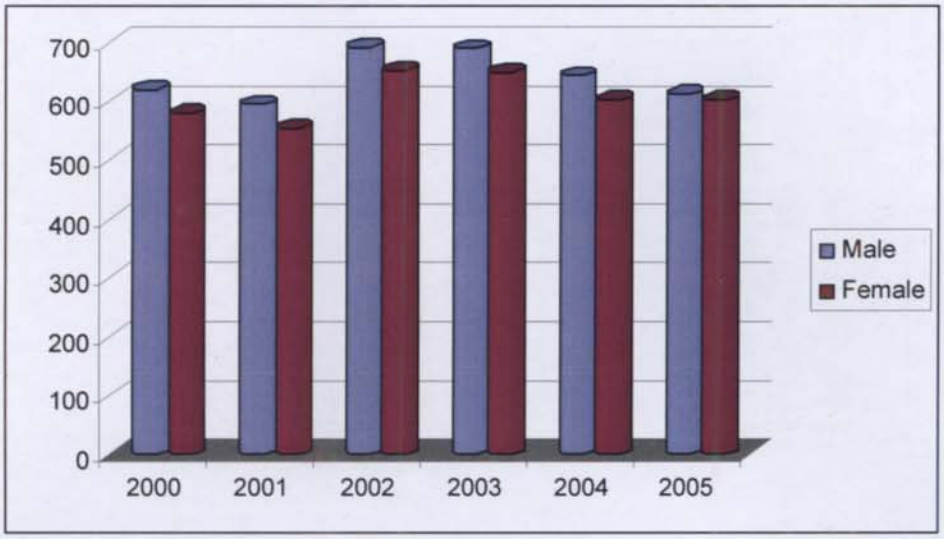
Fig: 2



As shown in fig 2, Out of 7497 patients with various types of fungal infection, 3853 were males (51 %) and 3644 were female patients (49 %). A relative high incidence of fungal infections was noticed in male populations.

SEX DISTRIBUTION OF 7497 PATIENTS (Year wise)

Fig: 3



As shown in fig 3. Sex distribution of 7497 patients with various fungal infections showed a high incidence of infection in the year 2002 and 2003. The incidences of fungal infections were higher in male than female populations.

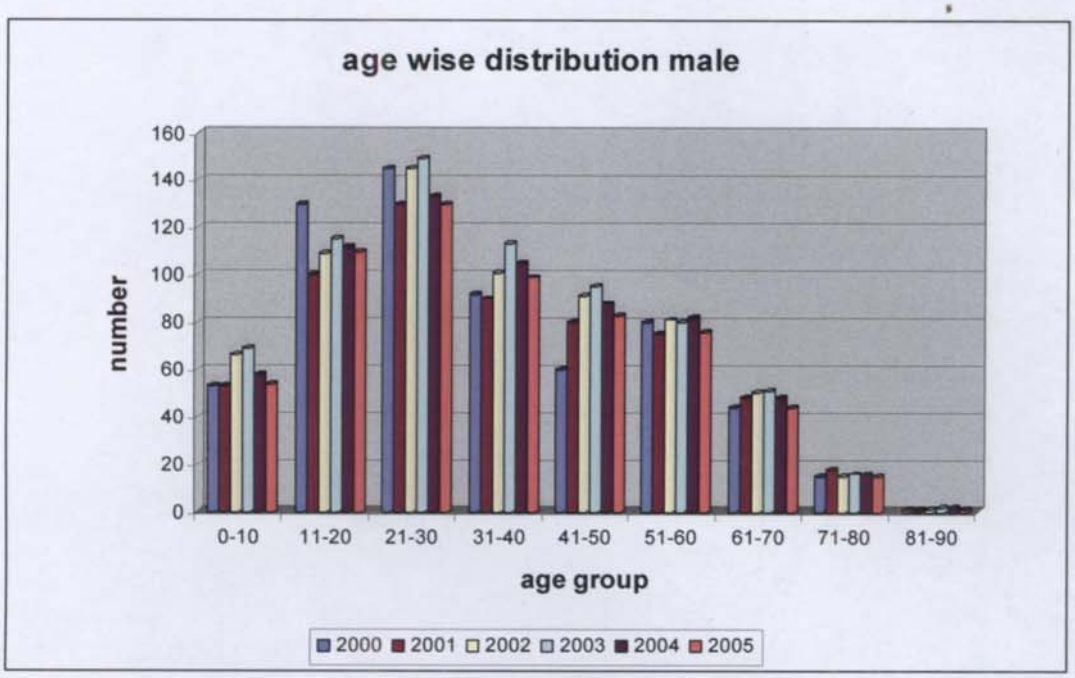
Table: 1

AGE WISE DISTRIBUTION OF MALE PATIENTS

	2000	2001	2002	2003	2004	2005	Total	%
0-10	53	53	66	69	58	54	353	9.16%
11-20	130	100	109	115	112	110	676	17.54%
21-30	145	130	145	149	133	130	832	21.59%
31-40	92	90	101	113	105	99	600	15.57%
41-50	60	80	91	95	88	83	497	12.89%
51-60	80	75	81	80	82	76	474	12.30%
61-70	44	48	50	51	48	44	285	7.39%
71-80	15	18	15	16	16	15	95	2.46%
81-90	1	1	1	2	2	1	8	0.20%
Total	620	595	**692**	**690**	644	612	3853	

As shown in table 1, higher incidences of fungal infections in male patients were noticed in the year 2002 followed by the year 2003.

Fig: 4



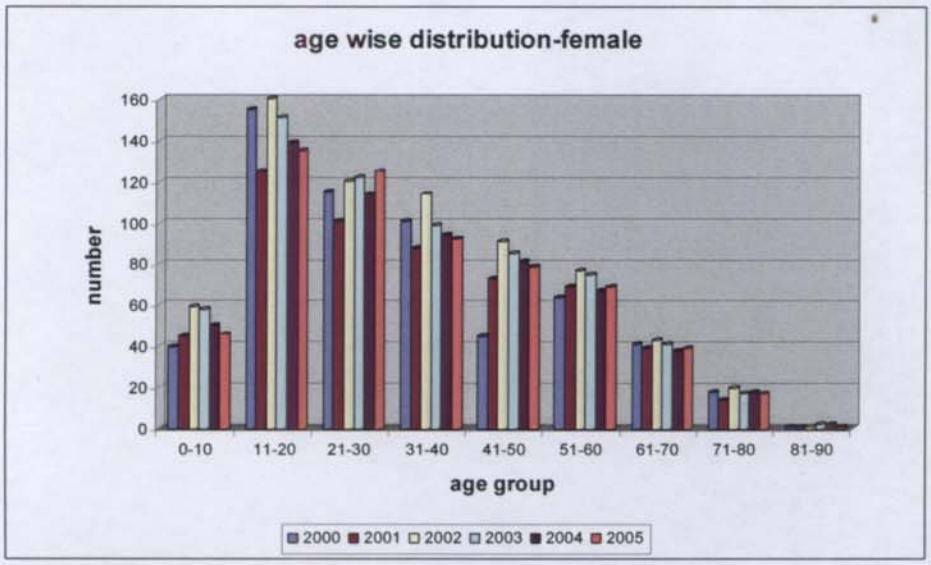
As shown in the Fig 4, the year wise distribution of 3853 male patients with various types of fungal infections for the period of six years from 2000 to 2005 showed a high incidence of infection in the age group 21-30 (21.59%), followed by 11-20 (17.5%).

Table: 2

AGE WISE DISTRIBUTION OF FEMALE PATIENTS IN 2000-2005

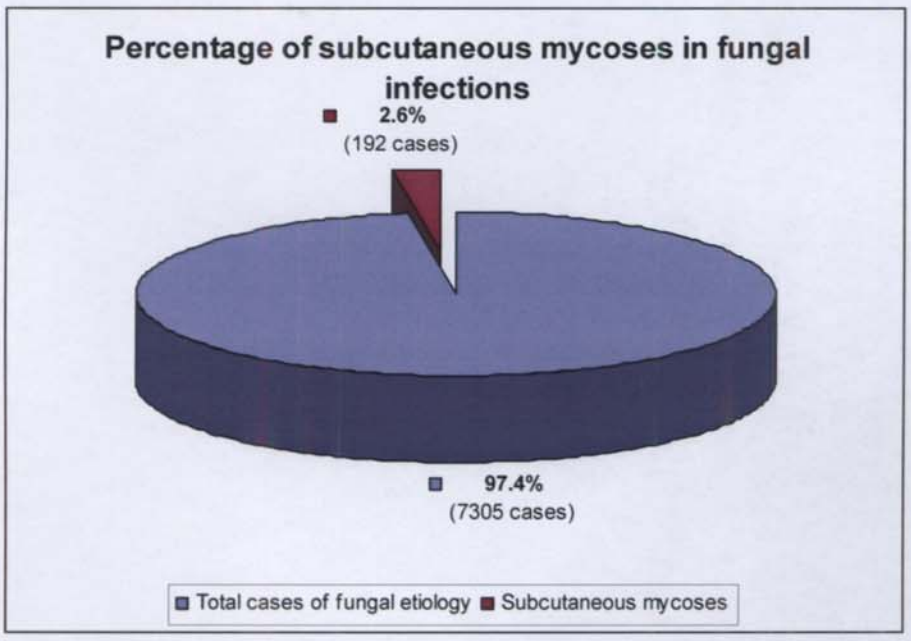
	2000	2001	2002	2003	2004	2005	Total	%
0-10	40	45	59	58	50	46	298	8.17%
11-20	155	125	160	151	139	135	865	23.73%
21-30	115	101	120	122	114	125	697	19.12%
31-40	101	88	114	99	94	92	588	16.13%
41-50	45	73	91	85	81	79	454	12.45%
51-60	64	69	77	75	67	69	421	11.55%
61-70	41	39	43	41	38	39	241	6.61%
71-80	18	14	20	17	18	17	104	2.85%
81-90	1	1	1	2	2	1	8	0.21%
Total	580	555	653	650	603	603	3644	

Fig: 5



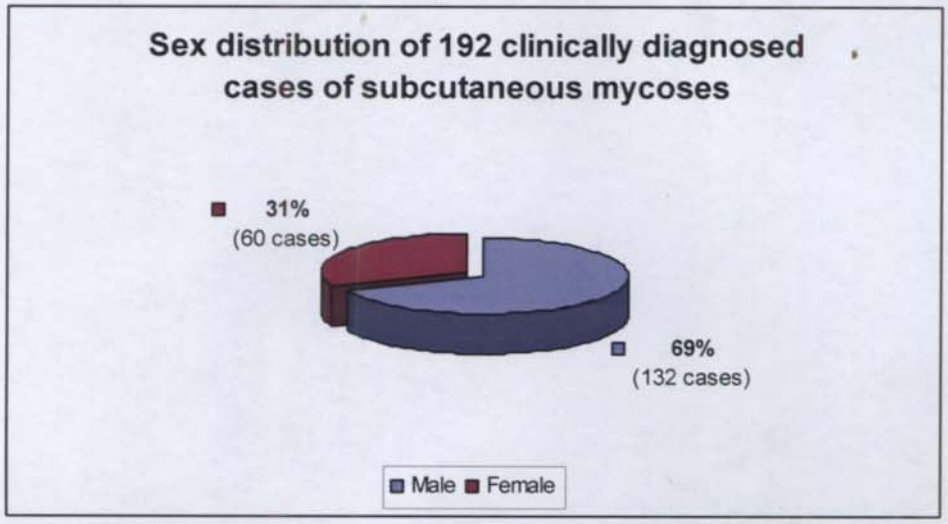
The year wise distribution of 3644 female patients with various types of fungal infections for the period of six years from 2000-2005 were shown in fig.5. Highest incidence of infections were noticed in the age group 11-20 (23.73%) followed by 21-30 (19.12%).

Fig: 6



As shown in fig.6, out of 7497 patients with various types of fungal infections, 192 were clinically diagnosed subcutaneous fungal infections (2.6%). 7305 cases (97.4%) were due to other fungal infections.

Fig: 7



Sex distribution of 192 cases of subcutaneous mycoses in study population is shown in fig.7. Of the 192 suspected subcutaneous mycosis 132 (69%) were male patients and 60 (31 %) were female.

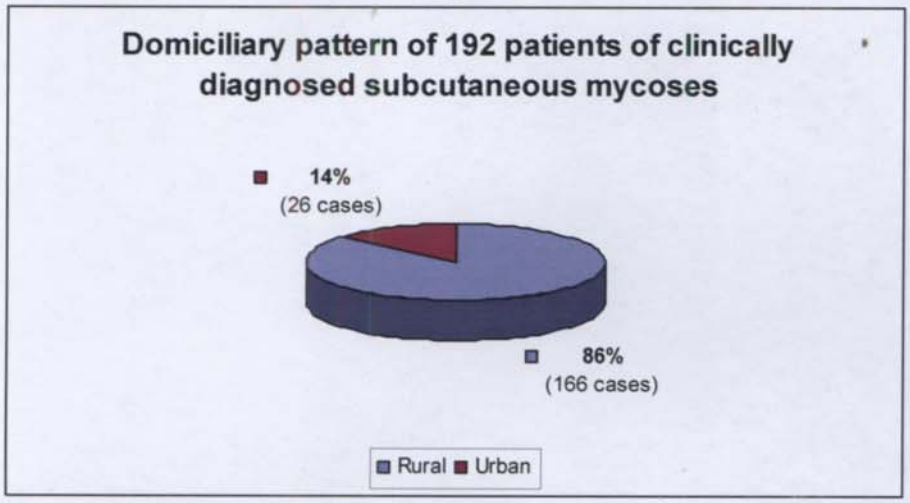
Table: 3

AGE AND SEX DISTRIBUTION OF 192 CLINICALLY DIAGNOSED PATIENTS WITH SUBCUTANEOUS MYCOSES

Age	Total	Male	Female
0-10	02	--	02
11-20	15	09	06
21-30	**33**	19	14
31-40	**39**	**25**	14
41-50	**37**	**27**	10
51-60	**33**	**26**	07
61-70	26	21	05
71-80	05	04	01
81-90	02	01	01
Total	192	132	60

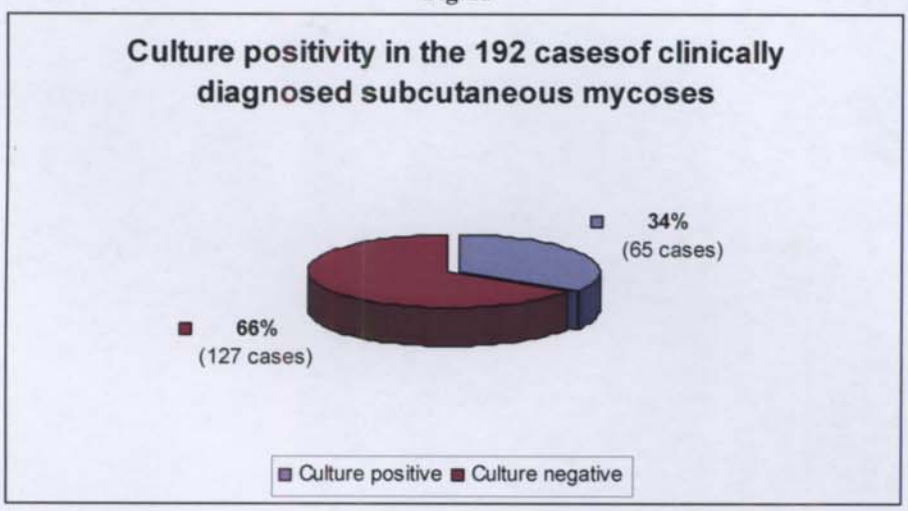
Age wise distribution of 192 patients with subcutaneous fungal infections for the period of six years (2000-2005) is shown in the table 6. A relatively high incidence of infections were noticed in the age group 31-40 *ie* 39 patients (20.3%). 37 patients were(19.2%) in the age group 41-50 and 33 patients each(17.1%) in the age group 21-30 and 51-60. In male incidence of subcutaneous fungal infections were highest in the age group 41-50 (27), followed by 51-60 (26) and 31-40 (25). In female highest incidence were obtained in the age group 21-30 (14 each).

Fig:10



Domiciliary pattern of 192 patients of clinically diagnosed subcutaneous mycoses is shown in fig 10. Of the 192 cases of subcutaneous mycosis 166 (86%) patients belonged to rural populations and 26 (14%) patients were in urban populations

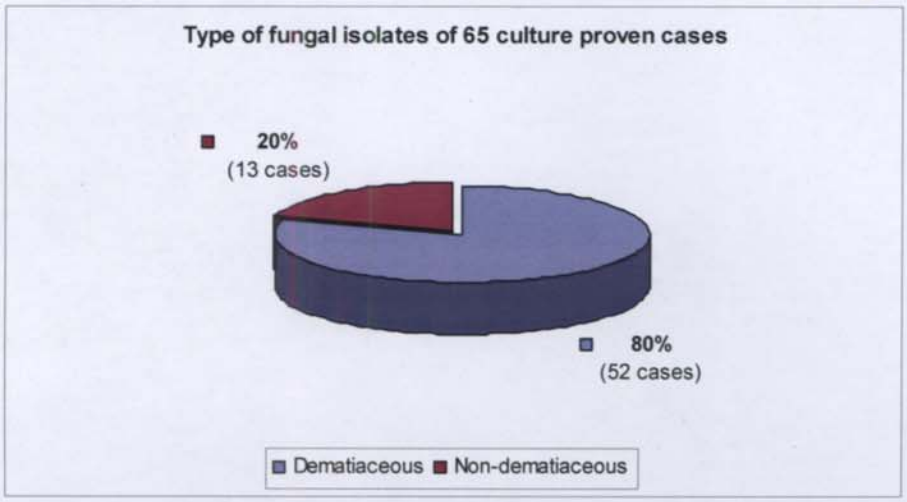
Fig:11



Culture positivity of 192 cases of subcutaneous mycoses is shown in fig 11. Of the 192 patient with subcutaneous mycoses, 65 cases were culture positive (34%) and 127 (66%) were culture negative. Out of 65 positive cultures 46 (71%) patients were male and 19 (29%) patients were female.

TYPE OF FUNGAL ISOLATES

Fig: 12



Type of fungal isolations obtained during the study period is shown in fig 12. Of the 65 fungal isolates, 52 (80 %) isolates were belonged to dematiaceous group of fungi and 13 (20%) isolates were non-dematiaceous group.

Table: 4

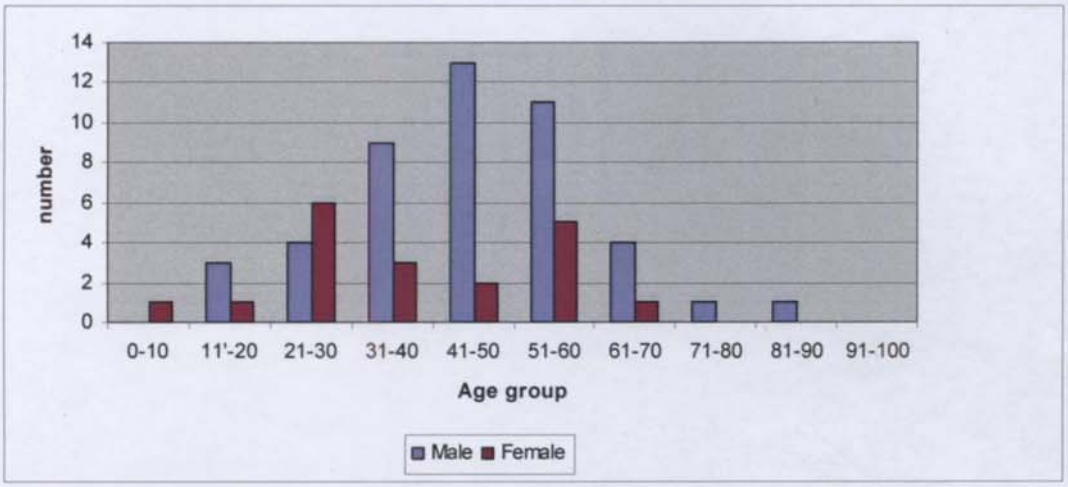
AGE AND SEX WISE DISTRIBUTION OF 65 CULTURE PROVEN CASES OF SUBCUTANEOUS MYCOSES:

Age	Total	Male	Female
0-10	01	--	01
11-20	04	03	01
21-30	10	04	**06**
31-40	**12**	**09**	**03**
41-50	**15**	**13**	02
51-60	**16**	**11**	05
61-70	05	04	01
71-80	01	01	--
81-90	01	01	--
Total	**65**	**46**	**19**

Age and sex wise distribution of 65 culture proven cases of subcutaneous mycoses is shown in table 7. A relatively high incidence of infections were noticed in the age group 51-60 *ie* 16 patients (24.6%). 15 patients were (23.%) in the age group 41-50 and 12 patients were in 31-40 age group(18.4%). In male incidence of subcutaneous fungal infections were highest in the age group 41-50 (13 cases), followed by 51-60 (11 cases) and 31-40 age group (9 cases). In female patient highest incidence of infections were obtained in the age group 21-30 (6 cases) and 31-40 (3 cases)

Fig: 13

AGE AND SEX DISTRIBUTION OF 65 CULTURE PROVEN CASES OF SUBCUTANEOUS MYCOSES



As shown in fig 13, the highest incidences of subcutaneous mycoses were noticed in the age group 41-50, followed by 51-60.

Table: 5

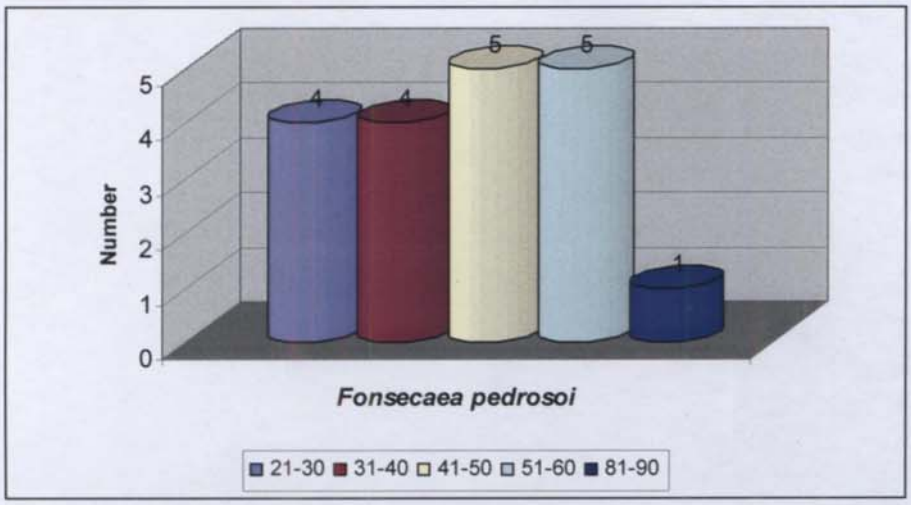
DEMATIACEOUS FUNGI ISOLATED DURING THE PERIOD OF STUDY (2000-2005):

	Total	Male	Female
Fonsecaea pedrosoi	**19**	**14**	5
Cladosporium carrionii	**15**	**13**	2
Aureobasidium pullulans	3	2	1
Phoma	3	2	1
Ramichloridium mackenziei	2	2	
Xylohypha bantiana	2	2	
Cheatomium	1	1	
Fonsecaea compacta	1		1
Curvularia	2	2	
Torula	1	1	
Black Yeast	1	1	
Fonsecaea dermatitidis	2	2	
TOTAL	**52**	**42**	**10**

As shown in table 5 *F.pedrosoi* (19 cases)and *Cladosporium carrionii*(15 cases) were the most common isolates. Less frequently isolated species were *Phoma, Ramichloridium* and *Xylohypha bantiana.*

Fig: 14

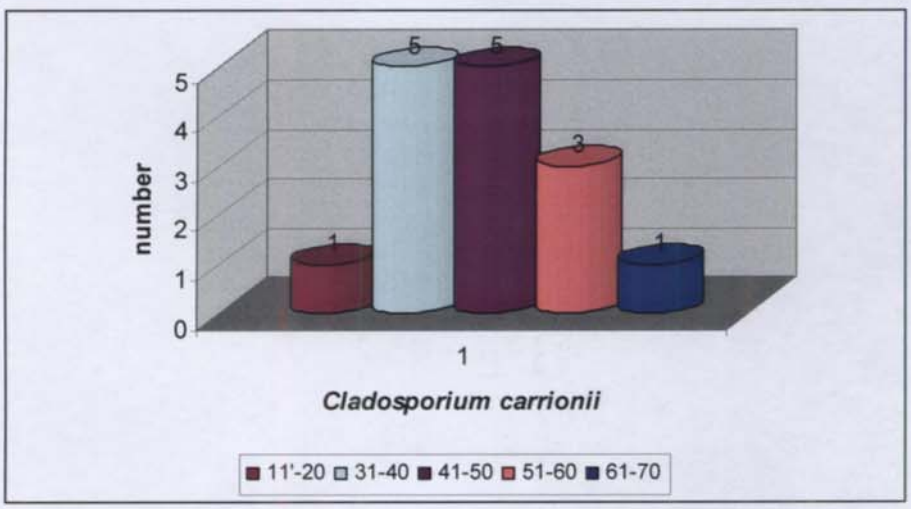
AGE GROUP OF PATIENTS FROM WHOM *F.PEDROSOI* WAS ISOLATED



The distribution of *F.pedrosoi* isolated in different age group is shown in fig 14. The highest incidence was noticed in the age group 41-50 and 51-60 (5 isolations each)

Fig: 15

AGE GROUP OF PATIENTS FROM WHOM *CLADOSPORIUM CARRIONII* WAS ISOLATED



The distribution of *Cladosporium carrionii* isolated in different age group is shown in fig 15. The highest incidence was noticed in the age group 31-40 and 41-50 (5 isolations each)

Table: 6

AGE WISE DISTRIBUTION OF DEMATIACEOUS FUNGI:

	F.pedrosoi	*Cladosporium*	*Aureobasidium*	*Phoma*	*F.dermatitidis*	*Curvularia*	*Cheatomium*	*F.compacta*	*Black yeast*	*X.bantlana*	*Torula*	*Ramichloridium*
0-10												
11-'20		1			1	1				1		
21-30	4		1	1								
31-40	4	**5**		1			1		1			
41-50	**5**	**5**										1
51-60	**5**	3	2	1	1	1		1				1
61-70		1								1	1	
71-80												
81-90	1											
91-100												
Total	**19**	**15**	**3**	**3**	**2**	**2**	**1**	**1**	**1**	**2**	**1**	**2**

Distribution of dematiaceous fungi in different age group is shown in table6. In *F.pedrosoi,* the highest incidence was noticed in the age group 41-50 and 51-60 (5 isolations each). In Cladosporium carrionii the highest incidence was noticed in the age group 31-40 and 41-50 (5 isolations each).

