

Original Research Article

A prospective clinico mycological study of deep mycoses in a tertiary centre in Tamil Nadu

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Received: 20 March 2018

Revised: 07 April 2018

Accepted: 09 April 2018

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ABSTRACT

Background: Deep mycoses which includes subcutaneous mycoses and systemic mycoses, accounts for about 1% of the all the fungal infections seen in human beings. Though rare, these infections assume significance due to the increased morbidity and mortality associated with them. The objective of the study was to study the incidence, clinical presentation, aetiological agents and histopathological findings of deep mycoses in patients attending the mycology section, department of dermatology of a tertiary centre in Chennai.

Methods: All Patients with clinical suspicion of deep mycoses who presented to mycology section during the period from November 2015 to September 2016 were screened. The samples from these patients were subjected to direct microscopy by potassium hydroxide wet mount, culture and histopathology.

Results: Among the 8250 patients who attended mycology OPD, 41 patients (0.5%) had deep mycoses. The commonly affected age group was 41-50 yrs (29.7%). Males (73.2%) were predominantly affected. Of the 41 patients, 26.8% were immunocompromised. 37 patients (90.2%) had subcutaneous infection and 4 (9.8%) had opportunistic mycoses. Mycetoma (43.2%) was the most common subcutaneous mycoses. Mucormycosis (75%) and aspergillosis (25%) were the opportunistic mycoses observed. KOH positivity was 100%, while culture positivity was 65.7%. *Madurella mycetomatis*, *Phialophora verrucosa*, *Rhizopus arrhizus* and *Aspergillus fumigatus* were the common organisms isolated in this study.

Conclusions: Mycetoma is the most common subcutaneous mycoses in this part of India. Eumycetoma is more common than actinomycetoma. Phaeohyphomycosis is on the rise. Simple KOH examination would pave way for an early diagnosis and prompt treatment of deep mycoses.

Keywords: Subcutaneous mycoses, Mycetoma, Phaeohyphomycosis, Mucormycosis, Aspergillosis, Immunosuppression

INTRODUCTION

Deep mycoses are rare fungal infections with varied clinical presentation, thus necessitating a high degree of clinical suspicion for diagnosis. These infections are known to cause significant morbidity and at times mortality. Cutaneous manifestations of deep fungal infections occur from primary infection of the skin or as cutaneous dissemination due to a systemic infection.

Deep mycoses comprise of two groups of infections namely the subcutaneous and systemic mycoses. Subcutaneous mycoses also known as implantation mycoses, refers to the fungal infections of skin, subcutaneous tissue and bones caused by inoculation of saprophytic fungi leading to progressive local disease and tissue destruction. These infections, which are acquired following a trivial trauma are common in tropical and sub-tropical countries and are characterized by long

incubation period.^{1,2} Among the subcutaneous mycoses, mycetoma, phaeohyphomycosis, entomophthoromycosis, and chromoblastomycosis are prevalent in South India. Rhinosporidiosis which is endemic in Southern India, is now considered as a pseudo fungal infection caused by protistan parasite, *Rhinosporidium seeberi*.

Systemic mycoses are classified as opportunistic mycoses and endemic respiratory mycoses.³ The opportunistic systemic mycoses like aspergillosis, systemic zygomycosis, systemic and deep candidiasis, and cryptococcosis occur in an immunocompromised host. The Endemic mycoses occur in well-marked areas of endemicity and are usually caused by inhalation of spores.⁴ The endemic mycoses are histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, and penicilliosis. This study was undertaken to study the clinico-mycological and histopathological correlation of deep mycoses and to know the current trend of deep mycoses in the prevailing scenario of increasing immunosuppression.