Table: 7

AGE GROUP OF PATIENTS FROM WHOM NON-DEMATIACEOUS FUNGI WAS ISOLATED:

		Male	Female	Male:Female ratio
Acremonium	4	3	1	3:1
Scedosporium apiospermum	3	1	2	0.5:1
Streptomyces	3	1	2	0.5:1
Penicillium marneffei	1	1	-	1:0
Aspergillus terreus	1	-	1	0:1
Histoplasma	1	1	-	1:0

As shown in table 7.*Acremonium* was the most commonest non-dematiaceous fungi isolated (4 cases) followed by *Scedosporium apiospermum* and *Streptomyces* three cases each.

Table: 8

AGE WISE DISTRIBUTION OF NON-DEMATIACEOUS FUNGI:

	Acremonium	*Scedosporium apiospermum*	*Streptomyces*	*Penicillium marneffei*	*Aspergillus terreus*	*Histoplasma*
0-10						
11-'20						
21-30						
31-40	1	1	1			1
41-50	**2**		1		1	
51-60						
61-70	1	1	1			
71-80				1		
81-90		1				
91-100						
Total	**4**	**3**	**3**	**1**	**1**	**1**

Distribution of non-dematiaceous fungi in different age group is shown in table 8. In case of *Acremonium,* the highest incidence was noticed in the age group 41-50.

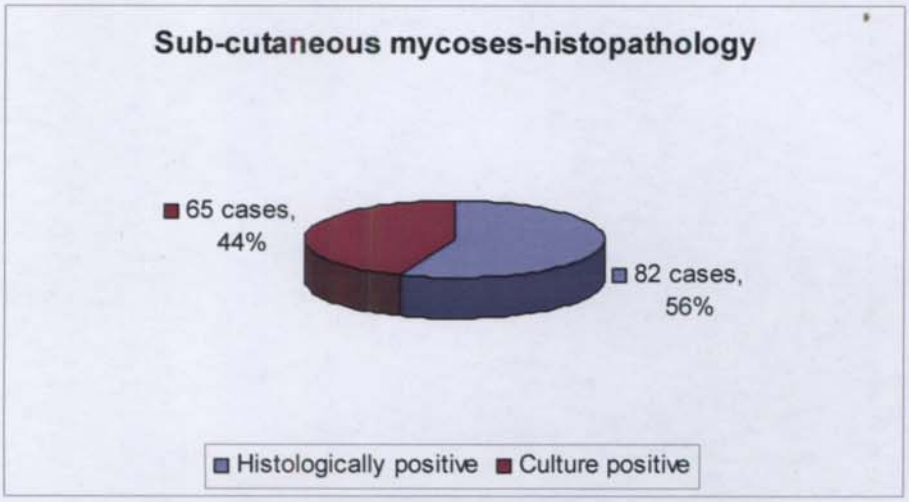
Table: 9

DIAGNOSTIC CRITERIA OF THE 192 CASES OF SUBCUTANEOUS MYCOSES

	Histopathologically positive	%	Culture positive	%
Chromoblastomycosis	**50**	**26%**	**40**	20.8%
Phaeohyphomycosis	**10**	**5.2%**	**10**	5.2%
Hyalohyphomycosis	8	4.2%	6	3.1%
Mycetoma	6	3.1%	6	3.1%
Rhinosporidiosis	3	1.7%	0	0%
Unusual site	5	2.6%	3	1.6%
Total cases	**82**		**65**	**33.8%**

Clinical distribution of 192 cases of subcutaneous mycosis is shown in table 9. Of the 192 cases of subcutaneous mycoses, 50 cases of (26%) chromoblastomycosis, 10 cases of (5.2%) phaeohyphomycosis were obtained.

Fig 16



As shown in Fig 16. Of the 192 clinically diagnosed subcutaneous mycoses 82 cases were histopathologically positive (56%) and 65 cases were culture positive (44%).

Table: 10

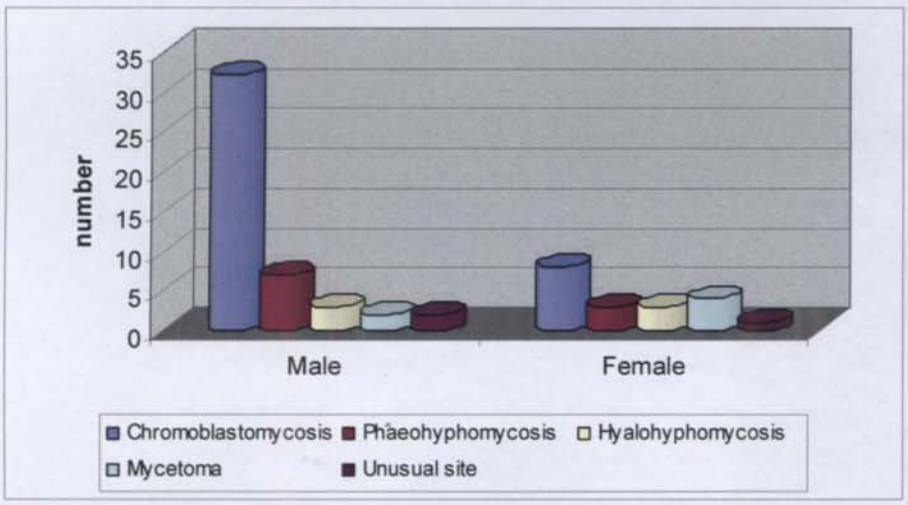
AGE GROUP & SEX WISE DISTRIBUTION OF CULTURE PROVEN CASES OF SUBCUTANEOUS MYCOSES:

	Chromoblastomycosis		Phaeohyphomycosis		Hyalohyphomycosis		Mycetoma		Unusual site		Total	
Age group	M	F	M	F	M	F	M	F	M	F	Male	Female
0-10												
11'-20	3								1		4	
21-30	2	2	2								4	2
31-40	**6**	**5**	**2**		2			1	1		11	6
41-50	**10**			1		3	1				11	4
51-60	**8**	**1**	**2**	**2**				1			10	4
61-70	2		1				1	1		1	4	2
71-80					1						1	
81-90	1							1			1	1
Total	**32**	**8**	**7**	**3**	**3**	**3**	**2**	**4**	**2**	**1**	**46**	**19**

Age group and sex wise distribution of 65 culture positive cases of subcutaneous mycoses is shown in table 10. In chromoblastomycosis highest isolations were obtained in the age group31-40 (11 isolates), followed by the age group 41-50 (10 isolates) and 51-60 (9 isolates). In phaeohyphomycosis 51-60 age group showed highest isolations (4 isolates).

Fig: 17

CASE WISE DISTRIBUTION OF SUBCUTANEOUS MYCOSES



Case wise distribution of subcutaneous mycoses is shown in fig 17. chromoblastomycosis was the most commonest subcutaneous mycoses (40 cases) followed by phaeohyphomycosis (8 cases). The incidences of subcutaneous mycoses were high in male populations except in mycetoma.

Table: 11

CHROMOBLASTOMYCOSIS

Details of culture positive Chromoblastomycosis cases during 2000-2005					
Year	No.	Sex	Age	Nature of lesion	Isolate
2000	1	Male	44	Ulcer foot	*Fonsecaea pedrosoi*
	1	Male	48	Ulcer foot	*Fonsecaea pedrosoi*
	1	Male	53	Ulcer hand	*Cladosporium carrionii*
	1	Male	52	Ulcer Big toe®	*Cladosporium carrionii*
	1	Male	53	Ulcer foot	*Fonsecaea dermatitidis*
	1	Female	35	Ulcer right hand	*Fonsecaea compacta*
	1	Female	29	Forearm(L)	*Fonsecaea pedrosoi*
2001	1	Male	55	Skin ulcer	*Fonsecaea pedrosoi*
	1	Male	50	Ulcer foot	*Fonsecaea pedrosoi*
	1	Male	50	Ulcer foot	*Cladosporium carrionii*
	1	Male	32	Ulcer foot	*Black Yeast*
	1	Male	15	Skin ulcer	*Fonsecaea dermatitidis*
	1	Male	12	Skin ulcer	*Xylohypha bantiana*
	1	Male	19	Nasopharynx	*Cladosporium carrionii*
2002	1	Male	60	Ulcer foot	*Fonsecaea pedrosoi*
	1	Female	56	Ulcer heel	*Fonsecaea pedrosoi*
	1	Male	48	Ulcer leg	*Cladosporium carrionii*
	1	Male	32	Ulcer (L)leg	*Cladosporium carrionii*
	1	Male	42	Ulcer (L)leg	*Cladosporium carrionii*
	1	Male	43	Warty plaques	*Fonsecaea pedrosoi*
	1	Male	35	Nodular ulcer	*Cladosporium carrionii*
	1	Male	34	Right foot	*Cladosporium carrionii*
2003	1	Male	25	Ulcer foot	*Fonsecaea pedrosoi*
	1	Male	36	Ulcer Knee	*Fonsecaea pedrosoi*
	1	Male	89	Ulcer hand	*Fonsecaea pedrosoi*
	1	Female	35	Ulcer right hand	*Fonsecaea pedrosoi*
	1	Male	60	Ulcer sole (L)	*Cladosporium carrionii*
	1	Male	37	Ulcer foot	*Cladosporium carrionii*
2004	1	Male	22	Ulcer foot	*Fonsecaea pedrosoi*
	1	Male	31	Ulcer chest	*Fonsecaea pedrosoi*
	1	Male	56	Ulcer foot	*Fonsecaea pedrosoi*
	1	Male	65	Ulcer ®leg	*Cladosporium carrionii*
	1	Female	39	Ulcer foot	*Cladosporium carrionii*
	1	Male	64	Ulcer foot	*Xylohypha. bantiana*
2005	1	Female	34	Knee Ulcer	*Fonsecaea pedrosoi*
	1	Male	57	Ulcer buttock	*Fonsecaea pedrosoi*
	1	Female	23	Ulcer hand	*Fonsecaea pedrosoi*
	1	Male	46	Ulcer foot	*Fonsecaea pedrosoi*
	1	Male	41	Ulcer leg	*Cladosporium carrionii*
	1	Male	50	Ulcer foot	*Cladosporium carrionii*

Nature of lesions in chromoblastomycosis and fungus isolated is shown in table 11

Table: 12

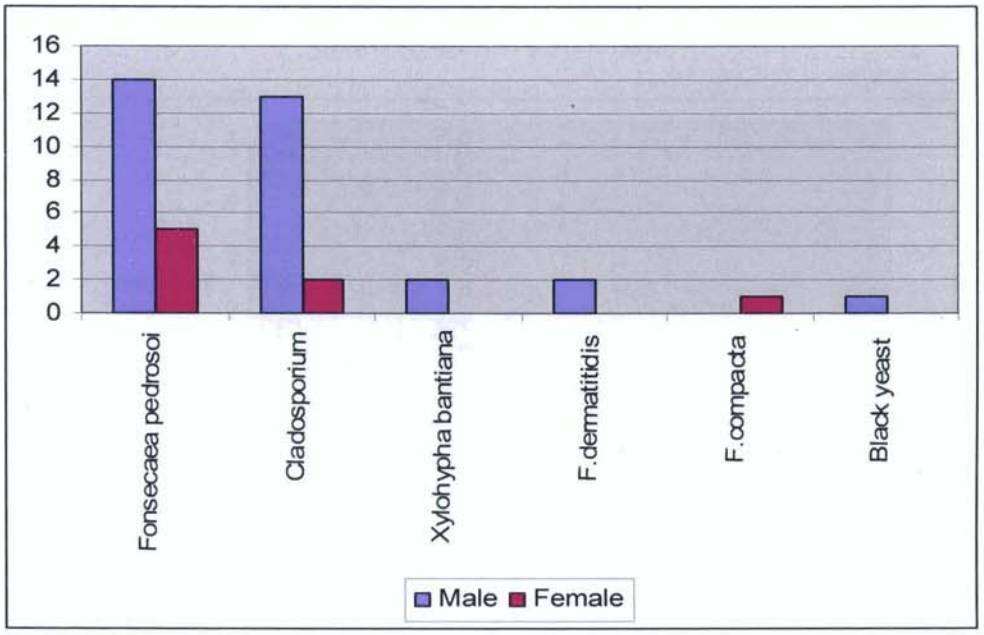
AGE GROUP, SEX & SPECIES WISE DISTRIBUTION IN CHROMOBLASTOMYCOSIS:

	Fonsecaea pedrosoi		*Cladosporium carrionii*		*F.dermatitidis*		*F.compacta*		*Black Yeast*		*Xylohypha*		Total	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0-10														
11'-20			1		1						1		3	
21-30	2	2											2	2
31-40	2	2	3	2				1	1				**6**	**5**
41-50	5		5										**10**	
51-60	4	1	3		1								**8**	**1**
61-70			1								1		2	
71-80														
81-90	1												1	
91-100														
	14	**5**	**13**	**2**	**2**			**1**	**1**		**1**		**32**	**8**

Species, age & sex distribution of chromoblastomycosis is shown in table 12. *Fonsecaea pedrosoi* and *Cladosporium carrionii* were the important fungal agents responsible for chromoblastomycosis. The age group distribution showed a highest incidence in the age group 31-40 (11 cases) followed by 41-50 (10 cases) and 51-60 (9 cases).

Fig: 18

SPECIES & NUMBER OF CASES IN CHROMOBLASTOMYCOSIS



Species, number of cases and sex distribution in chromoblastomycosis was shown in fig 18. *F.pedrosoi* and *Cladosporium carrionii* were the important isolates. The incidence of chromoblastomycosis was high in male populations except in one case of *F.compacta* which was isolated from a female patient.

Fig: 19

AGE & SEX DISTRIBUTION OF *FONSECAEA PEDROSOI* IN CHROMOBLASTOMYCOSIS

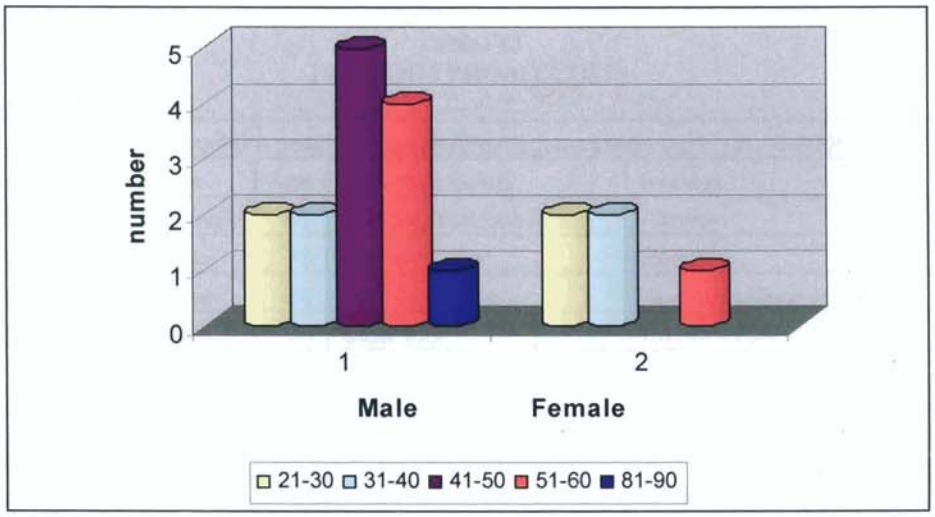


Fig 19 shows the age & sex distribution of F.pedrosoi in chromoblastomycosis. Highest incidence was noticed in male patients of age group 41-50 (5 number) followed by the age group 51-60 (4 number). In female highest incidence was noticed in the age group 21-30 & 31-40 (2 number each)

Fig: 20

AGE & SEX DISTRIBUTION OF *CLADOSPORIUM CARRIONII* IN CHROMOBLASTOMYCOSIS:

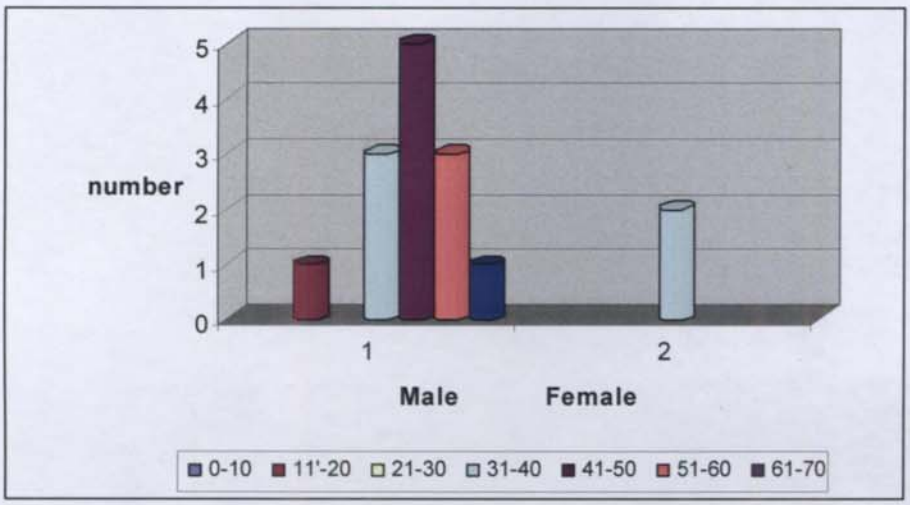


Fig 20 shows the age & sex distribution of *C.carrionii* in chromoblastomycosis. Highest incidence was noticed in male patients of age group 41-50 (5 number) followed by the age group 31-40 & 51-60 (3 number each). In female patients, highest incidence was noticed in the age group 31-40 (2 number)

Table: 13
PHAEOHYPHOMYCOSIS

Details of culture positive Phaeohyphomycosis cases during 2000-2005					
Year	No.	Sex	Age	Nature of lesion	Isolates
2001	1	Female	56	Vesiculo bulbous lesion	*Phoma*
2001	1	Male	33	Chronic Ulcer	*Phoma*
2002	1	Male	56	Warty plaques	*Aureobasidium pullulans*
2002	1	Male	36	Ulcer foot	*Cheatomium*
2003	1	Male	59	Nodular ulcer	*Curvularia*
2003	1	Female	50	Ulcer ® hand	*Ramichloridium mackenziei*
2004	1	Male	67	Ulcer leg	*Torula*
2004	1	Male	27	Ulcer foot	*Curvularia*
2004	1	Male	21	Ulcer (l) hand	*Aureobasidium pullulans*
2005	1	Female	52	Ulcer arm	*Ramichloridium mackenziei*

As shown in table 13, in phaeohyphomycosis, *Ramichloridium mackenziei, Curvularia, Phoma* and *Aureobasidium pullulans* species were isolated in two cases each.

Table: 14
HYALOHYPHOMYCOSIS

Details of culture positive Hyalohyphomycosis cases during 2000-2005					
Year	No.	Sex	Age	Nature of lesion	Isolate
2000	1	Male	32	Ulcer foot	*Acremonium*
2002	1	Female	43	Cysts	*Acremonium*
2003	1	Male	77	Nasopharynx	*Penicillium marneffei*
2003	1	Female	48	Ulcer neck	*Aspergillus terreus*
2004	1	Male	35	Left thumb	*Scedosporium apiospermum*
2004	1	Female	41	Elbow joint	*Streptomyces*

As shown in table 14, in hyalohyphomycosis *Acremonium* species was isolated in two cases.

Table: 15
MYCETOMA

Details of culture positive Mycetoma cases during 2000-2005					
Year	No.	Sex	Age	Nature of lesion	Isolate
2000	1	Female	82	Ulcer foot	*Scedosporium apiospermum*
2001	1	Male	43	Ulcer hand	*Acremonium*
2002	1	Female	66	Ulcer Forearm	*Scedosporium apiospermum*
2003	1	Male	38	Ulcer big toe(L)	*Streptomyces*
2005	1	Female	58	Ulcer foot	*Aureobasidium pullulans*
2005	1	Male	66	Ulcer buttock	*Acremonium*

As shown in table 15, in mycetoma *Scedosporium apiospermum* was isolated in two cases.

Table: 16
RHINOSPORIDIOSIS

Details of Rhinosporidiosis cases during 2000-2005				
Year	No.	Sex	Age	Site affected
2001	1	Male	24	Nasal cavity
2003	1	Male	58	Face and right eye
2004	1	Female	79	Nasal cavity

Table: 17

UNUSUAL SITE

Unusual sites for subcutaneous mycoses during 2000-2005					
Year	No.	Sex	Age	Site affected	Organism isolated
2001	1	Male	37	Skin upper arm	*Histoplasma*
2004	1	Female	67	Mandible	*Streptomyces*
2005	1	Male	17	Lip	*Phoma*

ANTIFUNGAL STUDIES OF TERBINAFINE SULPHATE AGAINST

FONSECAEA PEDROSOI & CLADOSPORIUM CARRIONII

Method : Agar dilution

Antifungal stock solution:

250 mg of drug in 5 ml
50 µg of drug in 1 µl

From stock
10 µl diluted in 1000µl
ie 1 µg of drug in 2 µl

Drug concentration starts from 2 µg/ml of the medium. Since SDA volume is 6 ml, 12µg/ml of drug is added.
ie for 12 µg/ml of drug 24µl of diluted drug solution is necessary

Table 18

Fonsecaea pedrosoi

No.of tube	Total amount of drug	Drug concentration	result
1 st tube	24µl of drug	2 µg/ml	Growth +
2 nd tube	48µl of drug	4µg/ml	Growth +
3 rd tube	96µl of drug	8µg/ml	No growth
4 th tube	192µl of drug	16µg/ml	No growth
5 th tube	384µl of drug	32µg/ml	No growth
6 th tube	768µl of drug	64µg/ml	No growth
Control tube	No drug	----	Growth present

RESULT: MIC of *Fonsecaea pedrosoi* is 8 µg/ml

Table 19

Cladosporium carrionii

No.of tube	Total amount of drug	Drug concentration	Result
1 st tube	24µl of drug	2 µg/ml	Growth +
2 nd tube	48µl of drug	4µg/ml	No Growth
3 rd tube	96µl of drug	8µg/ml	No growth
4 th tube	192µl of drug	16µg/ml	No growth
5 th tube	384µl of drug	32µg/ml	No growth
6 th tube	768µl of drug	64µg/ml	No growth
Control tube	No drug	----	Growth present

RESULT: MIC of *Cladosporium carrionii* is 4 µg/ml

Table 20

Number	Fungal isolate	MIC
1	*Fonsecaea pedrosoi*	8 µg/ml
2	*Fonsecaea pedrosoi*	4 µg/ml
3	*Fonsecaea pedrosoi*	2 µg/ml
4	*Fonsecaea pedrosoi*	8 µg/ml
5	*Fonsecaea pedrosoi*	4 µg/ml

MIC's of *Fonsecaea pedrosoi*

Table 21

Number	Fungal isolate	MIC
1	*Cladosporium carrionii*	8 µg/ml
2	*Cladosporium carrionii*	4 µg/ml
3	*Cladosporium carrionii*	2 µg/ml
4	*Cladosporium carrionii*	8 µg/ml
5	*Cladosporium carrionii*	4 µg/ml

MIC's of *Cladosporium carrionii*

Fig: 21

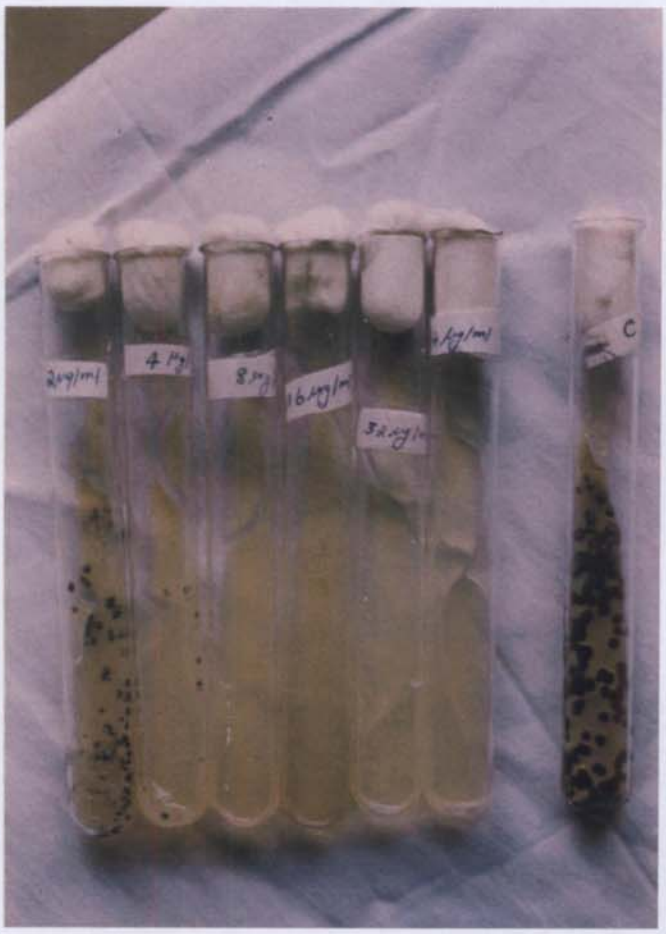
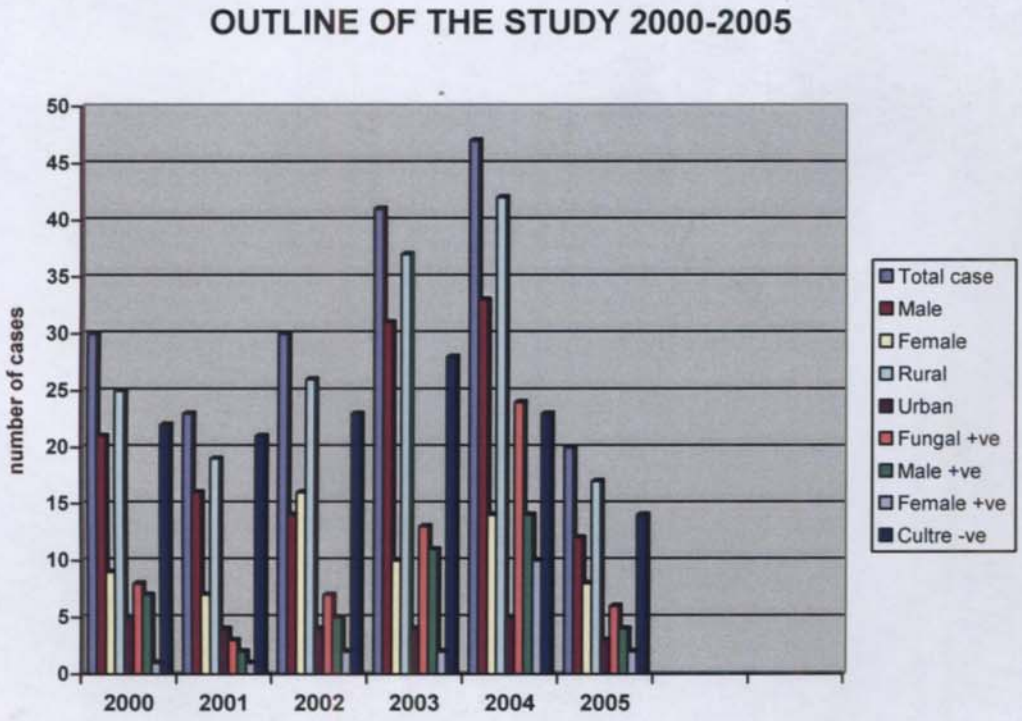


Table 22

OUTLINE OF THE STUDY 2000-2005

Year	2000	2001	2002	2003	2004	2005	Total
Total cases of fungal infection	1200	1150	1345	1340	1247	1215	7497
Male	620	595	692	690	644	612	3853
Female	580	555	653	650	603	603	3644
Total cases of subcutaneous mycosis	30	24	30	41	47	20	192
Male	21	16	20	31	33	12	133
Female	9	8	10	10	14	8	59
Rural	25	19	26	37	42	17	166
Urban	5	4	4	4	5	3	25
Fungal culture positive	8	7	7	13	24	6	65
Male	7	5	5	11	14	4	46
Female	1	2	2	2	10	2	19
Culture Negative	22	17	23	28	23	14	127

Fig: 22

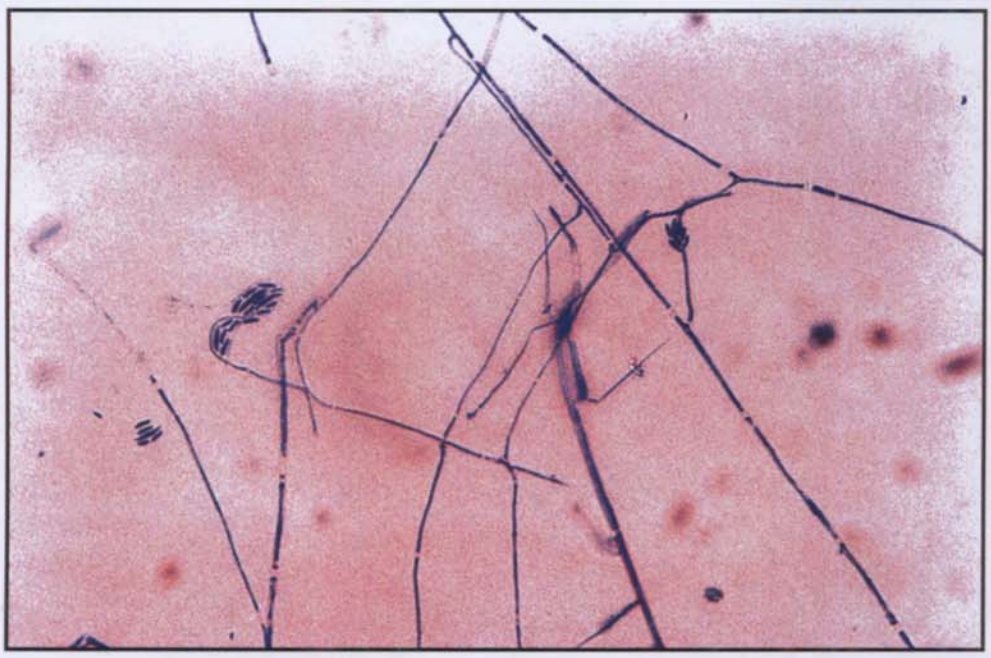


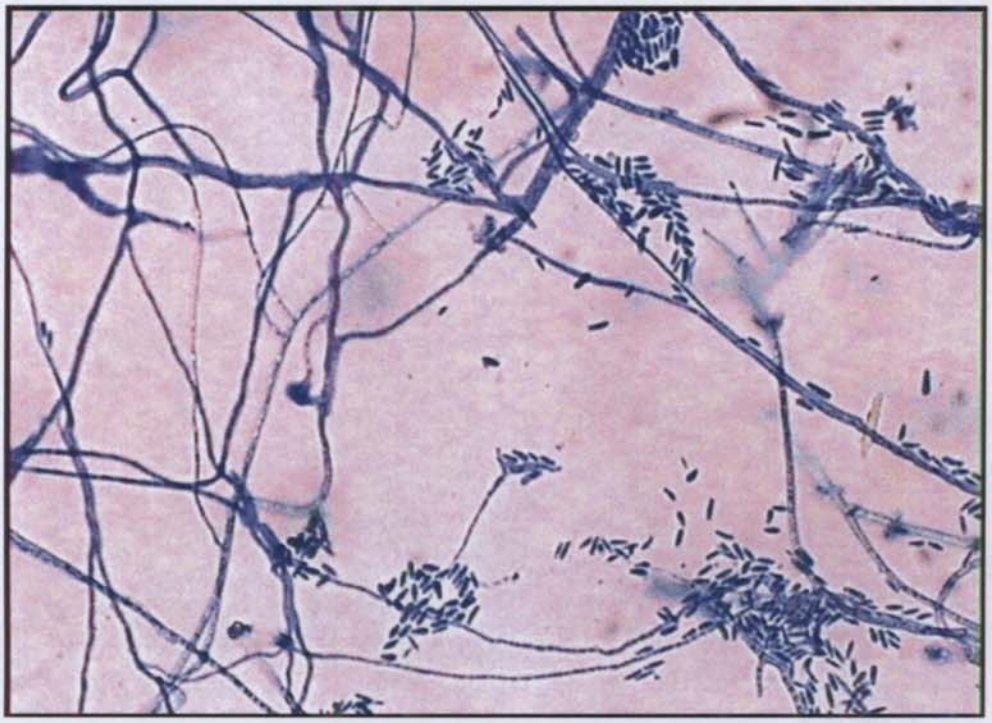
ACREMONIUM

Macroscopic morphology

The growth rate of *Acremonium* colonies is moderately rapid, maturing within 5 days. The diameter of the colony is 1-3 cm following incubation at 25°C for 7 days on potato glucose agar. The texture of the colony is compact, flat or folded, and occasionally raised in the center. It is glabrous, velvety, and membrane-like at the beginning. Powdery texture may also be observed. By aging, the surface of the colony may become cottony due to the overgrowth of loose hyphae. The color of the colony is white, pale grey or pale pink on the surface. The reverse side is either uncolored or a pink to rose colored pigment production is observed.

Microscopic morphology

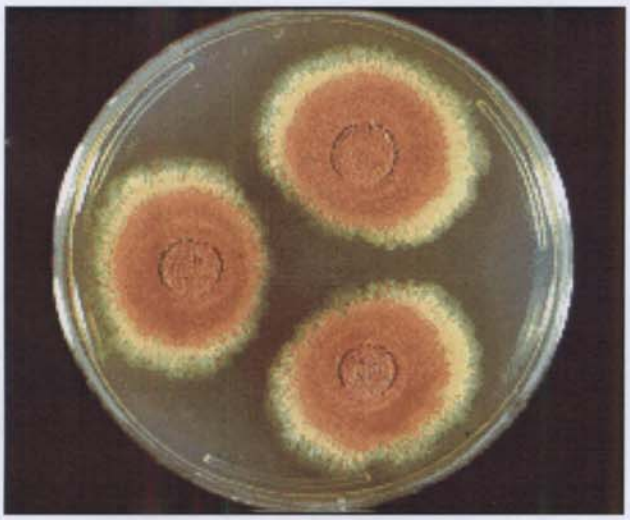




Acremonium spp. possess hyaline, septate hyphae which are typically very fine and narrow. Vegetative hyphae often form hyphal ropes. Unbranched, solitary, erect phialides are formed directly on the hyphal tips, the hyphal ropes, or both. The phialides are separated from hyphae by a septum and taper towards their apices. At the apices of the phialides are the hyaline conidia 2-3x4-8μm in size. They usually appear in clusters, in balls or rarely as fragile chains. The conidia are bound by a gelatinous material. They may be single or multicellular, fusiform with a slight curve or resemble a shallow crescent. These structural properties of conidia vary depending on the species. *Acremonium falciforme* usually produces crescentic, nonseptate conidia. Sometimes, 2- or 3-celled conidia may also be observed. *Acremonium kiliense*, on the other hand, has short straight conidia and the conidia of *Acremonium recifei* are usually crescentic and nonseptate.

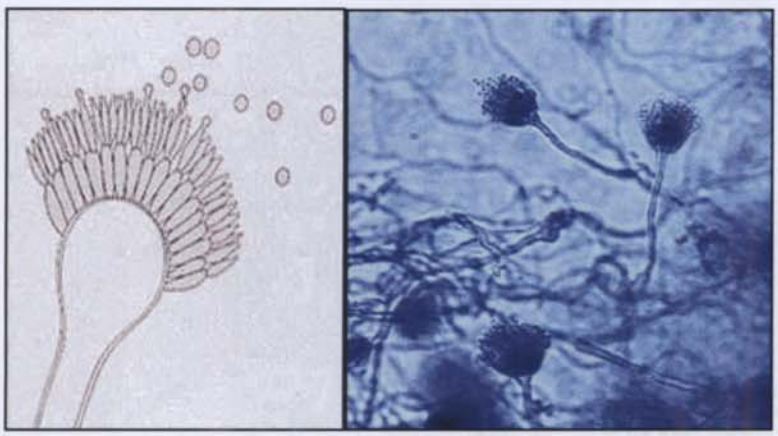
ASPERGILLUS TERREUS

Macroscopic morphology



On Czapek dox agar, colonies are typically suede-like and cinnamon-buff to sand brown in color with a yellow to deep dirty brown reverse.

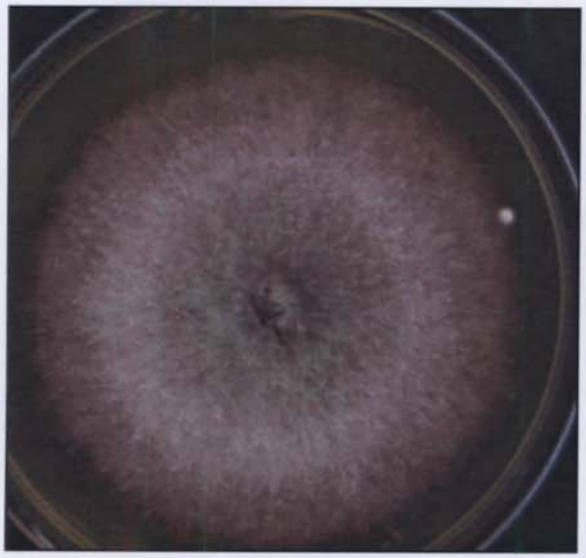
Microscopic morphology



Conidial heads are compact, columnar (up to 500 x 30-50 um in diameter) and biseriate. Conidiophores are hyaline,100-250μm long and 4.5-6μm wide, smooth walled, uncolored; vesicle dome shaped, 10-16 μm in diameter, phialides on upper half to two-third, head columber. Conidia are globose to ellipsoidal (1.5-2.5 um in diameter), hyaline to slightly yellow and smooth-walled.

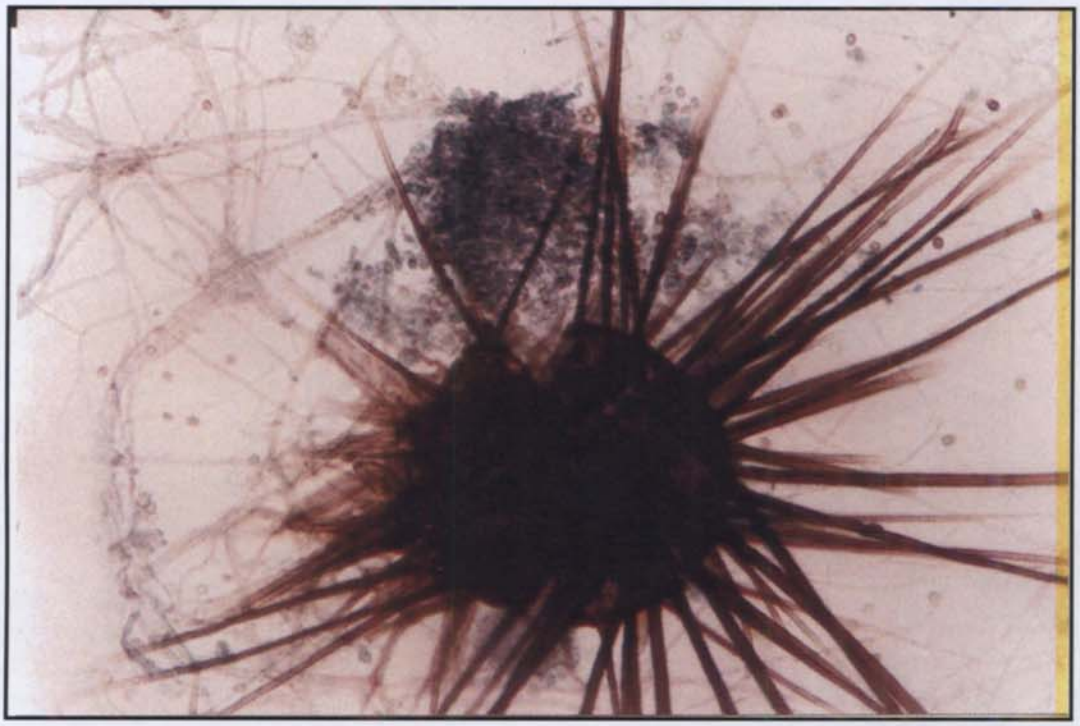
CHAETOMIUM

Macroscopic morphology



Chaetomium colonies are rapidly growing, cottony and white in color initially. Mature colonies become grey to olive in color. From the reverse, the color is tan to red or brown to black.

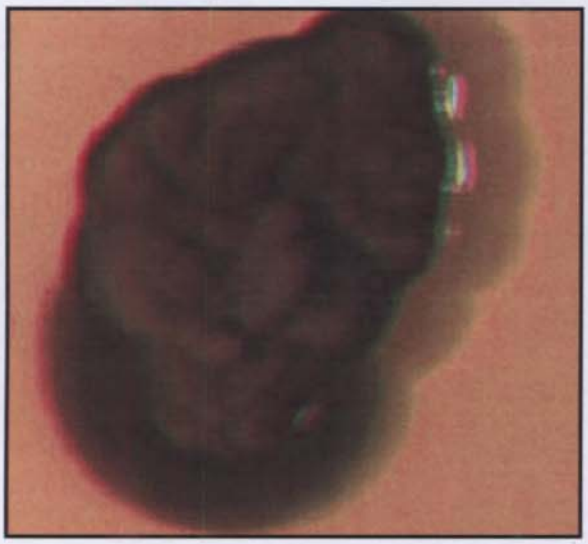
Microscopic morphology



Septate hyphae, perithecia, asci and ascospores are visualized. Perithecia are large, dark brown to black in color, fragile, globose to flask shaped and have filamentous, hair-like, brown to black appendages (setae) on their surface. Perithecia have ostioles (small rounded openings) and contain asci and ascospores inside. Asci are clavate to cylindrical in shape and rapidly dissolve to release their ascospores (4 to 8 in number). Ascospores are one-celled, olive brown in color, and lemon shaped. *Chaetomium strumarium* differs from other members of the genus chaetomium in forming ascocarps with pale thin walled flexuous hairs. Presence of pinkish exudates droplets/or crystals associated with hyphae or ascocarps; sometimes accompanied by a pinkish diffusible pigment; good growth at 42^0C; production of small conidia further distinguish this species.

XYLOHYPHA BANTIANA

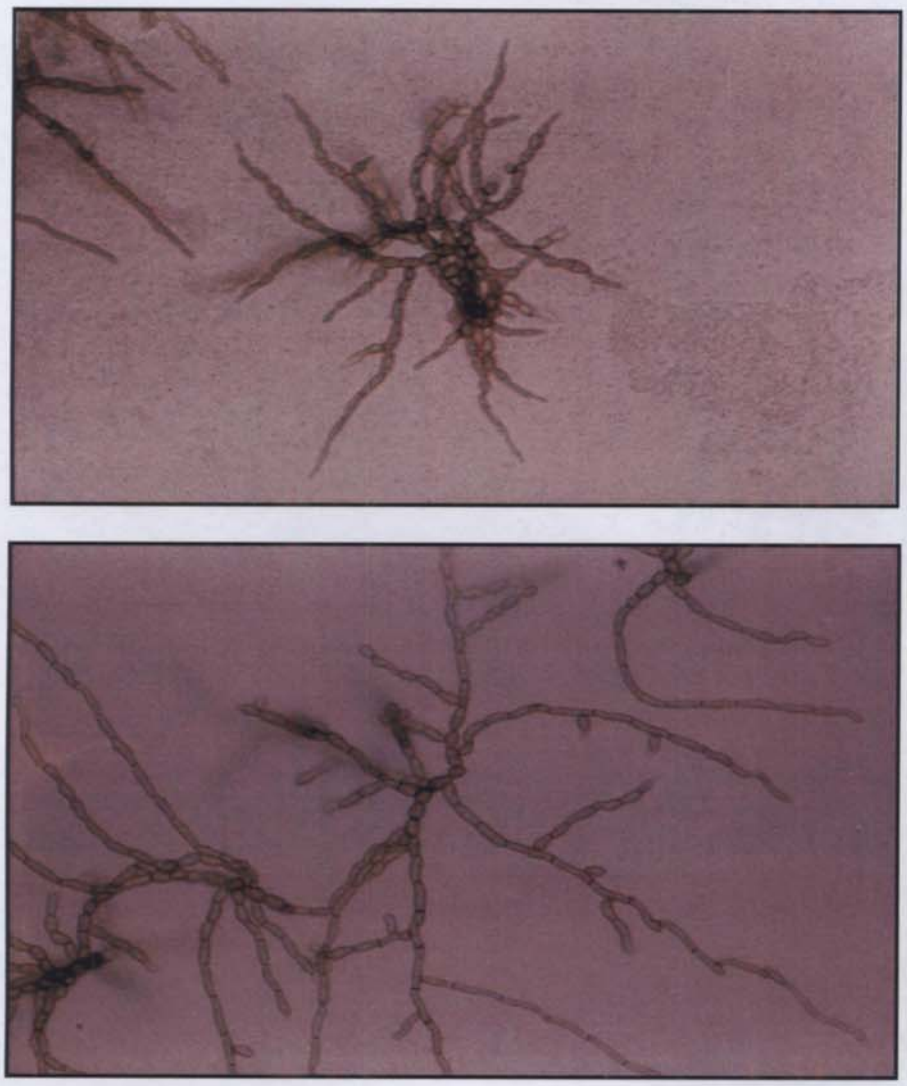
Macroscopic morphology



Colonies slow growing, moderately expanding, velvety to hairy, powery when sporulating abundantly, olivaceous gray to black; reverse olivaceous black. Conidiophores hyphalike, pale brown. Blastoconidia one celled, without evident darkly pigmented hila, occurring in long, sparsely branching chains that are poorly differentiated from conidiophore and vegetative hyphae. *X.bantiana* grows at 42-43 ^{o} C; conidia measures 2-2.5 by 4-7 μm

Colonies moderately expanding, velvety to hairy, powdery when sporulating abundantly, olivaceous green; reverse olivaceous black.

Microscopic morphology

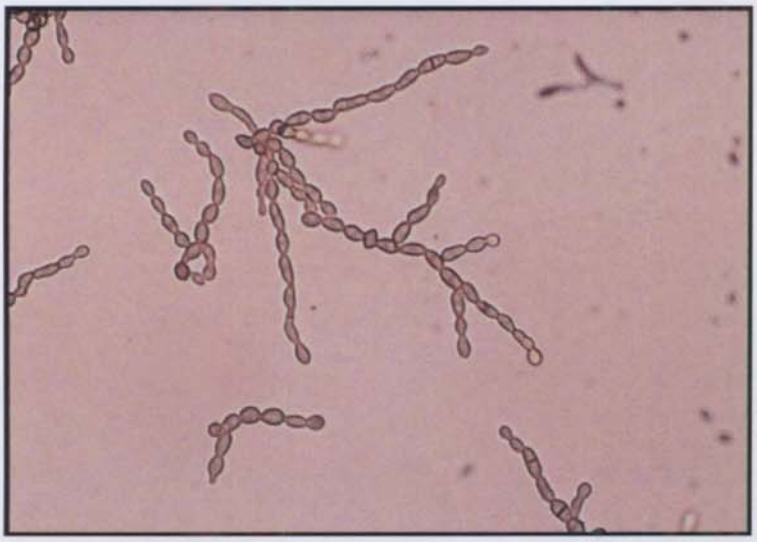


Conidia formed in long, strongly coherent, poorly branched, sessile, lateral or terminal chains on undifferentiated hyphae, pale olivaceous , ellipsoidal to fusiform or nearly cylindrical. Large chlamydospores occasionally present. *Cladophialophora bantiana* may be distinguished from *Cladosporium* species by the absence of conidia with distinctly pigmented hila, the absence of characteristic shield cells and by growth at 42^0C.Growth at 42^0C also differentiates it from *Cladophialophora carrionii* which has a maximum growth temperature of 35-36C.