METHODS

This prospective study was conducted at the Mycology section of Department of Dermatology at a tertiary centre in Chennai. All patients with clinical suspicion of deep mycoses attending the Mycology section during the period from November 2015–September 2016 were enrolled with inclusion criteria as follows: patients with clinical suspicion of subcutaneous or systemic mycoses and potassium hydroxide (KOH) positivity for fungus. The exclusion criteria were patients with KOH negativity for deep fungal infections except those with entomophthoromycosis and subcutaneous or systemic conditions due to a non-fungal etiology. Detailed history of the patients pertaining to age, sex, occupation, nativity, complaints, duration and comorbidities was taken. Astute clinical examination was done and relevant haematological and radiological investigations were carried out in all patients. Screening for HIV was done when indicated. Aspirate from cystic swelling, touch smear from sinuses/ulcers and scrapings from plaques were examined in 10% KOH, for the presence of fungal elements. All the specimens with KOH positivity were subjected to culture in modified Sabouraud dextrose agar (SDA) medium with chloramphenicol, cycloheximide and without cycloheximide when required and incubated at 25°C and 37°C. Macroscopic appearance of the cultures were examined daily for the first week and twice a week subsequently for 4-6 weeks. Microscopic morphology of the colony was examined in lacto phenol cotton blue (LPCB) stain. Incisional or excisional biopsy was done and subjected to haematoxylin and eosin (H&E) and special fungal stains such as Gomori methanamine silver (GMS) and periodic acid-Schiff (PAS) stains. Statistical tool used was SPSS version 16. Continuous data were analysed with mean and standard deviation and count data were analysed with frequency.

RESULTS

Among the 8250 patients who attended mycology section, 41 patients were diagnosed to have deep mycoses and the incidence was 0.5%.

Out of these 41 patients, 30 (73.2%) were males and 11 (26.8%) were females. Male to female ratio was 2.7:1. Among the 41 patients, five (12.2%) were children of which 3 were boys and 2 were girls. The mean age of patients was 38.4 years (ranging between 3 to 67 years).

Twenty six (63.4%) patients were from rural area, 6 (14.6%) from semi-urban and 9 (21.9) from urban location.

The most common occupation was farming, with 18 patients being farmers (50%), followed by housewives 9 (25%), while nine (25%) patients were involved in construction work, office job, hotel work and tailoring.

Duration of infection ranged from 1 month to 20 years. Mean duration was 2.15 years.

Of the 41 patients, 20 (48.8%) gave history of preceding trauma, while the others could not recall any trauma.

Eleven (26.8%) patients were immunocompromised while 30 (73.2%) were immunocompetent. The most common co-morbid condition associated in the immune compromised patients was diabetes mellitus seen in 7 (63.6%) patients, followed by renal transplantation in 5 (45.4%) patients and hepatitis c infection in 1 (9%) patient.

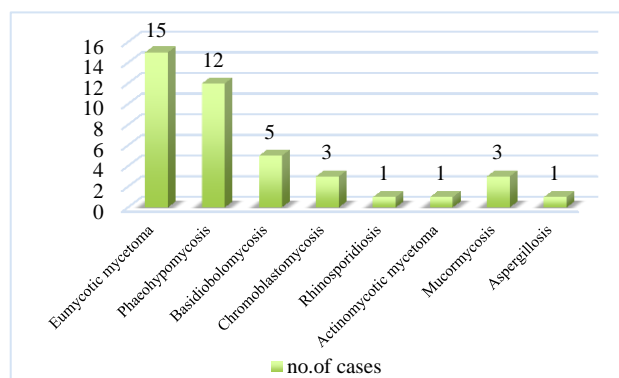


Figure 1: Various deep mycoses reported in this study.

Subcutaneous mycoses were present in 37 patients (90.2%), while opportunistic mycoses were seen in 4 (9.7%) patients. Mycetoma was the most common subcutaneous mycoses as seen in 16 (43.2%) patients, of which 15 (93.7%) of them had eumycotic and one (6.2%) had actinomycotic mycetoma. Phaeohyphomycosis was the second common subcutaneous infection seen in 12 (32.4%) patients followed by basidiobolomycosis in 5

(12.2%), chromoblastomycosis in 3 (7.3%) and rhinosporidiosis in 1 (6.2%) patient. Mucormycosis was the most common opportunistic fungal infection seen in 3

(75%) patients followed by aspergillosis in 1(25%) patient (Figure 1).

Table 1: Site of involvement in various mycoses.