CLADOSPORIUM CARRIONII

The growth rate of *Cladosporium* colonies is moderate on potato dextrose agar at 25°C and the texture is velvety to powdery. Similar to the other dematiaceous fungi, the color is olivaceous green to black from the front and black from the reverse. Most of the *Cladosporium* spp. do not grow at temperatures above 35°C.

Microscopic morphology



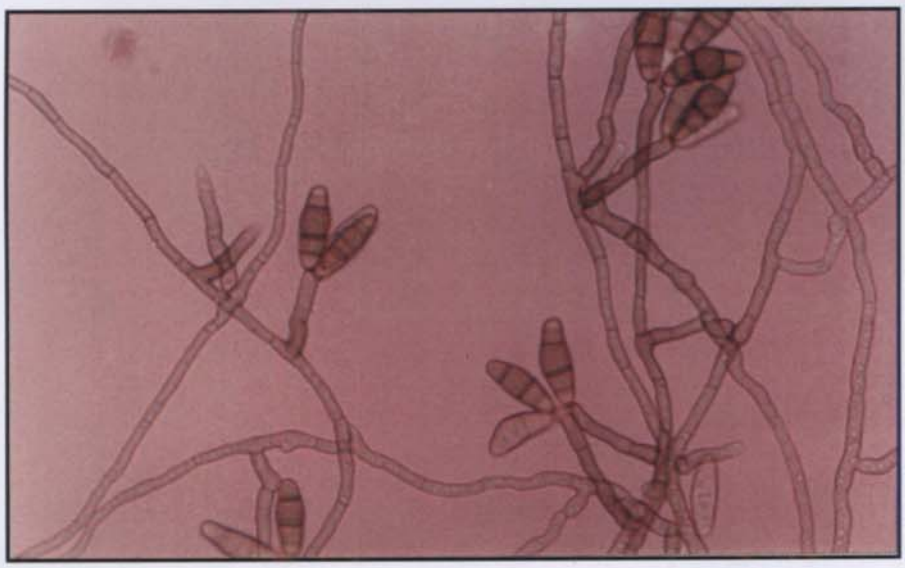
Cladosporium spp. produce septate brown hyphae, erect and pigmented conidiophores, and conidia. While the conidiophores of *Cladosporium cladosporioides* and *Cladosporium sphaerospermum* are not geniculate, those of *Cladosporium herbarum* have a geniculate appearance. In addition, conidiophores of *Cladosporium herbarum* bear terminal and intercalary swellings. Conidia of *Cladosporium* spp. in general are elliptical to cylindrical in shape, pale to dark brown in color and have dark hila. They occur in branching chains that readily disarticulate. Conidial wall is smooth or occasionally echinulate. *Cladosporium cladosporioides* produces unicellular conidia. On the other hand, those of *Cladosporium herbarum* are two- to four-celled. *Cladosporium sphaerospermum* produces elongate and septate shield cells which are also known as ramoconidia.

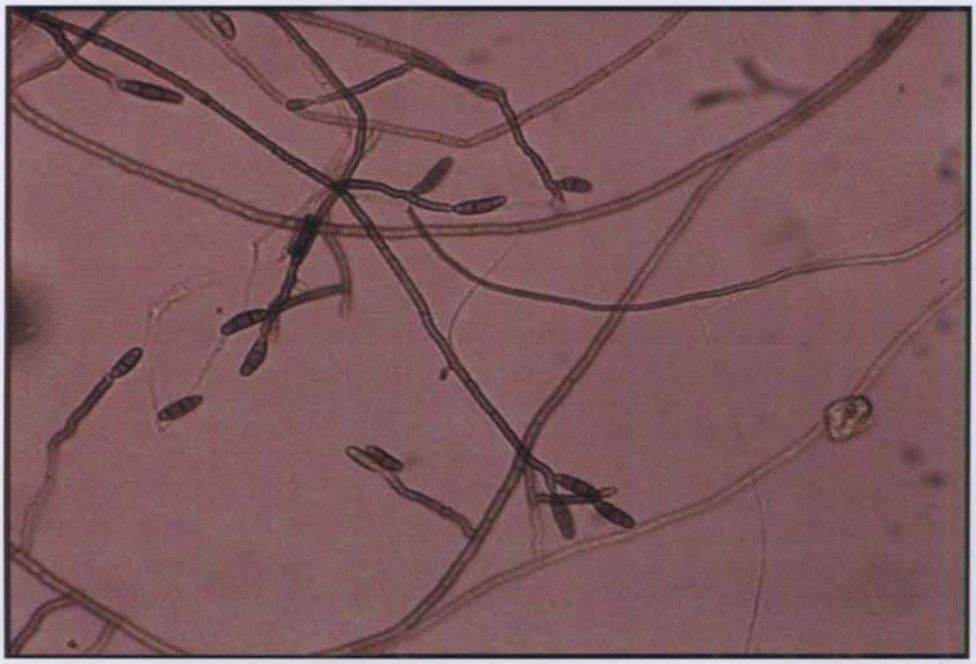
CURVULARIA

Macroscopic morphology

Curvularia produces rapidly growing, woolly colonies on potato dextrose agar at 25°C. From the front, the color of the colony is white to pinkish gray initially and turns to olive brown or black as the colony matures. From the reverse, it is dark brown to black.

Microscopic morphology





Septate, brown hyphae, brown conidiophores, and conidia are visualized. Conidiophores are simple or branched and are bent at the points where the conidia originate. This bending pattern is called sympodial geniculate growth. The conidia (8-14 x 21-35 μm), which are also called the poroconidia, are straight or pyriform, brown, multiseptate, and have dark basal protuberant hila. The septa are transverse and divide each conidium into multiple cells. The central cell is typically darker and enlarged compared to the end cells in the conidium. The central septum may also appear darker than the others. The swelling of the central cell usually gives the conidium a curved appearance. The number of the septa in the conidia, the shape of the conidia (straight or curved), the color of the conidia (dark vs pale brown), existence of dark median septum, and the prominence of geniculate growth pattern are the major microscopic features that help in differentiation of *Curvularia* spp. among each other. For instance, the conidia of *Curvularia lunata* have 3 septa and 4 cells, while those of *Curvularia geniculata* mostly have 4 septa and five cells.

FONSECAEA PEDROSOI

Macroscopic morphology

This species grows very slowly on Sabouraud's glucose agar, producing a heaped, brittle colony, dark brown to black in color. Colonies restricted, velvety to cottony. The reverse is normally jet black.

Microscopic morphology

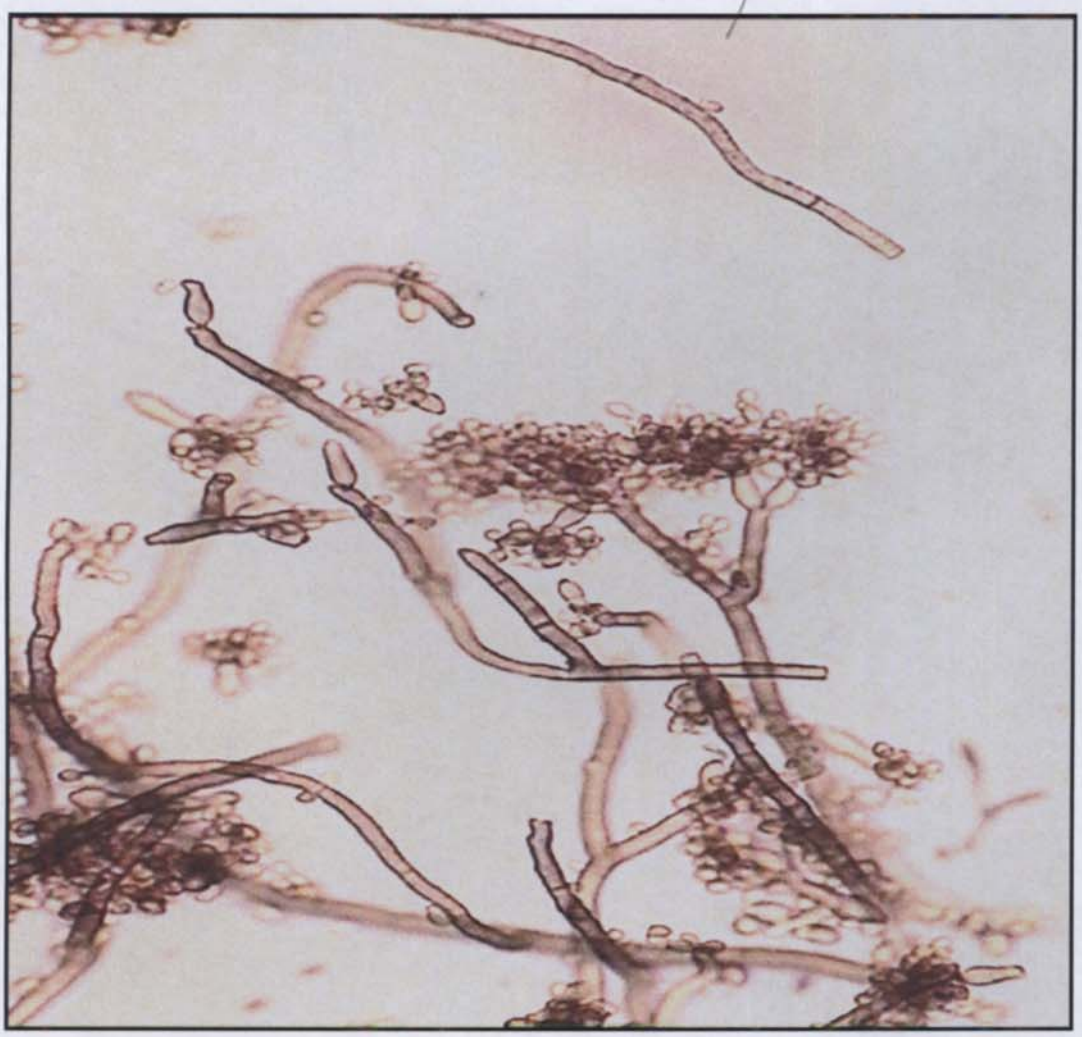
Conidiogenous cells pale olivaceous, in loosely branched systems, cylindrical, intercalary or terminal with clusters of prominent denticles .Conidia pale olivaceous, in short chains, sub hyaline, smooth and thin walled, clavate 3.5-5x1.5-2 μm ,locally ampulliform, dark olivaceous brown phialides with deep, funnel shaped collarettes may be present. yeast cells are produced at low pH.

FONSECAEA COMPACTA

Macroscopic morphology

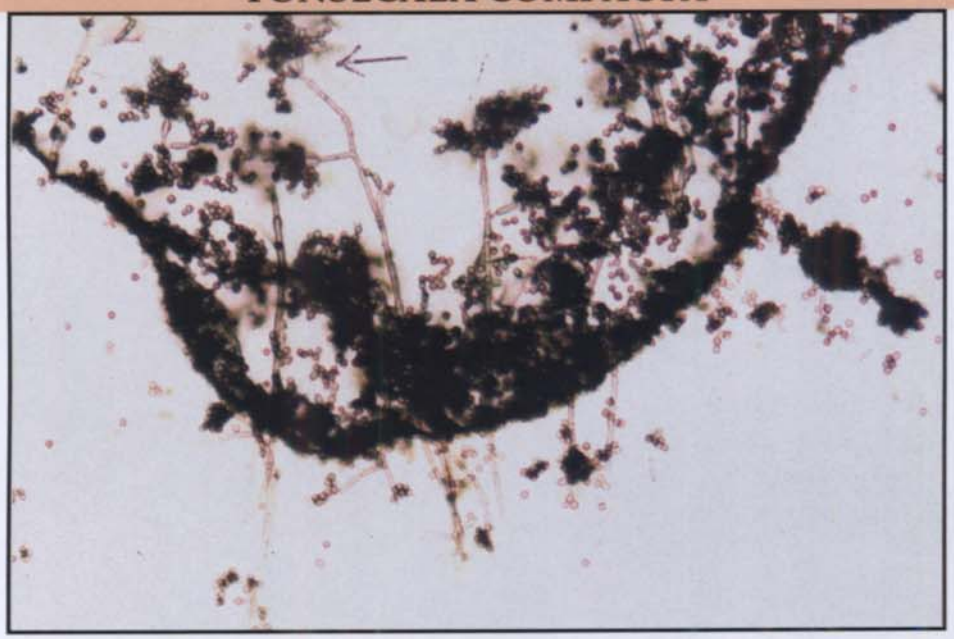
The colony grows slowly as folded, heaped, brittle colony with an irregular indented border. The surface becomes covered with a silver to gray velvety mycelium. The velvety mycelium contains the spores. Colonies often resembles those of *F.pedrosoi*, so differentiation on the basis of gross morphology is often not possible

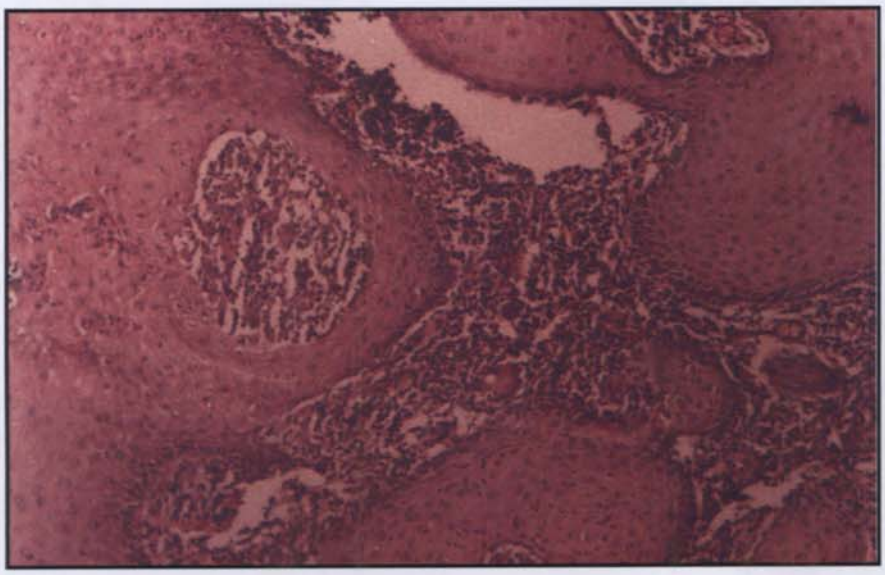
Microscopic morphology



FONSECAEA COMPACTA

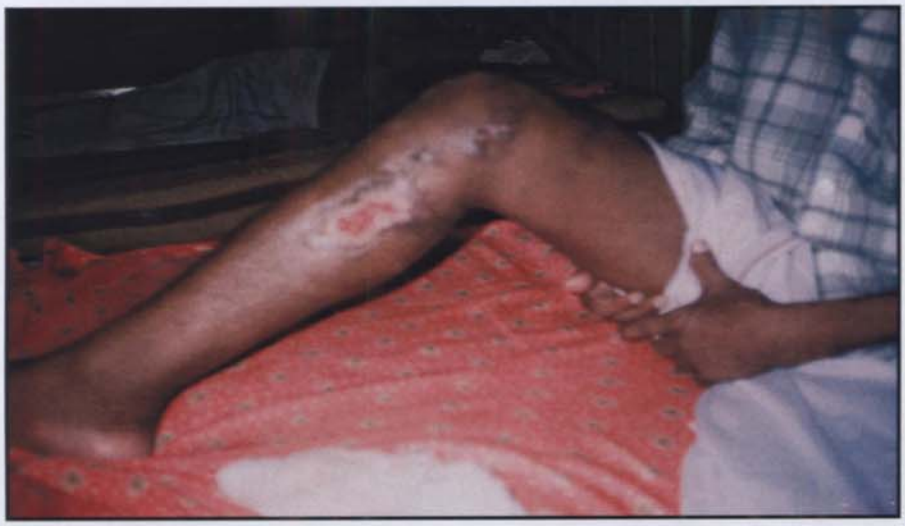
FONSECAEA COMPACTA



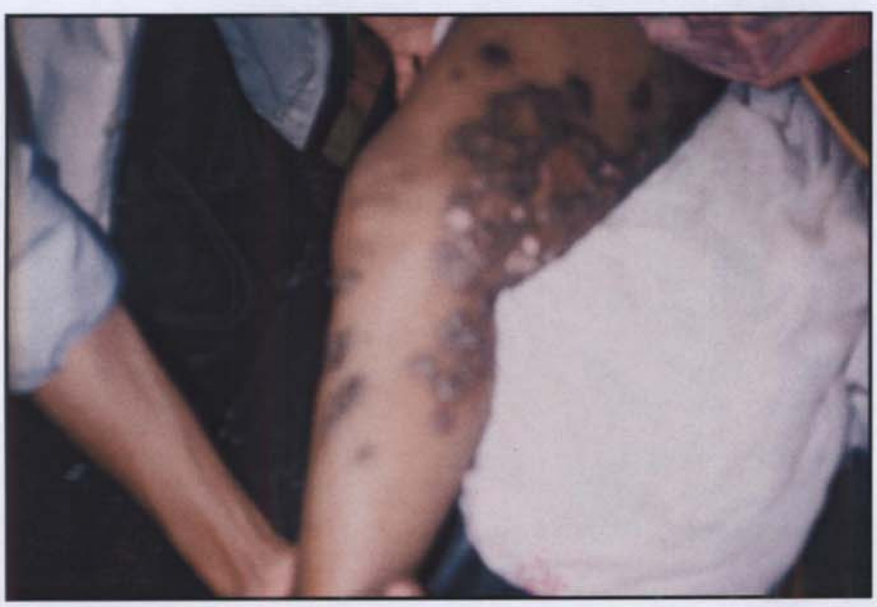


Characteristic tissue reaction in chromoblastomycosis

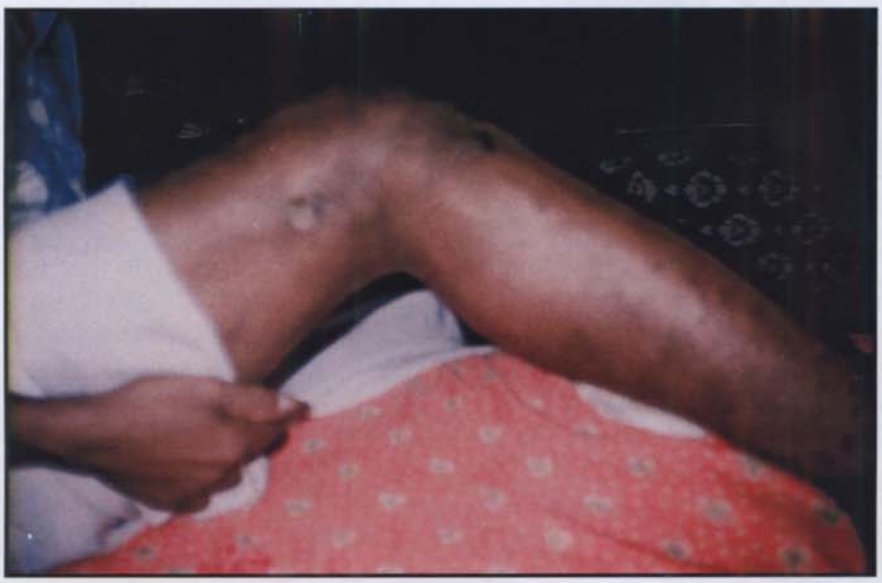
Tissue form of the fungus- Sclerotic/muriform body



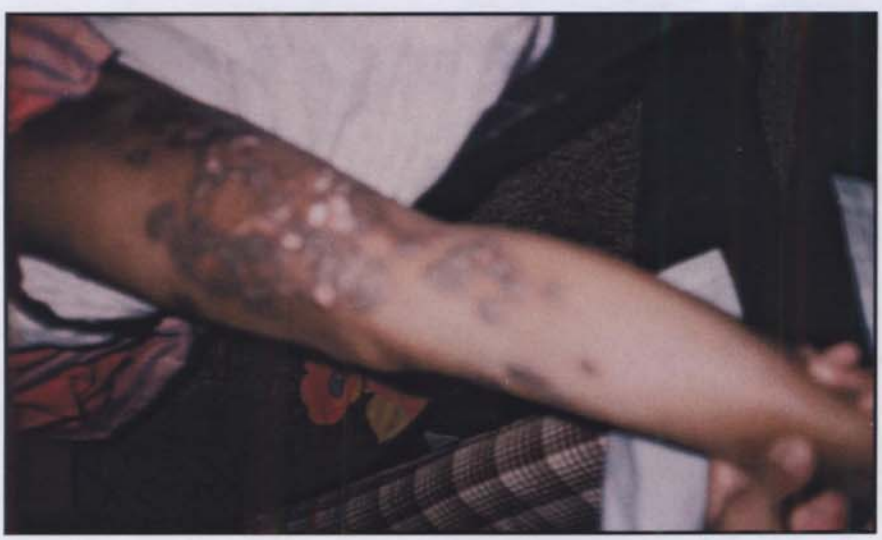
Chromoblastomycosis of leg



Cauliflower-like lesions in chromoblastomycosis



Verrucous nodular lesions in Chromoblastomycosis



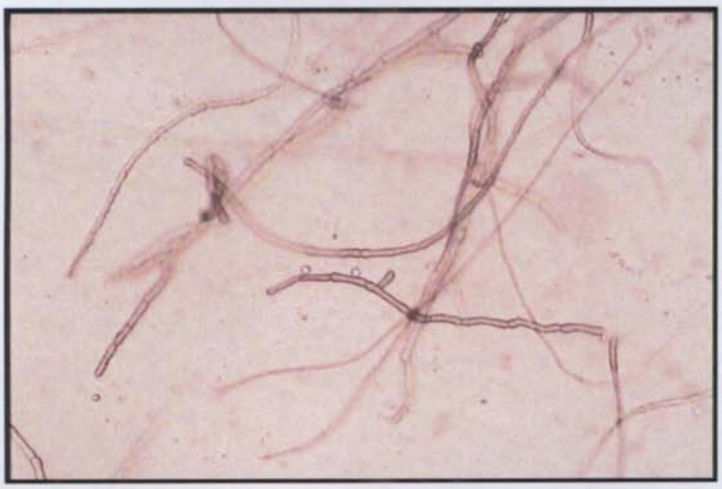
Chromoblastomycosis of upper arm

AUREOBASIDIUM PULLULANS

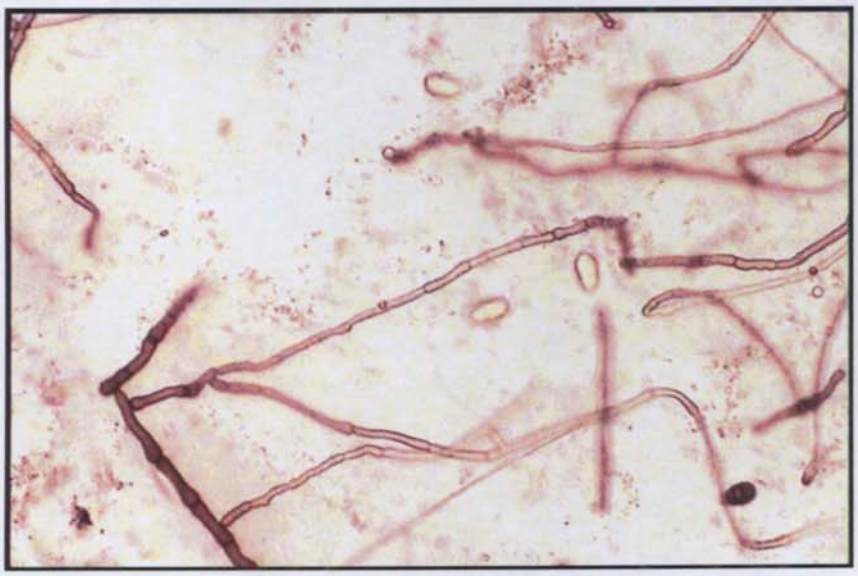
Macroscopic morphology

Colonies of *Aureobasidium* grow moderately rapid growing fungus matures to a black, leathery colony that is heaped and wrinkled with a grayish fringe of submerged mycelium. The initial isolants often appear as a white to pink yeast, turning to black. Hyphae not extensive, becoming multiseptate, with thick walls containing pigment. .

Microscopic morphology



Two types of hyphae develop. These are large, thick walled, black, cells that form the bulk of the mycelium and that may bud off several small pyriform conidia. Also thin walled , delicate hyphae that produce elliptical conidia from the hyphal walls may appear throughout the preparation.

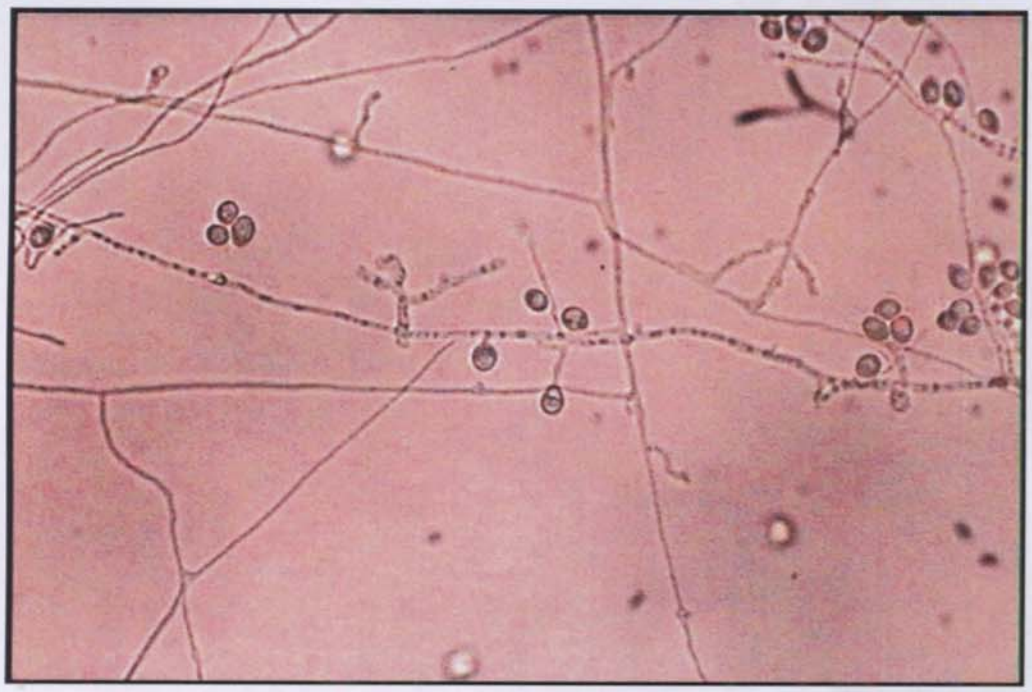


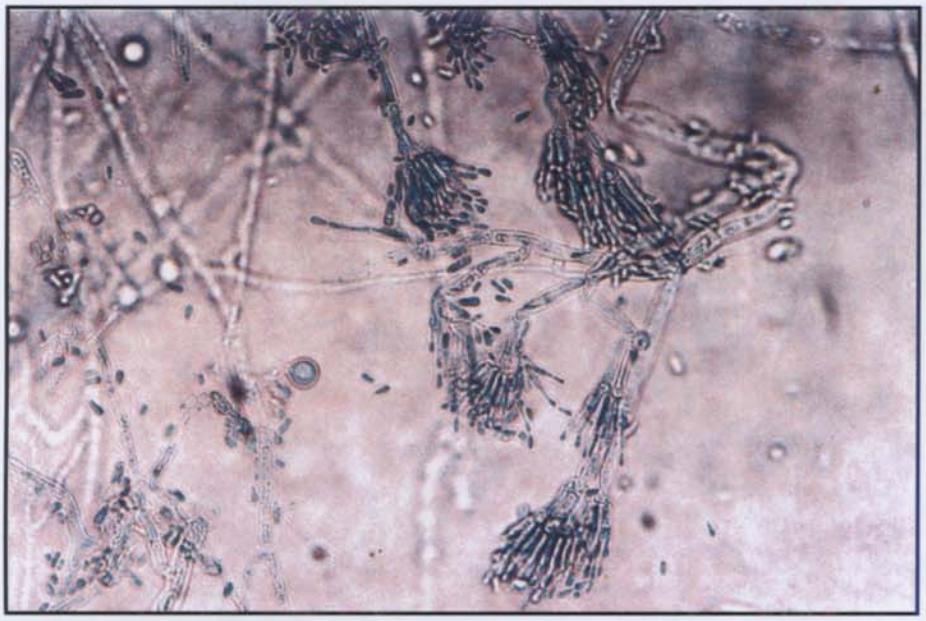
SCEDOSPORIUM APIOSPERMUM

Macroscopic morphology

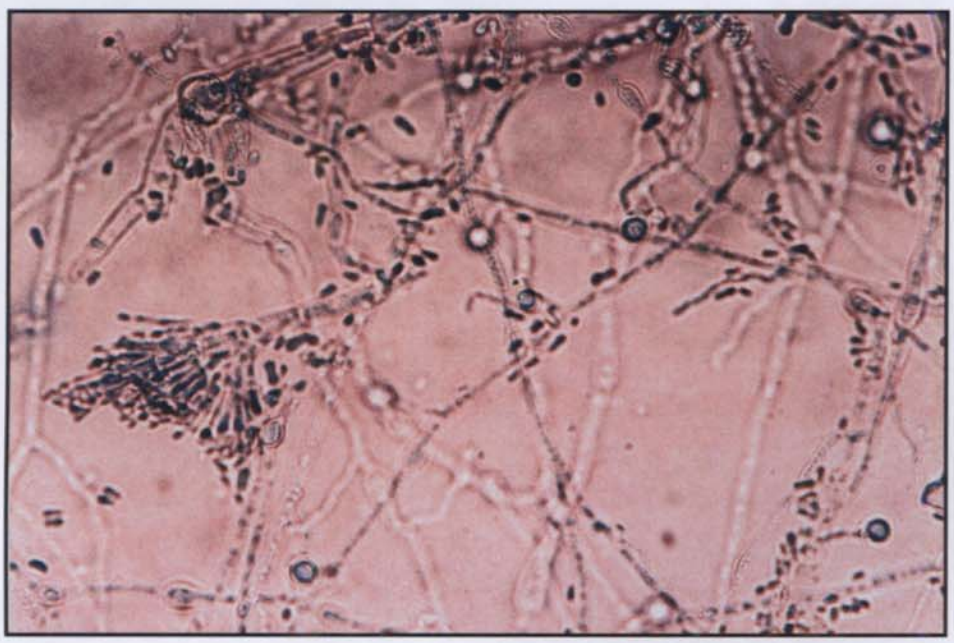
Grows rapidly on all laboratory media. There is an abundance of fluffy or tufted aerial mycelium, which is at first white but becomes brownish "house mouse" gray. The reverse of the colony shows areas of gray to black pigmentation. Rare strains are ivory colored and membranous, others are annular in growth, showing concentric rings, and some colonies are a rich tan or cinnamon brown and even pink.

Microscopic morphology





Elongate synnemata formation of the Graphium type conidiation



The hyphae are hyaline and 1 to 3 μm in diameter. Large pyriform annelloconidia are lemon shaped, born singly or in small groups on elongate, simple, or branched annellosphores, or laterally on hyphae. Tufts of conidiophores forming a synnemata are also found. Each annellophore ends in a single conidium.

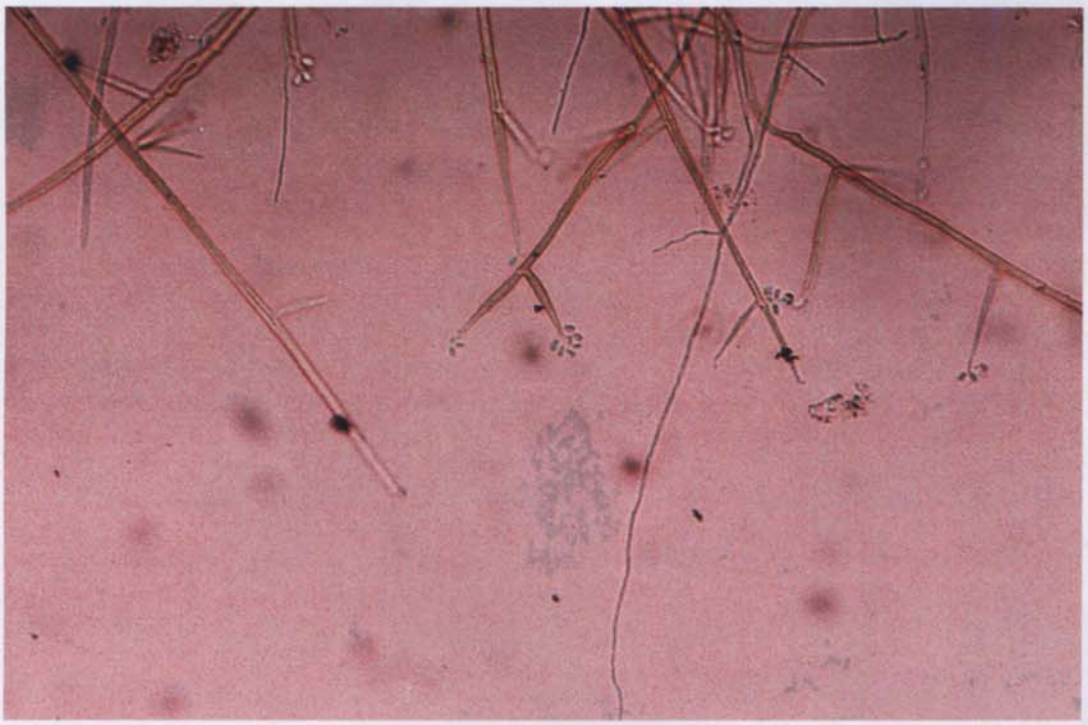
RAMICHLORIDIUM MACKENZIEI

Macroscopic morphology

Colonies rapidly growing, golden orange on protein medium; brownish- gray on cornmeal. The mycelial strands from biopsy specimen were golden

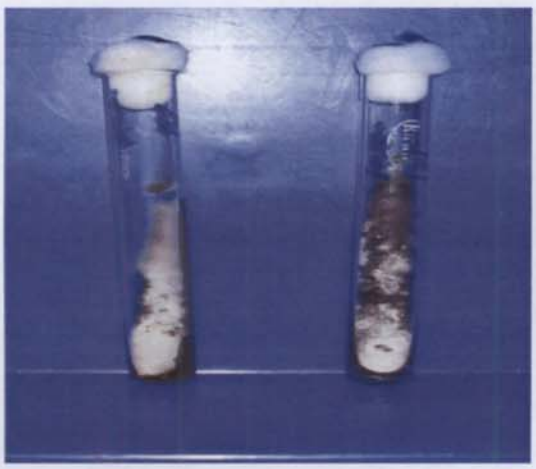
Microscopic morphology

Septate hyphae, conidiophores pale brown to midbrown, erect, sympodial, with denticles and elliptical.



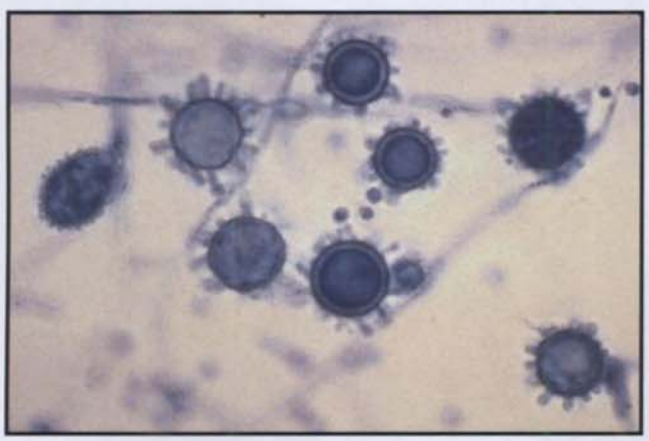
HISTOPLASMA CAPSULATUM

Macroscopic morphology

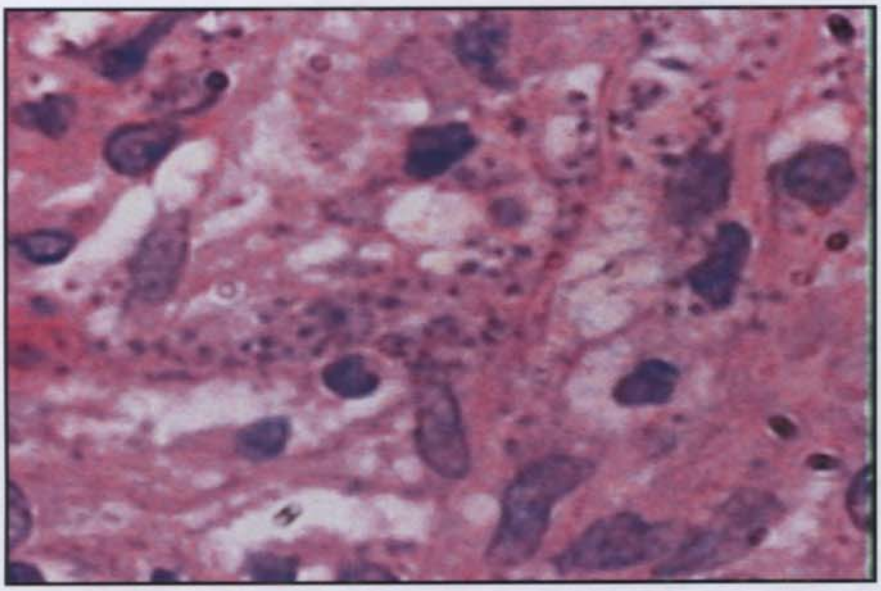


Colonies (SDA, 24^0C) expanding, granular to cottony, initially white, later becoming brownish. Reverse initially cream-coloured, becoming brownish with age.

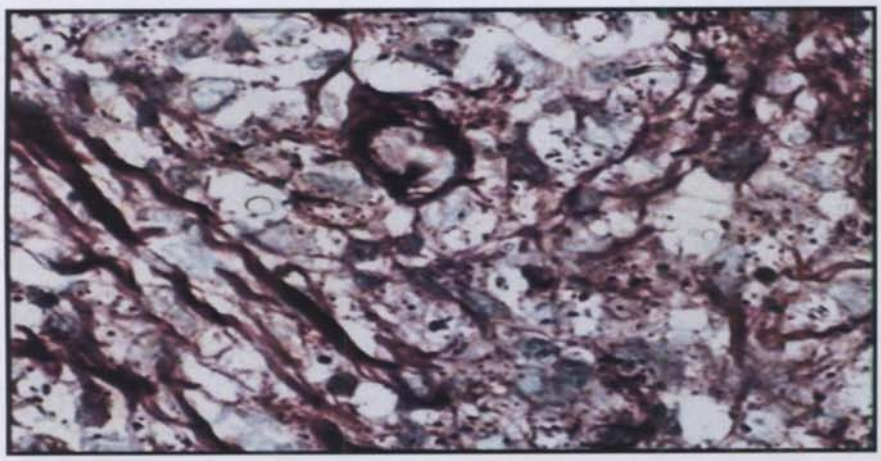
Microscopic morphology



Micro-as well as macro-conidia are produced. Microconidia (sub)hyline , sessile or arising on short stalks from undifferentiated hyphae , hyaline, smooth and thin-walled, 1-celled , pyriform to clavate, 1-4 x 2-6 µm. Chlamydospore-like macroconidia, brown, arising from short conidiophores, thick – walled, tuberculate or with cylindrical projections, 1-celled, spherical, 8-14 µm diam. At 37^0 C on BHI, a yeast-phase with budding cells up to 7 µm is formed.



Histoplasmosis: skin biopsy, H & E Stain



Histoplasmosis: skin biopsy, Methenamine Silver Stain

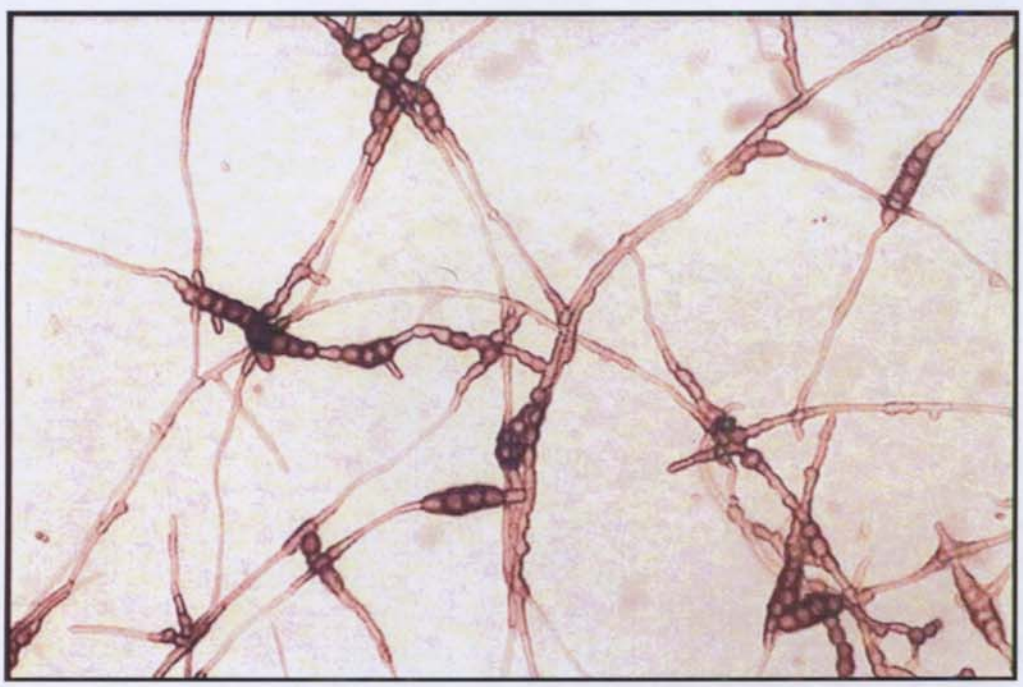
TORULA

Macroscopic morphology

Restricted shiny or hairy black colony. Mycelium sparse, may be whitish at first

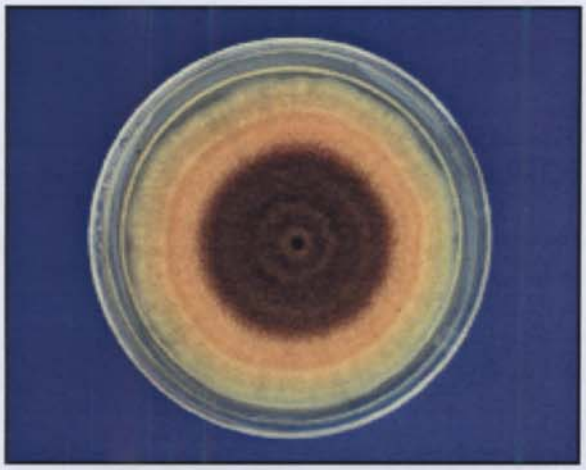
Microscopic morphology

Conidiophores are lacking or are short bulbous pegs. Conidia are produce directly on vegetative hyphae or on short, sometimes bulbous, conidiophores. Conidia sometimes form blastocatenulate chains with youngest at top.



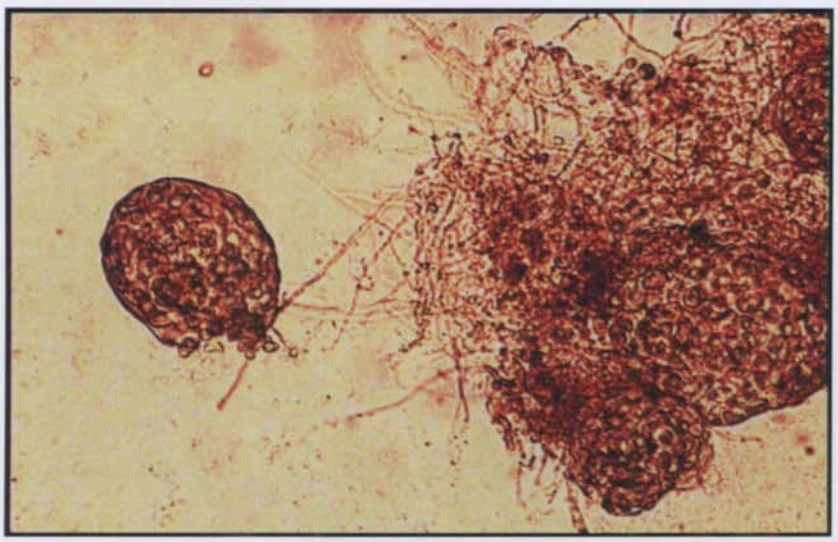
PHOMA

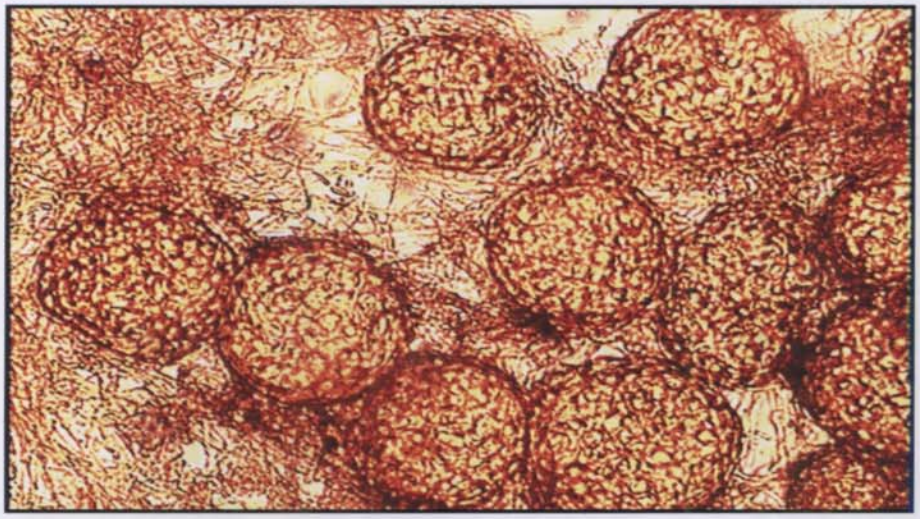
Macroscopic morphology

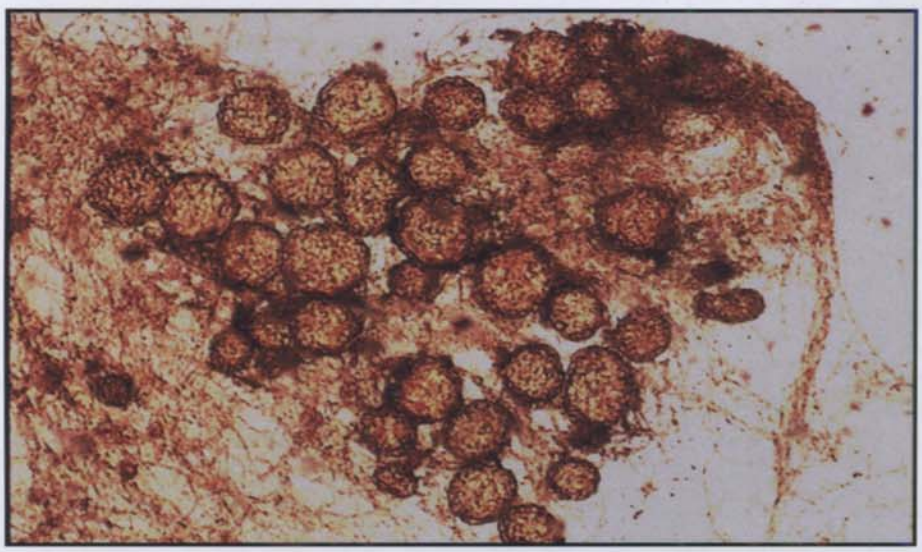


Colonies of *Phoma* grow rapidly. They are flat, spreading, powdery to velvety, and often largely submerged in the medium. From the front, the color is initially white and later becomes olive grey with an occasional tint of pink. From the reverse, it is dark brown to black. Some species (particularly, *Phoma cruris-hominis* and *Phoma herbarum*) produce a reddish-purple to yellowish-brown diffusable pigment which is readily visible from the reverse.

Microscopic morphology



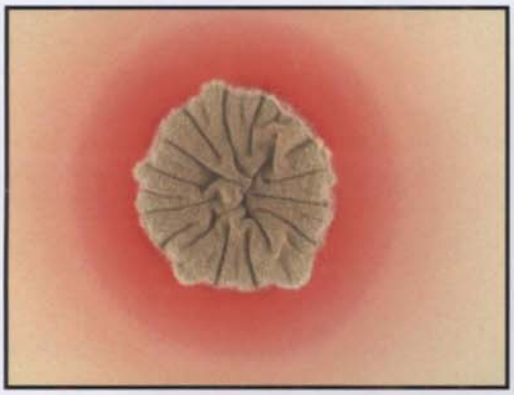




Septate hyphae, pycnidia, conidia, and chlamydospores (for some species only) are visualized. The hyphae are hyaline to brown. Pycnidia are the large, round to pyriform, asexual fruiting bodies which are 70-100 μm in diameter. They are dark in color and bear phialides at their inner lining. Pycnidia have one to several openings (ostioles) on their surface from which the conidia are released outside. Conidia are unicellular, hyaline, and oval-shaped. Each conidium typically has two oil droplets inside. Some *Phoma* species produce brown chlamydospores that are arranged singly or in chains. These chlamydospores may be unicellular or multicellular and "alternarioid" (resembling *Alternaria*) in appearance.

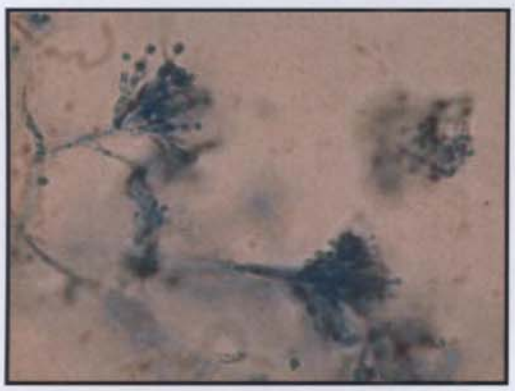
PENICILLIUM MARNEFFEI

Macroscopic morphology

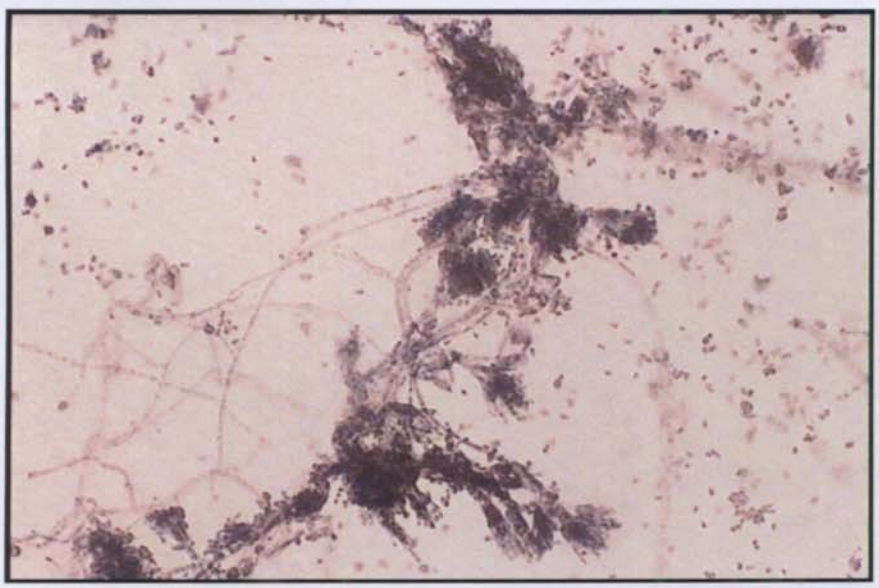


On Sabouraud's dextrose agar at 25C, colonies are fast growing, suede-like to downy, white with yellowish-green conidial heads. Colonies become greyish-pink to brown with age and produce a diffusible brownish-red to wine red-pigment.

Microscopic morphology



Conidiophores are hyaline, smooth walled and bear terminal verticils of 3-5 metulae, each bearing 3-7 phialides. Conidia are globose to subglobose, 2-3μm in diameter, smooth walled and are produce in basipetal succession from phialides. At 37^0C, yeast-cells are spherical to ellipsoidal, 2 to 6 um in diameter, and divide by fission rather than budding. Numerous short hyphal elements are also present.

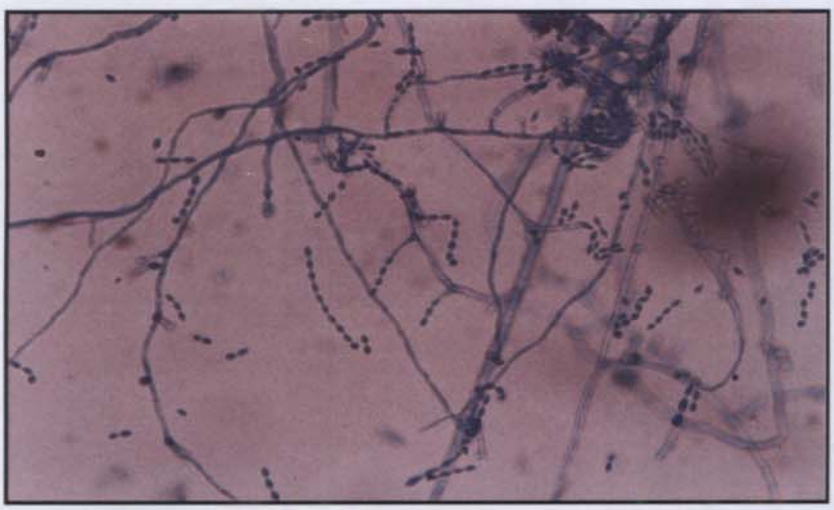


STREPTOMYCES

Macroscopic morphology

Colonies are slow growing, aerobic, Gram-positive, nonacid-fast, glabrous or chalky, heaped and folded, and white, tan, gray, brown, or black in color.

Microscopic morphology



Isolates of *Streptomyces* are frequently recognized by their chalky appearance and earthy odour. Colonies often have an earthy odor. Filaments are extensively branched; aerial filaments are abundant and usually produce long chains of spores formed by fragmentation of the filaments.

STUDY ON
THE PREVALENCE OF SUBCUTANEOUS MYCOSES IN NORTH KERALA

Thesis submitted
in partial fulfilment of the requirements
for the award of the degree of

DOCTOR OF PHILOSOPHY

IN MICROBIOLOGY (MEDICAL FACULTY)

BY

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AUGUST- 2006

DISCUSSION

Subcutaneous mycoses consist of a heterogeneous group of infections caused by a broad spectrum of taxonomically diverse environmental organisms. The infections are predominantly tropical or subtropical and occur sporadically, except in the case of sporotrichosis, wherein clustering of cases may occur suggesting exposure to a common source. The fungi reach the subcutaneous tissue, usually through a penetrating injury, where they remain in localized micro-environmental niches associated with abscess formation. The tissue damage is variable and the immune system recognizes the fungi. The focus of infection in subcutaneous mycoses is dermis and sub cutis and lesions seldom spread to distal sites or metastasize. However, they may spread to contiguous structures. The spectrum of infection includes mycetoma, sporotrichosis, chromoblastomycosis and rhinosporidiosis. Less commonly, infections like hyalohyphomycosis, phaeohyphomycosis, subcutaneous zygomycosis, lobomycosis etc, may also be encountered. These infections are seldom lethal, but they can cause considerable morbidity. Although uncommon, primary subcutaneous infections without dissemination caused by systemic pathogens like *Blastomyces dermatitidis* and *Histoplasma capsulatum* are on record. These infections are world wide in distribution and the incidence varies geographically.

Although subcutaneous mycoses are widespread around the world, it is seen most often in those countries where workmen are exposed to thorn or splinter puncture wound while working without the protection of shoes. These subcutaneous mycoses are characterized by the development of lesions at the site of inoculation. The prevalence of subcutaneous mycoses varies considerably with geographic location and climatic conditions. India is a large sub continent with remarkably varied topography. It is situated within the tropical and sub tropical belts, which incidentally are the regions with a high prevalence of mycotic infections.

The present study was undertaken to determine the prevalence of subcutaneous mycosis in North Kerala and also the important fungal species responsible for the subcutaneous mycoses.

The study group consisted of a total of 161542 patients with various types of skin infections who attended Dermatology, Surgery and ENT departments of Calicut Medical College Hospital for a period of five years from January 2005 to January 2005. In the above study group a total of 7497 patients (4.64%) with different types of fungal infections were found. The important skin diseases other than fungal infections in this area are Henson disease of various types, chronic dermatitis, psoriasis, lichen planus, Discoid lupus erythematosus and lupus vulgaris of descending order. Even though high incidences of various skin infections were prevalent in this area (96%), the diseases due to fungal agents are very low (4.64%). Of the 7497 cases of fungal infections, 3853 (51.4%) were males and 3644 were females (48.6 %), Male to female ratio was 1.05: 1. In male patients, a high incidence of infection was noticed in the age group of 21-30 (21.59%) followed by 11-20 (17.54%), 31-40 (15.57%), 41-50 (12.89%), 51-60 (12.30%), 0-10 (9.16%), 61-70(7.39%), 71-80(2.46%) and 91-100(0.20%). Of the 3644 female patients, high incidence was seen in the age group 11-20(23.73%) followed by 21-30 (19.12%), 31-40 (16.13%), 41-50 (12.45%), 51-60 (11.55%), 0-10(8.17%), 61-70(6.61%), 71-80(2.85%) and 81-90(0.21%). In both sex groups 21-30 and 11-20 were shown to have a higher incidence for fungal infection.

In the present study, of the 7497 cases of fungal infections, 192 cases of clinically diagnosed subcutaneous mycoses were identified. The incidence of subcutaneous mycoses was higher in male patients. Among the 192 patients of subcutaneous mycoses, 132 (69 %) patients were males and 60 (31%) were females.

Male to female ratio was 2.4:1. The increased incidence in male patients in India is probably due to the greater outdoor exposure of men, being the sole earning members of the family, often found it difficult to leave their work to seek medical advice and treatment. The review of Indian literature by Sharma N.L etal 2000 on chromoblastomycosis had found male to female ratio 6.2:1. In 2002 Sharma N.L etal reviewed subcutaneous phaeohyphomycosis in India and found male to female ratio 1.3:1.

A high incidence of fungal infections were noticed in the age group 31-40 (39 numbers) followed by the age group 41-50 (37 numbers), 21-30 and 51-60 (33 numbers), 61-70 (26 numbers), 11-20 (15 numbers) and 81-90 and 0-10 (2 numbers each). The incidence of subcutaneous mycoses was high in male patients in the age group 41-50 (27 number) followed by the age group 51-60 (26), 31-40 (25), 61-70 (21), 21-30 (19), 11-20(09), 71-80 (04) and 81-90 (01). Of the 60 female patients, the incidence was high in the age group 31-41 (14) and 21-30 (14), followed by the age group 41-50 (10), 51-60 (07), 11-20(06), 61-70 (05), 0-10 (02), 71-80 (1) and 81-90 (1). In the present study the age of the patients ranged from 12 to 89, this is very similar to Indian literature, where the age of the patients ranged from 12 to 80 (Sharma N.L etal 2000). In the present study 186 patients (87.5%) were belonged in the age group 20-70.

Of the 192 cases (2.6 %) of subcutaneous mycoses the incidence were high in the rural populations *ie* 166 cases (86.54%) and in the urban population it was 25 cases (13.02%). The reasons for high incidence of subcutaneous mycoses in rural populations of both males and females are that they were involved in various types of agricultural work and also they did not use protective measures to prevent wounds and scratches during their agricultural works. In India 70% of the population lives in

rural areas, engaged either fully or partly in agriculture. Since in most of these places the requisite expertise and facilities to make a proper diagnosis are lacking, many patients are left undiagnosed or misdiagnosed. Similar to reports from other parts of the world, (Hollick G.E 1993. & Rippon J.W, 1982) most of the patients here were aged between 20 and 70 years old and, being the sole earning member of the family, often found it difficult to leave their work to seek medical advice and treatment in a center situated far away from their home.

The statistics obtained from clinics may not exactly reflect the natural incidence of subcutaneous mycoses in the population at large. Actually the incidence of subcutaneous mycoses is much higher because the symptoms produced by these infections are trivial and all patients with the disease may not seek medical advice. They usually come for medical advice, only when the lesions are troublesome. Similar to reports from other parts of the world (Vollum D, 1977 & Rippon J.W, 1982) most of the patients were between 20 and 50 years age and being the sole earning members of the family, often found it difficult to leave their work to seek medical advice and treatment.

In this study, among 192 cases of clinically diagnosed subcutaneous mycoses, 65 cases (34%) were culture positive and 127 cases (66%) were culture negative. Repeated biopsies were taken from culture negative patients. The reasons for a negative culture were probably 1) samples may not be taken from the actual site. 2) Slow growing nature of some of the fungi or their inability to grow on artificial culture medium. All the above patients were well responded to antifungal treatment.

Out of the 65 culture proven cases, 52 (80%) isolates belonged to dematiaceous group of fungi and 19 (20%) were of non- dematiaceous fungi. In the present study, dematiaceous fungi were the predominant fungal pathogens isolated

from subcutaneous mycoses. In a similar study by Sharma N.L, 2000, the fungal isolations were made in 24 cases (72%). *F.pedrosoi* was isolated in eight cases, *F.compacta* in five, *Phialophora verrucosa* in three, and *Cladosporium carrionii* in five. In the present series, 65 strains were isolated, 52 were dematiaceous fungi. *Fonsecaea* species were isolated from a total of 22 (44.8 %) cases which includes *F. pedrosoi* in 19 (38.8 %), *F. dermatitidis* in two (4.1 %) and *F.compacta* in one case (2%) respectively. *Cladosporium* species was isolated in 15 (30.6 %) cases. *Aureobasidium pullulans* and *Phoma spp* were isolated in three cases each (6.1%). *Xylohypha bantiana, Ramichloridium* and *Curvularia* spp were isolated in two cases each (4.1%). *Torula, Black yeast*, and *Cheatomium spp* were isolated in one cases each. The distribution of *F.pedrosoi* isolated in different age group showed a highest incidence in the age group 41-50 and 51-60 (5 isolations each). In *Cladosporium carrionii,* highest incidence was noticed in the age group 31-40 and 41-50 (5 isolations each).

Non dematiaceous fungi constituted 13 (20%) of the total 65 fungal isolates. They include *Acremonium* in four cases, *Scedosporium apiospermum* and *Streptomyces* in three cases. *Penicillium marneffei, Aspergillus terreus* and *Histoplasma were* isolated in one case each. The distribution of *Acremonium* isolated in different age group showed a highest incidence in the age group 41-50.

The age and sex distribution of 65 culture positive patients showed an increased incidence of infection in the age group 51-60 (16 number) followed by 41-50 (15 number), 31-40 (12 number), 21-30(10 number), 61-7-(5 number), 11-20(4 number) and one each at the age groups of 0-10, 71-80, and 81-90. In male patients, high incidences was noticed at an age group of 41-50 (13 number) followed by 51-60(11 number), 31-40 (9 number), 21-30 and 61-70 (four each), 11-20 (3 number)

and one each in 71-80 and 81-90. In female patients high incidence was noticed in the age group of 21-30 (6 numbers) followed by 51-60 (5 numbers), 31-40 (3 numbers), 41-50 (2 numbers) and one each in the age group of 1-10, 11-20 and 61-70. The male female ratio of Fonsecaea *pedrosoi* was 2.8:1 and *Cladosporium carrionii* was 6.5:1.

Out of the 192 clinically diagnosed cases of subcutaneous mycoses, 82 (56%) were positive by KOH and histopathologically. 65 fungal isolates were obtained from 192 cases. The percentage of isolation was 44%. In the present study, four fungal isolations were obtained with out histopathological evidence. Repeated isolation of fungus from the site and response of patient with antifungal therapy confirmed the diagnosis. In a study by Nand Lal Sharma etal 1999, three cases of chromoblastomycosis were identified without the evidence of histopathology and the fungus was isolated repeatedly by culture (Aikat, B.k etal 1973, Venkataramiah, N.R 1973 and Mukerjee, 1975).

Among the 192 subcutaneous mycoses 50 cases of chromoblastomycosis (26%), 10 cases of phaeohyphomycosis (5.2%), 8 cases of hyalohyphomycosis (4.1%), 6 cases of mycetoma (3.1%), 3 cases of unusual site infections (1.5%) and 3 cases of rhinosporidiosis (1.5%) were obtained. Among the 40 cases of chromoblastomycosis highest isolations were obtained in the age group 31-40 (11 isolates),followed by the age group 41-50 (10 isolates) and 51-60 (9 isolates). In phaeohyphomycosis 51-60 age group showed highest isolation (3 isolates). In hyalohyphomycosis highest isolations was obtained in the age group 41-50 (3 isolates) and all the three patient were females

Among the 192 patients with subcutaneous mycosis, 50 patients were histologically diagnosed as chromoblastomycosis (26%). The histopathologic examination showed a hyperkeratitic epidermis with small neutrophilic abscesses and

tubercule formation in the dermis composed of epithelioid cells and multinucleated giant cells. Within the abscess sclerotic bodies of chromoblastomycosis were seen. Cultures were positive in 40 cases (80 %) and sclerotic bodies were seen in 50 cases by direct KOH smear and in histopathology sections. The fungi isolated in this study were those that represent the major and adequately documented agents of chromoblastomycosis. *Fonsecaea pedrosoi* was isolated in 19 (38 %) of the 50 cases which is by far the most common species responsible for chromoblastomycosis. The next common species was *Cladosporium carrionii*, in 15 (30 %) of 50 cases, followed by *F.dermatitidis* and X.*bantiana* in two cases (4%) each. *F.compacta* and, *Black yeast* were isolated in one each (2%).

In all patients routine clinical investigations were within normal limit in all cases, except for an elevated erythrocyte sedimentation rate. Of the 50 histologically diagnosed chromoblastomycosis the onset of disease before the age of 20 was seen in 3 cases. In the present study 47 patients were involved in agriculture (21%). The age of the patients ranged from 12 to 80 years with male to female ratio 4:1. Culture was positive in 40 (80 %) cases and sclerotic bodies were observed in 50 cases. A relatively higher prevalence (38%) of *Fonsecaea pedrosoi* was observed. Lower extremities were involved in 27 cases (54 %) and were localized in one limb. Involvement of upper arm and extremities were observed in 8 cases (16 %). In 3 cases (6 %) of skin ulcers and one case (2 %) of ulcer buttock and ulcer chest were identified.

A review of 36 cases of chromoblastomycosis in India by Nand Lal Sharma *etal*, 1999 found that the duration of disease at the time of presentation varies from a few months to years. Except for one mineworker, two housewives, and two students, all these patients were involved in agriculture directly or indirectly. The lesions were

localized to one limb in 21 cases. Two distant sites were involved in two cases and disseminated disease was seen in four cases. The site affected were the lower limb 22 cases (66%), upper limb (7 cases) face (4 cases), and trunk (3 cases). The vulva and penile shaft were involved in one case each. Lesion were mostly asymptomatic, except for seven cases that experienced moderate to severe pruritus. Extracutaneous spread was seen in eight patients involving the regional lymph nodes, pleural cavity, oropharynx, trachea, ileocecal region, and probably bones and respiratory parenchyma. Except for the last two sites, the fungus was demonstrated in all of these organs either by culture (four cases) or by histopathology (three cases). This is in contrast with the study from Venezuela where the trunk is the most commonly involved site (Rippon J.W, 1988). No extra cutaneous spread was detected in this study and it was very low in chromoblastomycosis (Fukushiro R 1983), however 24 % extra cutaneous involvement was reported from Indian literature (Rippon J.W, 1988). Overlapping infection with another fungus, *Geotrichum candidum*, was also seen in one case. The lower extremities are believed to be involved commonly in developing countries (Rippon J.W 1988) but rarely so in developed countries. Though the incidence is not very high, the disease is not uncommon. So far 36 cases have been reported (Thomas E *etal* 1957) from this country between 1957 and 1997. The Indian cases were last reviewed in 1967 by Mohapatra *etal*..

Out of 36 cases, 19 were from the sub- Himalayan belt, eight from the western coast, and nine from the eastern coast. All these areas have warm and humid conditions, while the central and northwestern arid zones of the country seem to be free of the disease. The age of the patients ranged from 12 to 80 years. The average age was 42 years (male, 44 years; female, 34 years). The onset of disease before the age of 15 years was seen in two cases, between 15 and 20 years in six cases, between

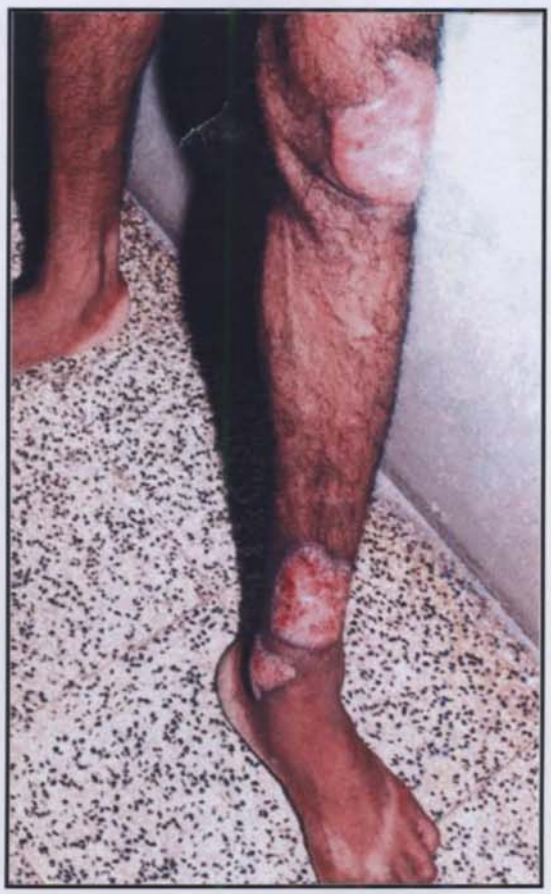
20 and 50 years in 19 cases and above 50 years in six cases. There were five females in the series giving a male to female ratio of 6.2:1. Sclerotic bodies were seen in 28 cases, either in direct KOH smears or in histopathology sections. In three cases, in which they were not seen, the fungus was isolated by culture. The fungus was grown in 26 (72%) patients: *Fonsecaea pedrosoi* in ten cases, *Fonsecaea compacta* in five, *Phialophora verrucosa* in three, *Cladosporium carrioni* in five; the species was not mentioned in three cases (Phialophora in one and *Cladosporium* in two). From the sub Himalayan belt, *Fonsecaea pedrosoi* and *Cladosporium* were isolated in four cases each and *Fonsecaea compacta* and *Phialophora* in two cases each. From the western coast, *Fonsecaea compacta* as well as *Fonsecaea pedrosoi* were seen in two cases each. The eastern coast showed *Cladosporium* in three cases, *Fonsecaea pedrosoi* and *Phialophora* in two cases each, and *Fonsecaea compacta* in one case. In two patients, bone lesions were observed; (osteomyelitis of the tibia (Battu V *etal* 1986) and a defect in the olecranon process (Verma K.C *etal* 1977).

During the study period, three unusual presentations of chromoblastomycosis were also noticed.

Case 1

In the present study, two cases of cutaneous chromoblastomycosis due to unusual fungus, *Xylohypha bantiana*, which is known mainly for cerebral chromoblastomycosis, was identified. A 53 year old male rubber tapper presented with multiple reddish plaques on the left lower limb of 45 years duration. The lesion first started on the left knee as a small papule which gradually increased in size. Another lesion on the left lower leg started 25 years after the initial one. Five years later he developed a similar lesion on the ankle. The patient had no history of trauma except for the ankle lesion. He ignored the lesions for a long period due to lack of

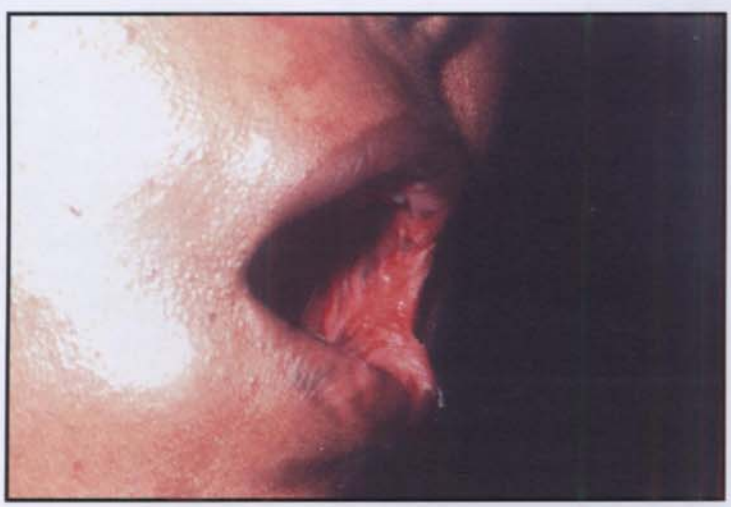
response to previous medications. The lesion consisted of well defined erythematous verrucous plaques on the (L) knee and ankle of size 10 x 8 cm and 5 x 3 cm, another similar plaque with central atrophy and telangiectasia on the left lower leg and of size 8 x 6 cm. The surface of the lesions showed multiple punctuate brownish scaly spots. Mucosae, hair and nails and other systems were normal. All the laboratory investigations were within normal limits except an increased ESR of 40 mm/hr. Biopsy from the lesion was taken for culture and histopathology. Microscopic examination of isolated fungus showed smooth, elliptical conidia that is poorly differentiated from the vegetative hyphae and forming long chains as many as 35 or more blastoconidia. These infections are often seen in immunocompromised individuals. In world literature seven cases of primary subcutaneous phaeohyphomycosis caused by *Xylohypha bantiana* (Cladophialophora *bantiana*) are reported. (Gugnani *etal* 1977, Amma *etal* 1979, Padhye *etal* 1988, Patterson *etal* 1999, Jain *etal* 2003, and Sean M *etal* 2005).



Case 2

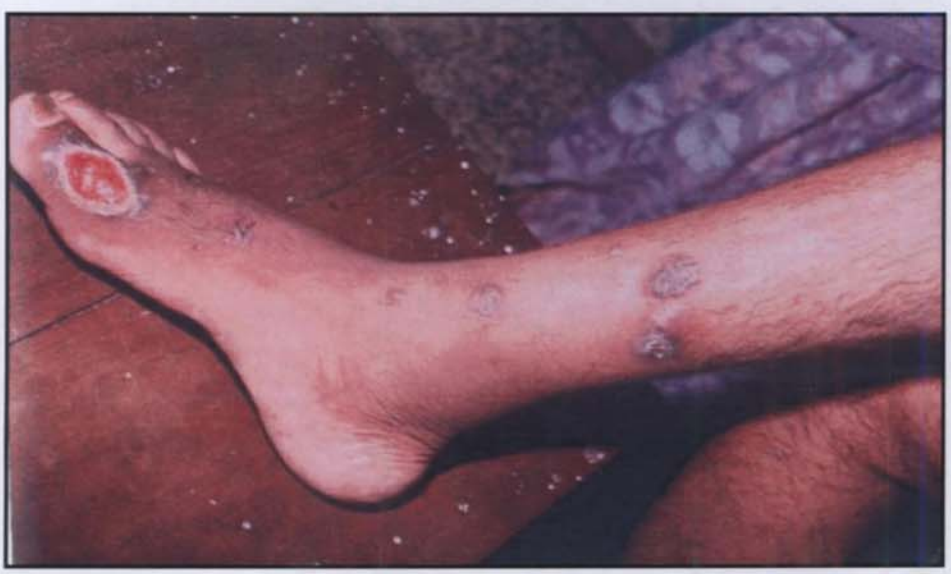
The second case was a 35 year old Indian tribal lady, presented with crusted plaque involving the left angle of the mouth and adjacent parts of upper and lower lips of two year duration. The lesion started as a small non tender nodule on the upper lip

which later ulcerated and spread to lower lip. The adjacent buccal mucosa also showed extension of the lesion with erosion. The regional lymph nodes were not significantly enlarged. There was no history of trauma to that site and no past history of pulmonary tuberculosis. With this clinical picture, it was considered as lupus vulgaris with a differential diagnosis of cutaneous leishmaniasis and chromoblastomycosis. The patient was investigated further. Her basic haemogram and urine analysis were normal. The chest radiograph was within normal limits and mantoux test was negative. The non specific (VDRL) and specific (TPHA) tests for syphilis were non reactive and HIV screening was negative. Direct microscopic examination of scrapings and tissue smear from biopsy in Potassium hydroxide mount was unremarkable. The skin biopsy specimen taken from the lesion on two occasions showed a chronic inflammatory cell infiltrate with predominant plasma cells and no organisms or granuloma was seen. Both occasions fungal culture on Sabouraud's medium yielded *F.pedrosoi,* with a velvety dark gray to brown colonies with a black reverse. Microscopic examination of culture showed acrogenous conidia with short branching chains confirming the diagnosis of chromoblastomycosis caused by *F.pedrosoi.* The patient was given an oral itraconazole 100 mg daily but with partial response after months.



Case 3.

The third case was a forty year old male agricultural worker who presented with a non healing ulcer on the left big toe and multiple nodules over the anterior aspect left leg and foot duration arranged in a linear fashion of one year duration. It started as non tender nodules on the dorsum of the left big toe which later ulcerated. Subsequently multiple nodules started to appear in a linear fashion on the left foot and leg. He gave history of trauma to the site of lesion on the left big toe. With this clinical picture it was considered to be sporotrichosis with a differential diagnosis of chromoblastomycosis. His base line blood and urine examination were normal. The renal and liver function tests were normal. Scarping from the lesion and tissue smear did not show any organism. Histopathology of the lesion showed pseudoepithelomatous hyperplasia and sub epithelial region showing epithelioid cells with Langhan's giant cells and sclerotic bodies in the granuloma, both intracellularly as well as extracellularly. Fungal culture showed velvety dark gray to brown colonies with a jet black reverse. Microscopic examination of the cultured organism showed acrogenous conidia with short branching chains, characteristic of *F.pedrosoi*, thus confirming chromoblastomycosis. The patient was given oral itraconazole 100 mg daily, the skin lesion showed good response to this treatment.



The subcutaneous fungal diseases, especially chromoblastomycosis affect numerous people worldwide, especially those living in tropical, subtropical countries, notably in Mexico, Cuba, Brazil, and Dominican Republic (Rippon J.W, 1982, Brygoo E.R, 1975, Al-Doory Y.1972). In Japan, large numbers of cases have been reported during recent years, particularly since 1970 (Fukushiro R, 1983). The most frequently isolated, fungi from case of chromoblastomycosis are *Fonsecaea pedrosoi*, *Phialophora verrucosa*, and *Cladosporium carrionii* (Brygoo ER and Destombes P, 1975. AL-Doory Y 1972). Chromoblastomycosis was first reported from India in 1957. Initially two cases were reported from Assam, an eastern state, and in both cases Fonsecaea compacta were isolated (Thomas E *etal* 1957 and Kakoti L.M, Dey N.C, 1957). The third and fourth cases reported from the country were from Punjab (Rajan R.V *etal* 1958) and Maharashtra, (Gokhale B.B *etal* 1959) northern and western states, respectively. The isolated fungus were *Fonsecaea dermatitidis* and *Fonsecaea species* respectively. The prevalence of chromoblastomycosis in the southern part of India, the belt that is now known for the highest number of chromoblastomycosis cases reported in India, was documented from a case report from Kerala in 1966 (Meenakshi L.V *etal* 1966).The isolated fungus was *F.pedrosoi*. More cases were documented subsequently from different parts of India (Dube B, Dube R.1966).

The details of chromoblastomycosis reported in India is given below

Table 23. Summary of the cases of chromoblastomycosis reported in India (cases diagnosed without cultural or histologic evidences are excluded)

Year	Place	No.of cases	Sex		Age	Site affected	organism involved
			M	F			
1957	Assam (Thomas E,1957)	1	1	-	35	Foot	*F.compacta*
1957	Assam (Kakoti L.M 1957	1	-	1	34	Vulava with external genitala	*F.compacta*
1958	Punjab (Rajam R.V, 1985)	1	1	-	7	Face,trunk,extremities etc	*Wangiella dermatitidis*
1959	Maharastra (Gokhale, B.B 1959)	1	-	-	-	Above umbilicus	*F.compacta*
1966	Kerala (Meenakshi L.V,1966)	1	1	-	35	Leg	*F.pedrosoi*
1966	Karnataka (Dube B, 1966)	2	2	-	30,30	Forearm, elbow	*F.compacta*
1967	Utter Pradesh (Mohapatra L.N, 1967)	2	1	1	30,31	Foot,foot	*Cladosporium carrionii*
1967	Andhra Pradesh (Radhakrishnamurthy K, 1967)	1	1	-	55	Leg	*Cladosporium sp.*
1970	Tamil Nadu (Bhaktaviziam C,1970)	2	1	1	42,43	Foot, face	*F.compacta*
1972	Andhra Pradesh (Naidu P.S, 1972)	1	1	-	25	Face,trunk,extremities etc	*F.pedrosoi*
1973	Karnataka (Venkataramiah N.R,1973)	1	1	-	43	Elbow joints	-
1974	Karnataka (Nagarkatti P.S,1974)	1	1	-	33	leg	*F.pedrosoi*
1977	Haryana (Varma K.C, 1977)	1	1	-	40	Leg	-
1978	Punjab (Kumar K, 1978)	2	2	-	12,26	Face, face	*F.pedrosoi/ C.carrionii*
1978	Karnataka (Sivananda P.G, 1978)	1	1	-	21	Parotid region	*F.pedrosoi*
1981	Andhra Pradesh (Radhakrishnamurthy K, 1967)	3	3	-	40,40,60	Leg, foot,foot	*F.pedrosoi*
1984	Pondicherry (Sardari Lal, 1984)	1	-	1	10	Face, Nasopharynx.	*Exophiala jeanselmei*
1985	Chandigarh (Sharma V.K, 1985)	1	1	-	35	Extremities	*C.carrionii*
1986	Meghalaya (Battu V, 1986)	1	1	-	65	Penis, leg	*F.pedrosoi*
1988	Kerala (Pavithran K, 1988)	1	1	-	50	Knee	*C.carrionii*
	Tamil Nadu (Jacob M,1988)	1	1	-	46	Upper extremity	*C.cladosporioides*
1991	Chandigarh (Bushan Kumar,1991)	2	2	-	49	Foot	*F.pedrosoi*
1991	Kerala (Pavithran K, 1991)	1	1	-	42	Face,trunk,extremities, oral cavity	*C.carrionii*
1993	Punjab (Nair S.P, 1993)	1	1	-	21	Hand, forearm	-
1994	Tamil Nadu (Harshani V, 1994)	1	1	-	40	leg	*Philophora verucosa*
1997	Jammu &Kashmir (Rajendran C, 1997)	1	1	-	45	Ulcer buttock	*F.pedrosoi*
1986	Bihar (Rajendran C, 1997)	1	1	-	40	Ulcer left elbow	*F.pedrosoi*
1997	Utter Pradesh (Rajendran C, 1997)	1	-	1	30	Ulcer ® foot	*F.pedrosoi*
1997	Assam (Rajendran C, 1997)	1			40	Ulcer ® leg	*F.pedrosoi*
	Total	36					

Chromoblastomycosis, caused by several species of dematiaceous fungi is usually confined to one of the lower extremities and affects only the skin and subcutaneous tissue, though the lymph glands draining the diseased focus may participate in the pathological process. Rare cases have been reported affecting the hand, arm, face and buttocks (Rajendran etal, 1997). Mucous membrane is usually not involved, but invasion of the conjunctiva and nasal septum have been reported (Sardari Lal etal 1984). The chromoblastomycosis lesion may be verrucous with central scarring (tuberculoid), extremely scarred with a serpigenous border (syphiloid), scaly (psoriasifiorm) or indurated with fistula (mycetomatoid) (Vollum DI 1977). The diagnosis of chromoblastomycosis should be confirmed either by direct microscopy of the scrapings from the lesion in 20 % KOH, when thick walled dark brown tissue form of the fungus (sclerotic bodies/muriform bodies/copper pennie body) are seen; (Zaias N and Rebell G, 1973) or by histological examination of a biopsy specimen when the granulomatous reaction and spore are diagnostic; or by culture of scrapings or biopsy materials.

Chromoblastomycosis is a common disease among rural workers in tropical and subtropical countries of Central and South America and Africa. This may occur occasionally in temperate zones but it is most frequently encountered in warmer climates where people go barefoot and wear minimal clothing. These fungi are widely distributed as saprobic in soil and decaying vegetation in all types of climates. The mode of infection is by inoculation of phaeoid fungi into the cutaneous tissue through trauma. Like mycetoma, chromoblastomycosis is also seen most often among males residing in rural areas being relatively more common in the rural than urban population. In Japan, however, the incidence is found to be equal in both sexes (Fukushiro, R 1983). This infection has also been reported in animals like dogs and horses. The infection is not transmitted from animals or between humans.

Chromoblastomycosis is a chronic mycosis of the skin and subcutaneous tissue characterized by a brown-walled, round, nonbudding form of causative fungus in the tissue. This uncommon disease has been reported from almost all the continents, but the majority of cases are from tropical and subtropical regions. The text book on medical mycology names only China, Japan, Philippines, and Malaysia as the countries of Asia having this disease (Rippon JW 1988, Kwo-Chung K.J & Bennett 1992), and India is not mentioned, probably because of the limited circulation of Indian Journals.

In the present study 19 isolates of *F.pedrosoi* (38%) and 15 isolates of *Cladosporium carrionii* (30%) were isolated from chromoblastomycosis. *Fonsecaea dermatitidis and Xylohypha bantiana* were isolated in two cases. *Black yeast* and *F.compacta* were isolated in one cases each. Of the two main pathogens, *Fonsecaea pedrosoi* is seen more in humid tropical climates (Kwon-Chung K.J & Bennett J.E, 1992) while *Cladosporium carrioni* is thought to be endemic in semiarid zones. *Cladosporium carrioni* is the commonest pathogen in Australia and Africa and *Fonsecaea pedrosoi* in Columbia, Venezuela, Cuba and Japan (Brygoo E.R & Destombes P,1975, Al-Doory Y,1972, Rippon J.W, 1982). In the present series, both of these agents were seen in equal frequency and no particular distribution pattern is evident for any of the agents isolated. *F.compacta* is said to be quite rare (Rippon JW, 1988). It was isolated from one case. Good hygiene and adequate nutrition may help the individual abort a potential infection (Kwon-Chung K.J & Bennett J.E, 1992). Poverty and malnutrition in Indian children may be responsible for the early development of clinical infection.

The peculiar distribution of disease in the sub-Himalayan belt and the coastal areas appears to be due to the hot and humid climates of these areas, which are

essential for growth of these fungi. The northwest arid zone of the country is free from disease. In contrast to the Indian figure of a six times higher incidence in males, females made up only 4.8% in the series of Azulay and Azulay(1959), while in that of Fukushiro(1983, Japan) women outnumbered men. In the present series, females formed only 15 % of the cases, and this figure is comparable with that (13%) of Coulanges and Locheron from Medagascar in a series of 891 cases. Rural males from an agricultural background were predominantly affected, which is the common pattern of disease worldwide (Kwon-Chung K.J & Bennett J.E 1992)

Therapeutic modalities used were surgical excision, heat therapy, cryotherapy, thiabendazole, amphotericin-B, and azoles, singly or in combination. The outcome was unsatisfactory in the majority, except in the case of surgical excision of localized lesions. Ketaconazole was found to be disappointing in our limited experience. Newer azoles, however, do achieve clinical cure, but the doses and duration of therapy seem to be high and prolonged. Flucanazole appears to be preferable in serious cavity infections because of its water solubility. A combination of the two azoles needs further evaluations..

Immunocompromised patients are at risk of contracting serious fungal infections. The emergence of acquired resistance to azole treatment by opportunistic fungal organisms is increasing and poses a major therapeutic challenge. Treatment of some deep cutaneous and subcutaneous mycoses remains unresolved, relapse are frequent, lack of tolerability of the antifungal drugs becomes an obstacle, and unfortunately surgery is in some cases, the last option. The development of allylamine antifungals, of which terbinafine is most effective to date, may help to resolve this situation. Invitro terbinafine is highly active against a broad spectrum of pathogenic fungi. Clinical studies have shown that terbinafine is effective in the treatment of both

cutaneous and lymphocutaneous sporotrichosis, chromoblastomycosis, phaeohyphomycosis and maduromycosis. These results (Perez A, 1999) suggest that the therapeutic potential of terbinafine extends well beyond its currently licensed applications to include a range of serious and life threatening subcutaneous and systemic mycoses. Sevig G.M *etal*, 2000, reported a patient with chromoblastomycosis due to *F.pedrosoi*, who was treated 8 months of terbinafine 250 mg by mouth daily with histologic and mycologic cure. In the present study terbinafine showed a high invitro activity against *Fonsecaea pedrosoi* and *Cladosporium carrioni*

Another report by C.Rajendran *etal,* 1997 identified a wider distribution of the disease, chromoblastomycosis in India and reviewed in the Indian literature. A total of 30 cases of chromoblastomycosis were reported from India from 1957 to 1994. Males accounted for 25 and females five. In India about 70 % of the population lives in rural areas, engaged either fully or partly in agriculture. Since in most of these places the requisite expertise and facilities to make a proper diagnosis are lacking, many patients are undiagnosed or misdiagnosed. Similar to reports from other parts of the world, most of the patients here were aged between 20-50 years old and, being the sole earning member of the family, often found it difficult to leave their work to seek medical advice and treatment in a center situated far away from their home. So the data given above may not reflect the true picture; considering the fact that the casual agents of chromoblastomycosis are known to survive under diverse climatic conditions prevailing here, the disease could be more widely prevalent than indicated by published reports.

Of the body sites affected in India, the most common areas were leg, foot, and hand, (Thomas E *etal*,1957 and Nair S.P and Sarojini P.A, 1993) although other areas

like the buttocks and face have also been reported (Kumar K and Sarin R.C 1978). The lesions were nodular, verrucous, plaque-like, or cicatrical. The commonest fungal isolates were *F.pedrosoi* and *Cladosporium carrioni*. This is similar to reports from other countries like Mexico, Cuba, and Dominican Republic, Venezuela, Australia, and South Africa (Brygoo E.R. Destombes P 1975, Londero A.T, Ramos C.D 1976, Leslie D.F, Beardmore G.L, 1979, Simson F.W, 1946)

Although subcutaneous mycoses were more prevalent in tropical and subtropical regions, only a few reports are available from Sri Lanka or from Asia. Majority of infections have been caused by *Fonsecaea pedrosoi*. During the period from 1952 to 1962 twelve culture proven cases of this disease caused by *F.pedrosoi* had been recorded from Sri Lanka. Similar study conducted by Attapattu in 1997 from 71 Sri-Lankan patients with chromoblastomycosis for the 16 year period from 1978 to 1993. Culture identification was made in 69 cases. Three fungal species were identified *F.pedrosoi* (64), *Phialophora verrucosa* (3) and a fungus morphologically compatible with *F.compacta*.(2).

Jayalakshmi-P,1990,reported 9 histologically diagnosed chromoblastomycosis from Malaysia. All the patients were males and ranged in age from 56 to 65 years and the duration of symptoms varied from 5 months to 13 years. All the lesions were noted in the lower limbs. Malignancy was suspected in 5 cases. Diagnosis was established by the characteristic muriform cells in the tissue. Woodgyer A.J, 1992 isolated four endemic cases of chromoblastomycosis from New Zealand. All these cases were due to *F.pedrosoi*. Esterre P 1990 studied retrospectively forty years of confirmed cases of chromoblastomycosis in Medagascar from 1955 to 1994. The total number of cases reported was 1343 of which 98.5% were confirmed histopathologically. Only 30.8 % of cases showed positive culture on mycological media. *F.pedrosoi* was identified from 61.8 % of the fungus strains.

Another study by Silva J.P, *etal* (1998-99) presented 325 cases of chromoblastomycosis diagnosed during the last 55 years where the data obtained showed that: a) the main age group affected by the diseases ranged from 41 to 70 years old, b) 86.1 % of the patients were agriculture workers, c) 93.2 % of them were male and d) 80.7 % showed lesions on the lower limbs (feet and legs). The diagnosis of 62 % of the cases was confirmed by laboratory studies considering the tissue form in the histopathological analysis. In 24 % of patients (78 cases) the etiological agent was isolated and identified through culture. *F.pedrosoi* was present in 77 cases and *Phialophora verrucosa* in only one case. Huerre M *etal* (1991) studied deep mycoses observed in New Caledonia, France between 1975 and 1989. During the period, two cases of histoplasmosis, 4 of actinomycosis, 3 sporotrichosis, 5 mycetoma and 5 chromoblastomycosis were identified. Diagnosis was supported by histopathological examination and thus, for future studies it is vital that specific mycological culture is essential.

Diaz Almeida J.G, *etal* (1978), studied chromoblastomycosis in Cuba, retrospective clinical and epidemiologic studies on 72 patients. The study was conducted during the period 1963-1973. *F.pedrosoi* was the prevalent pathogen.Bonifaz A *etal* (1991) studied 51 cases of chromoblastomycosis detected in a 17 year period where all of which were clinically and mycological proven by direct examinations, culture and biopsies. Most cases were male (36 of 51; 70%), the mean age was 35 years and farmers predominated (74%), with the most frequent lesions in lower limb (54%). Major clinical presentation was nodular (41%) and verrucous (26%). The principal etiological agent isolated was *F.pedrosoi* (90%) and result showed 31% cure, 57 % improved and 21% failed. The best results were obtained

with cryosurgery for small lesions, with itraconazole for large ones, and in some cases the combination of both treatments. Deshpande S (1993) isolated two cases of chromomycosis from Bombay during the year 1980-1989 and the isolated fungus was *Fonsecaea pedrosoi.*

Phaeohyphomycosis encompasses a diverse group of dematiaceous fungal infections characterized by presence of pigmented hyphae in tissue. The involvement of the skin and subcutaneous tissue is termed subcutaneous phaeohyphomycosis (Kwon-Chung K.J and Bennett J.E, 1992) and is characterized by a nodule, cyst, or pyogranuloma. The list of phaeohyphomycosis- producing fungi in 1992 included 28 genera with 59 species, and has now been expanded further (Kwon-Chung K.J and Bennett J.E, 1992).

In the present study out of a total 192 cases of subcutaneous mycoses, 10 cases of phaeohyphomycosis (5.2 %) were obtained by observing pigmented fungal hyphae in tissue sections histopathologically. Fungal isolations were obtained in all the cases. The important fungal isolates were *Aureobasidium* and *Ramichloridium in* two cases. *Phoma, Cheatomium, Curvularia* and *Torula* were isolated in one case each. The age of the patients ranged from 21 to 67 years, with a male to female ratio 6:1. A relatively late onset was observed in this study. A history of prior injury was noticed in five patients. The lower extremities were involved in 3 cases, one nodular cyst in the lumbar region and ulcer in the right upper arm.

Table 24	Subcutaneous Phaeohyphomycosis in India				
Patients	Reference	Sex	isolate	Site affected	lesion
1	Rajam R.V etal, 1958	M	*Phialophora dermatidis*	Disseminated	Warty and cystic
2	Dube R,Gupta K, 1973	M	Not known	Not known	Abscess
3	Agarwal S, Chouhan, 1966	M	Not known	Not known	Abscess
4	Amma S.M etal, 1979	M	*Cladosporium bantianum*	Foot	Nodule
5	Amma S.M etal, 1980	M	*Phialophora dermatidis*	Leg	Chronic ulcer
6	Amma S.M etal, 1980	M	*Phialophora dermatidis*	Leg	Chronic ulcer
7	Amma S.M etal, 1980	M	*Phialophora dermatidis*	Leg	Chronic ulcer
8	Prabhakar V etal, 1983	F	*Exophiala jeanselmei*	Disseminated	Cysts,warty plaques
9	Lal S, etal, 1984	F	*Exophiala jeanselmei*	Disseminated	Papuloplaques
10	Jacob M etal, 1988	M	*C.cladosporoides*	Arm	Warty plaques
11	Sharma N.L etal,1990	F	*Phialophora gougerotii*	Arm	Cysts
12	Singh S.M etal, 1990	M	*Alternaria alternata*	Toe and nail	Scaly & dystropic
13	Ramani R etal, 1992	F	*Alternaria chlamydospora*	Disseminated	Scaly
14	Singh S.M etal, 1992	F	*C.cladosporoides*	Foot	Verrucous growth
15	Singh S.M etal, 1992	F	*Phialophora richardsiae*	Waist & buttock	Kerotic, macerated
16	Singh S.M etal, 1992	F	*Phialophora richardsiae*	Waist & buttock	Kerotic, macerated
17	Deshpande S etal, 1993	M	*Fonsecaea compactum*	Below mandible	Sinuses
18	Agarwal A, Singh S.M, 1995	M	*Curvularia pallescens*	Both feet & thigh	Ulcer
19	Agarwal A, Singh S.M, 1995	M	*Curvularia pallescens*	Left thumb	Ulcer
20	Somani V.K etal, 1996	M	*Curvularia lunata*	Leg	Nodule
21	Tendolkar U.M etal, 1996	F	*Phialophora verrucosa*	Shin	Ulcer
22	Dhindsa M.K, etal, 1998	F	*Nattrassia mangiferae*	Hand	Scaly lesion
23	Sharma N.L etal,2002	F	*Fonsecaea pedrosoi*	Left lumbar region	Ulcer

Similar study conducted by Nand Lal Sharma *etal* (2002) reviewed phaeohyphomycosis in India. A total 23 patients with subcutaneous

phaeohyphomycosis have been reported, distributed throughout the country in the belt from north to south, sparing the western and eastern regions. The age of the patients ranged from 3 to 60 years, with a male to female ratio 1.3:1. A relatively early age of onset was observed. A history of prior injury was recalled by five patients. The lower extremities were involved in eight cases, upper extremities in five, gluteal region in two, lumbar area and submandibular area in one, face in two and disseminated disease was seen in four cases. Three of the cases died during follow up. Osteomyelitis was observed in two cases, hepatosplenomegaly in one, and lymph node involvement in two. Thirteen species from seven genera of dematiaceous fungi were isolated. *Phialophora dermatidis* was the most common isolate.

Subcutaneous phaeohyphomycosis occurs throughout the world in all climates. The Indian subcontinent has climates ranging from tropical to temperate, and the disease has been reported from the extreme south through to the extreme north, but sparing the western and eastern region. Indian patients are afflicted at a relatively early age. More than half of the patients were less than 30 years of age, and about 19 % developed the disease in the first decade of life. In one series of 78 published cases, 86 % of the patients were 30 years or above, 60 % were male, and about 85 % of the lesions were seen on the extremities. In this review, about 66 % patients had lesions on the limbs. The slight preponderance of male patients in India is probably due to the greater outdoor exposure of men. The duration of the disease was mostly one year or more, and in one case it was 33 years, with subsequent development of squamous cell carcinoma. Thus it can be inferred that relatively asymptomatic nature of the disease resulted in a delay in seeking treatment. None of the cases showed foreign body, as compared to 24 % of cases reported by Connor etal, 1982. *Exophiala dermatidis* seems to be associated with more fatalities, and

E.jeanselmei usually produces a cystic form of the disease. *Cladosporium cladosporoides* infection showed warty lesions.

Hyalohyphomycosis is an infection in which the basic tissue form of fungi is hyaline, hyphal elements, without any pigment in their cell walls (Padhye A.A, 1988). Till date, there are 74 species of fungi belonging to 20 genera that meet the requirements and qualify as agents of hyalohyphomycosis and have been proved to cause infection in humans and animals. Important human pathogens of this clinical entity *are Pseudallescheria, Fusarium*, and *Penicillium* species. Other fungi which can cause opportunistic hyalohyphomycosis include *Acremonium, Paecilomyces*, *Geotrichum*, and *Chrysosporium* species. The emerging basidiomycetes pathogens are *Schizophyllum commune*, *Coprinus cinereus, Hormographiella aspergillate* and *Tilletiopsis minor* which are found associated with various hyalohyphomycosis (Jagdish Chander, 2002).

In the present study 8 cases of hyalohyphomycosis were obtained (4.1%) by histpathologically. Fungal isolations were obtained in 6 cases. The important fungal isolates were *Acremonium* in two cases (Zaitz C etal, 1995)and *Scedosporium apiospermum, Streptomyces* and *Penicillium marneffei* in one case each.

Mycetoma is a localized, chronic, progressive and granulomatous inflammatory lesion caused by certain fungi or bacteria. It is characterized by gradually increasing painless tumor like swelling with appearance of multiple sinuses, discharging pus with granules. In the present study, 6 cases of mycetoma (3.1 %) were obtained histopathologically and fungal isolations were obtained in all the cases. The important fungal isolates were *Scedosporium apiospermum* in two cases and, *Aureobasidium pullulans*, *Acremonium*, and *Streptomyces* in one case each. . The age of the patients ranged from 38 to 82 years, with a male to female ratio 0.6:1.

The prevalence of etiological agent of mycetoma varies from place to place. The eumycetes accounts for about 40 % and actinomycetes for 60% of mycetoma in the world. In the Southeast Asia, India and Pakistan and neighboring countries eumycotic and actinomycotic mycetoma ratio is about 35% and 56%, respectively except in Rajasthan (Singh H 1979). In Mexico 98% of mycetoma is caused by actinomycetes and 2% by eumycetes, while in Europe actinomycetes accounts for about 30 % and eumycetes for 70% of the reported cases of mycetoma (Hay R.J *etal* 1992). In Mexico, 98% of mycetoma are caused by actinomycetes, of which 86% are caused by *Nocardia brasiliensis*, followed by *Actinomadura madurae*, *Streptomyces somaliensis, Nocardia asteroids, Actinomadura pelletieri*, and *Nocardia caviae*. In Venezuela most frequent etiologic agent of eumycetoma are *Pyrenochaeta mackinnonii*, *P.remeeroi* and *Madurella grisea*; actinomycetoma are *Actinomadura madurae* and *Nocardia brasiliensis*. In Africa, eumycetoma are more common and extensive (Jagdish Chander 2002). Sporadic cases of mycetoma may also be found elsewhere in the world. In northern India including Rajasthan eumycetoma is more prevalent with commonest agent as *Madurella mycetomatis* (Joshi K.R, *etal* 1987) whereas actinomycetoma is more common in South India with a marginal difference. From eastern India *Streptomyces viridis* has also been claimed to be found in a patient of actinomycetoma, which is first of its kind. The reported incidence of the two types of mycetoma varies not only in different parts of the country but also within the same geographical area.

Dasgupta *etal* 1974 and Taralakshmi *etal* 1977 have reported actinomycetoma as the common type in South India while Murthi & Padmavathy,1963 have reported eumycetoma as the common type. Desai *etal* 1970 found actinomycetoma more prevalent in Bombay while Mankodi & Kanavinde 1970 reported eumycetoma as

common in Ahamedabad. Similarly Mohapatra & Bhargava 1967 reported eumycetoma from Delhi while Talwar and Sehgal 1979 reported actinomycetoma to be the common type in North India. These studies indicate that the concept of preponderance in the incidence of certain species in different geographical regions is slowly fading. The etiological agents appear to be present ubiquitously and they await suitable opportunities to manifest themselves.

Similar work by P.K. Maiti *etal* 2001 from Kolkata, India, made a retrospective analysis of 264 cases of mycetoma in West Bengal between 1981 and 2000. The ratio of actinomycetomas and eumycetomas was 197:67; Male to female ratio was 183:81. Ninety four cases occurred in the 1980s and 170 in 1990s with significantly more infections of *Actinomadura spp* and fewer with *Nocardia caviae* during the last decade. Pricking was the most common injury associated with eumycetomas. A total of 196 infections were in exposed body parts and 68 in covered areas. Overall prevalence of the disease during the period between 1980 to 1990 increased, nearly six fold in the case of actinomadura infections, although the prevalence of *N.caviae* infections decreased by a factor of three. This may be because of the rapid geo-ecological changes in eastern India during the last 2-3 decades after green revolution, when agricultural practices changed resulting in more intense use of ground water. A similar retrospective study in an area of low Mycetoma prevalence in the hills of North West India (Chakrabarti & Singh 1998) found no marked changes in the prevalence.

Farmers are at greater risk of injuries that permit infection with saprophytic agents. But trauma is not the only factor for the outcome of the disease; viability of the organisms and local wound conditions can also modify the outcome (Mackinnon 1962). As the nature of trauma in covered areas of the body is usually minor, only

those organisms which can colonize short term shallow wounds are likely to infect these areas. The chance of soil contact with these injuries is also smaller. The conditions related to the precipitating trauma, associated bacterial flora (Gonzalez & Gonzalez- Mendoza 1960) and the temperature of the anatomical region are great importance for the disease outcome. This subcutaneous mycosis essentially requires a predisposing trauma to create a point of entry for the casual saprophytic micro-organisms. The interval between the incidence of trauma and the appearance of overt disease can thus be taken as the incubation period for the isolates, provided the patients can recall the exact incidence of trauma, however minor it may have been.

In another review of 333 cases, the variation of the duration of illness before confirmation of the disease was 3 months to 20 years, although most cases were detected within 2-3 years (Mahgoub & Murray 1973). This depends upon patient's availability for diagnostic facilities. In the above study most cases were diagnosed with in 3-4 years indicating the need for better diagnostic facilities for mycotic diseases in tropical developing countries like India.

Another study by Hazra B *etal*, 1998 analyzed forty cases of mycetoma with respect to clinico-epidemiological, histopathological and radiological features. The age of the patients ranged from 17 to 57 mean being 32.4 +/-8.68. The disease was equally distributed amongst the sexes. Most of the patients had a rural background and the disease occurred mainly among farmers and housewives. Actinomycetes (32 cases. 80 %) were found to be the main pathogenic organism and Eumycetes in 4 cases (20%) only. Clinical features were more or less the same irrespective of the etiologic agent, consisting of local swelling with discharging sinuses. History of trauma was present in only 20 % cases and 80% were incidental. Foot (80%) was the most commonest site of infection.

Another study by Chakraborthi A and Singh K, 1998, mycetoma in Chandigarh and surrounding areas analyzed 23 cases of mycetoma in sixteen years (1980-96). Actinomycotic agents were isolated in 56.5% while eumycotic agents were responsible for 43.5% of mycetoma cases. *Madurella mycetomatis* was the agent most frequently isolated. The other agents isolated were *N.asteroides, A.madurae, M.grisea* and *Streptomyces spp.* Foot is the common site of infection. Males and females were found to be equally affected. Luiz Carlos Severo etal 1999 in Brazil, reviewed 14 world literature of *Exophiala jeanselmei* mycetoma. Out of these, three cases were from India.

Sporotrichosis is generally a chronic mycotic infection characterized by nodular and ulcerative lesions of cutaneous, subcutaneous or lymphatic tissues. It has a world wide distribution but more common in temperate and tropical zones with high temperature and humidity. The etiologic agent is a dimorphic fungus, *Sporothrix schenckii*, which occurs as a saprobe on a variety of dead or decaying plant materials. The infection is usually acquired by traumatic implantation of the fungus from the environment. In India, Sporotrichosis is known to be endemic in the sub-Himalayan region, *ie.*; in Assam, West Bengal in the north-east and Himachal Pradesh in the north-west. It has not been reported so far in the residents of Delhi area and the adjoining plains of Uttar Pradesh, Hariyana and Rajasthan states. An update of sporotrichosis is based upon literature review (1932-2001). Of the 205 cases reported to date from the literature, 91 (44%) came from West Bengal, 56 (28%) from Himachal Pradesh and 45 from Assam whereas the remaining 13 (6.3%) occurred sporadically in other states, including Bihar, Punjab and Karnataka (Gosh A *etal,* 1999). One case from Delhi and another one from Kerala. The first authentic case was reported from the north-western region by Kini S, Pal D *etal* 1988. It was a female

from Utter Pradesh. Sporotrichosis in Himachal Pradesh (North India) By Gosh A *etal* 1999; in a one year study period (1996-97), 25 cases of Sporotrichosis were diagnosed. 16 cases were referred by doctors from local area and 9 were from cases suspected during the skin test survey. This study identified Kanga district and adjoining areas in Himachal Pradesh as an endemic region for sporotrichosis.

Another report from Bangalore by Nagasaki Mohan *etal* 2004 analyzed sporotrichosis for a period of 15 years. Result of the study identified seven cases of sporotrichosis in South India. In our five year study we could not isolate any case of sporotrichosis from these areas. Sporotrichosis is considered to occur only sporadically in Southern India and is very rare in this area. In Kerala a case of cutaneous sporotrichosis was reported by Sumangala Bai *etal* (1998) from a 52 years old male working in a rubber plantation who presented with a case of chronic non healing plantar ulcer which failed to respond to anti tuberculosis treatment. The culture yielded *Sporothrix schenckii* and was subsequently successfully treated with itraconazole. In the present series no case of sporotrichosis was diagnosed.

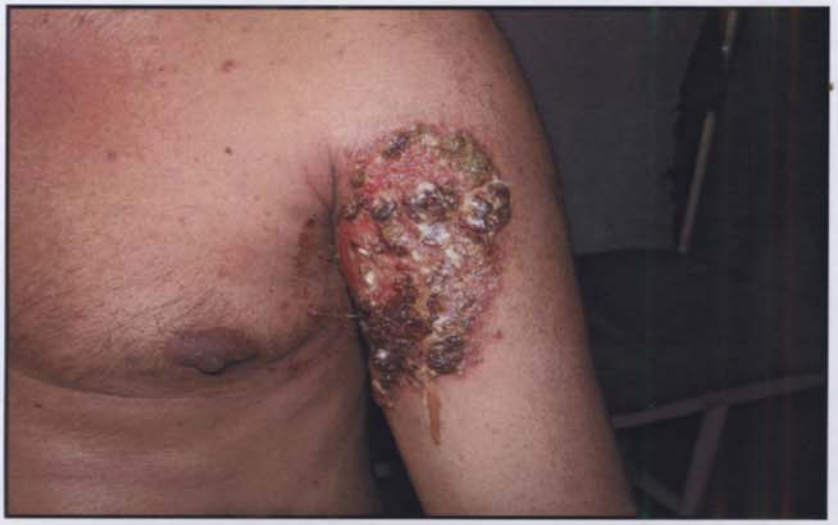
In the present study three proven cases of rhinosporidiosis (1.56%) were identified histopathologically. Although this disease is endemic in Kerala only three cases have been identified during the six year period of study (2000-2005). The organism causing rhinosporidiosis can not be cultured so the diagnosis is confirmed by biopsy. Sporangia can be seen on direct microscopy or hematoxylin and eosin-stained sections and have multiple basophilic spores in a clear material. The sub mucosa shows chronic inflammation and spherical sporangia in different stages of development and ranging in size from 250 to 300 μm in diameter. When compared with the spherules of *Coccidioides immitis*, the mature sporangia of rhinosporidiosis are larger and do not have a thick outer wall.

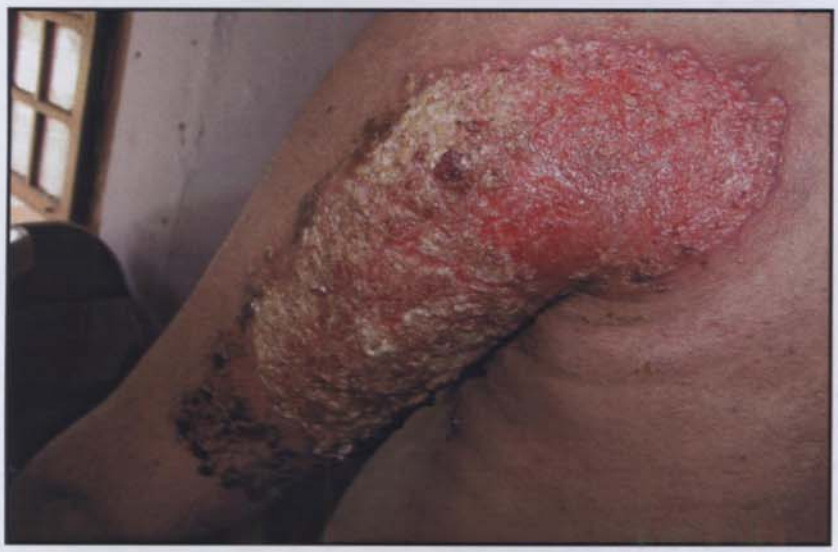
Rhinosporidiosis occurs in healthy people, as there are no predisposing factors besides exposure to freshwater. Rhinosporidiosis is a chronic granulomatous infection of the mucous membranes that usually manifests as vascular friable polyps that arise from the nasal mucosa or external structures of the eye, initially described by Seeker in 1900 in an individual from Argentina. Rhinosporidiosis is endemic in India, Sri Lanka, South America, and Africa (Snood.N.N&Rao S.N, 1969) Many cases were reported from the United States and Southeast Asia, as well as scattered occurrences throughout the world have been reported (Cohen J etal 1997). Infection of the nose and nasopharynx is observed in 70 % of persons with rhinosporidiosis; infection of the palpebral conjunctivae or associated structures (including the lacrimal apparatus) is observed in 15 %. Other structures of the mouth and upper airway may be sites of disease. Disease of the skin, ear, genitals, and rectum has also been described. Genital disease has been described in the vagina, penile urethra or meatus, and scrotum. Dissemination has been described rarely. Rhinosporidiosis is not responsive to treatment. Anecdotal treatment of three patients with year long course of dapsone has been reported, no controlled studies have been performed. The best management for rhinosporidiosis is excision because there is no medical treatment known to be effective. To prevent the 20 % of cases that recur after surgery, the base of the lesion can be cauterized after excision.

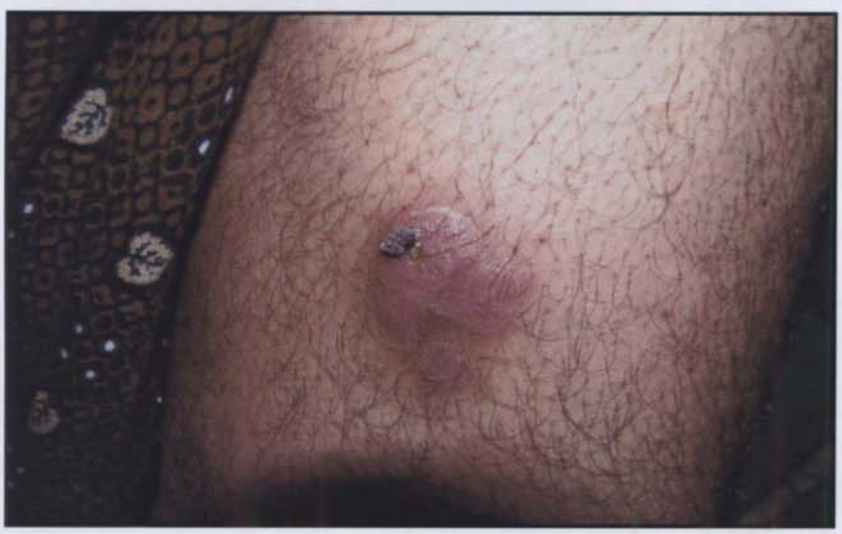
Inspite of high endemicity in India, the distribution of the disease throughout country is not uniform. It has been extensively reported from Tamil Nadu, Kerala, Pondicherry, Andhra Pradesh, West Bengal and Chattisgarh (Ratnakar C *etal* 1992). Isolated cases have been reported from other states either in patients who had migrated from endemic states or as original cases. The states of Tamil Nadu and Kerala are hyper endemic areas of this disease. The cases have been reported from

Eastern and Northern parts but it is rare in central India. From Bankura 116 (Samaddar R.R &Sen M.K, 1990) cases have been reported over a period of five years. Prabhakar and Bhatnagar (1983) have reported 36 cases from Amristar. Moses and colleagues (1988, 1990) have reported 19 and 112 cases respectively from Kanyakumari district of Tamil Nadu. Mohan *etal* (1995) reported a male patient from Kerala, who had been residing in Chandigarh for the last four years, with nasal rhinosporidiosis. Thakar A *etal* 2001 reported four illustrative cases from India.
During the study three unusual site infections caused by *Histoplasma, Streptomyces* and *Acremonium* have also been identified. One case of sub cutaneous histoplasmosis was identified during the study period.

A 32 year old male from Wynad, working in Gulf for the past two years had complaints of progressively increasing erythematous firm non tender plaque of 12 x 15 cm encircling (L) upper arm, studded with pustules and papules of one year duration. Another oval erythematous plaques over ® thigh was seen after three month. One week later ulceration of plaque of (L) arm and fluctuation in plaque on thigh was observed. General and systemic examination of the patient was with in normal limit The patient had no history of trauma and patient had mild burning pain. Biopsy was taken from the infected site. Histopathology revealed granulomatous infiltrate in dermis and subcutaneous tissue, with macrophage and scattered giant cells. Small round organism with clear halo intracellularly and extracellularly. GMS staining reveled budding oval yeast (2-4 μ) . Fungal culture on SDA showed white cottony growth of histoplasma.







Skin lesions in histoplasma

There are several organisms that cause subcutaneous zygomycosis, which is an uncommon tropical infection. Fungi of the orders Mucorales and Entomophthorales (Conidiobolus species and Basidiobolus species) are localized to different areas of the body. The subcutaneous zygomycoses (subcutaneous phycomycosis, entamophthoromycosis) caused by *Basidiobolus ranarum* is most common in Africa,(Kelly, 1980) . The other form of subcutaneous zygomycete infection is caused by *Conidiobolus coronatus* is found in tropical areas. Most of the infections were seen in West Africa and also in South America and India (Koshi G etal 1992). But in the present study we could not isolate any zygomycete.

The rare chronic infection, lobomycosis, is localized in tropical areas of central South America and is hyper endemic in areas of Brazil. The organism that causes the infection, *Lobo lobo* which comes from an unknown source, has not been isolated invitro, but has been found in freshwater dolphins (Miami Gretel 1983). Implantation is believed to be the route of infection. In the present study, no cases of lobomycosis could be identified.

STUDY ON
THE PREVALENCE OF SUBCUTANEOUS MYCOSES IN NORTH KERALA

Thesis submitted
in partial fulfilment of the requirements
for the award of the degree of

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BY

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SUMMARY & CONCLUSION

Subcutaneous mycoses comprise a heterogeneous group of fungal infections which are characterized by development of lesions at the site of implantation of etiological agents in the subcutaneous tissues. These infections primarily involve the dermis and subcutaneous tissue and rarely disseminate into systemic diseases. These infections usually develop from the implantation of ubiquitous organisms into the skin through trauma and are most commonly found in tropical areas. Similar to other mycoses, immunosuppressed patients are at increased risk for these infections. The main subcutaneous mycoses are chromoblastomycosis, phaeohyphomycosis, mycetoma and sporotrichosis. Other less common subcutaneous mycoses are zygomycosis, lobo mycosis, and rhinosporidiosis. These uncommon diseases have been reported from almost all the continents, but majority of cases are from tropical and subtropical regions

In the present study period, out of total 161542 cases of skin diseases, 7497 cases of fungal etiology were noticed. It was 4.64 % of total study group. From the 7497 patients with various fungal etiologies, only 192 patients were clinically diagnosed as subcutaneous mycosis. (2.56%) of total population. Higher incidence of infections were noticed in the age group of 31-40(39), followed by the age group, 41-50(37). In this study male patients outnumbered (132) female patients (60). In male patients, high incidence of infections were noticed in the age group of 41-50 (27), followed by 51-60(26) and 31-40 (25). In female patients, a higher incidence were was in the age group of 21-30 and 31-40 (10 each).

Of the 192 cases of subcutaneous mycosis, 50 cases of chromoblastomycosis (26%) were identified. Fungal isolations were obtained in 40 cases (80 %) Phaeohyphomycosis were identified in 10 cases (5.2 %) and fungal isolations were positive in all cases .Hyalohyphomycosis were identified in 8 cases (4.16 %) and fungal isolations were obtained in 6 cases (3.1 %). Mycetoma was identified in six

cases (4.16 %) and fungal isolations were obtained in all cases Unusual site infections were seen in five cases (2.6 %) and rhinosporidiosis was confirmed in three cases (1.56 %).

So according to the present study, the commonest subcutaneous mycoses prevalent in the hilly areas of north Kerala is chromoblastomycosis followed by phaeohyphomycosis. In chromoblastomycosis *Fonsecaea pedrosoi* is the common infecting agent and it is isolated in 19 cases (38%), followed by *Cladosporium carrionii* in 15 (30 %) cases. So according to this study, chromoblastomycosis due to *F. pedrosoi* is prevalent in this area. The next common species is *Cladosporium carrionii.. Fonsecaea dermatitidis* and *Xylohypha bantiana* were isolated in two cases each. *F.compacta and Black yeast* were isolated in one case each.

Compared to chromoblastomycosis, phaeohyphomycosis is less prevalent in this area. Total ten cases have been reported during the study period (5.2%). Fungal isolations were obtained in eight cases (80 %). The important fungal isolates were *Aureobasidium* and *Ramichloridium* in two cases. *Curvularia, Cheatomium, Torula and Phoma were isolated* in one case each. In the present study, eight cases of hyalohyphomycosis were identified (4.1%). Fungal isolations were obtained in five cases (62.5 %). The important fungal isolates were *Acremonium* in two cases. *Scedosporium apiospermum, Penicillium marneffei* and *Streptomyces* in one case each.

During the study period, six cases of mycetoma cases were identified (3.1%). Fungal isolations were obtained in all cases. The important isolates were *Scedosporium apiospermum* and *Acremonium* in two cases, *Streptomyces* and *Aureobasidium* in one case each. During the study period three cases of rhinosporidiosis were identified.

Invitro antifungal studies showed, terbinafine have good antifungal activity against *F.pedrosoi* and *Cladosporium carrioni* and patients showed good response.

STUDY ON
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BY

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