Infection (n=41)	Most common site	Numbers	Percentage (%)	Less common site	Numbers
Mycetoma (16)	Feet	12	75%	Hand, gluteal region, face	4
Phaeohyphomycosis (12)	Lower leg/feet	8	66.6%	Lumbar region, hand	4
Basidiobolomycosis (5)	Gluteal region, thighs	3	60%	Genitalia, lower abdomen and thighs	2
Chromoblastomycosis (3)	Legs	2	66.6%	Forearm	1
Mucormycosis (3)	Paranasal sinuses	2	66.6%	Hard palate	1
Aspergillosis (1)	Nasal cavity and maxillary sinuses				
Rhinosporidiosis (1)	Back, both lower legs, left palpebral conjunctiva, nasal and laryngeal mucosa				

Table 2: Findings observed in KOH wet mount in various mycoses.

Infections	KOH findings	Number
Eumycotic mycetoma	Black grain	15
Actinomycotic mycetoma	White grain	1
Phaeohyphomycosis	Moniliform hyphae	12
	Dematiaceous, moniliform hyphae	8
Chromoblastomycosis	Sclerotic bodies	3
Rhinosporidiosis	Sporangia with endospores	1
Mucormycosis	Mucorales-broad ceonocytic hyphae	3
Aspergillosis	Hyaline thin branched septate hyphae	1

Table 3: Culture results in this study

Organisms isolated	Numbers	Macroscopic appearance [modified SDA agar]	Microscopic appearance in LPCB mount
<i>Madurella mycetomatis</i>	7	The colony colour ranged from white to yellowish brown, powdery with brown diffusible pigment into the media. Reverse was dark brown. [Figure 4A]	Septate hyphae with numerous chlamydo spores [Figure 4B]
<i>Madurella grisea</i>	1	Dark grey in colour with radial grooves on the surface. Reverse was dark brown.	Wide branching septate hyphae with few chlamydo spores.
<i>Actinomyces madurae</i>	1	White to cream coloured colonies with wrinkled surface.	Thin branching filaments with conidia.
<i>Phialophora verrucosa</i>	9	Colour of colonies varied from dark greyish white to brown or brownish black. Surface was either granular, folded or flat. Reverse was black	Branched septate vase shaped conidiophore bearing masses of oval conidia
<i>Phialophora compactum</i>	1	Black heaped brittle colonies with reverse black pigmentation	Branched septate, cladosporium type of conidiophore with chains and masses of round conidia
<i>Aeuriobasidium pullulans</i>	1	Greyish black granular colonies with reverse black pigmentation	Thick walled, dark, closely septate with few budding elliptical conidia
<i>Rhizopus arrhizus</i>	2	Dense grey cottony mycelia which quickly covered the agar surface. Reverse was white.	Rhizoids at the point where stolon meets the sporangiophore. Sporangium terminated in sporangium containing columella and pores.
<i>Aspergillus fumigatus</i>	1	Smoky green powdery colonies. Reverse was red brown.	Smooth conidiophores, phialides in single row covering half of the vesicle [Figure 4C].

Table 4: Comparison of various studies of phaeohyphomycosis infection in renal transplant recipients.

Variables	Schieffelin et al study(USA) (1988-2009) ¹⁶	George et al study (CMC Vellore) (2002-2003) ¹⁷	This study (2015-2016)
Incidence of phaeohyphomycosis in renal transplant recipients	3.6%	1.5%	7%
Average duration between renal transplantation and phaeohyphomycosis	20 months	30 months	36 months
Most common organism	<i>Exophiala jeanselmi</i>	-	<i>Phialophora verrucosa</i>
Most common site of involvement	Hand	Foot	Foot
Most common clinical presentation	Nodules or cyst	Cyst	Cystic swelling



Figure 2: (A) Eumycotic mycetoma- swelling of right foot with nodules and draining sinuses; (B) Eumycotic mycetoma- overnight saline gauze showing serosanguinous discharge and black grains; (C) Actinomycotic mycetoma- X- ray right hand- osteosclerosis in 2nd, 3rd and 4th metacarpal bones; (D) Phaeohyphomycotic cysts over left hand; (E) Chromoblastomycosis- multiple verrucous plaques in right forearm; (F) Basidiobolomycosis- swelling of right gluteal region and thigh with scar of previous incision and drainage; (G) Rhinosporidiosis- erythematous polypoidal plaque in right leg; (H) Mucormycosis- palatal perforation with eschar; (I) Aspergillus fungal sinusitis- hypertelorism due to broadening of the nose.

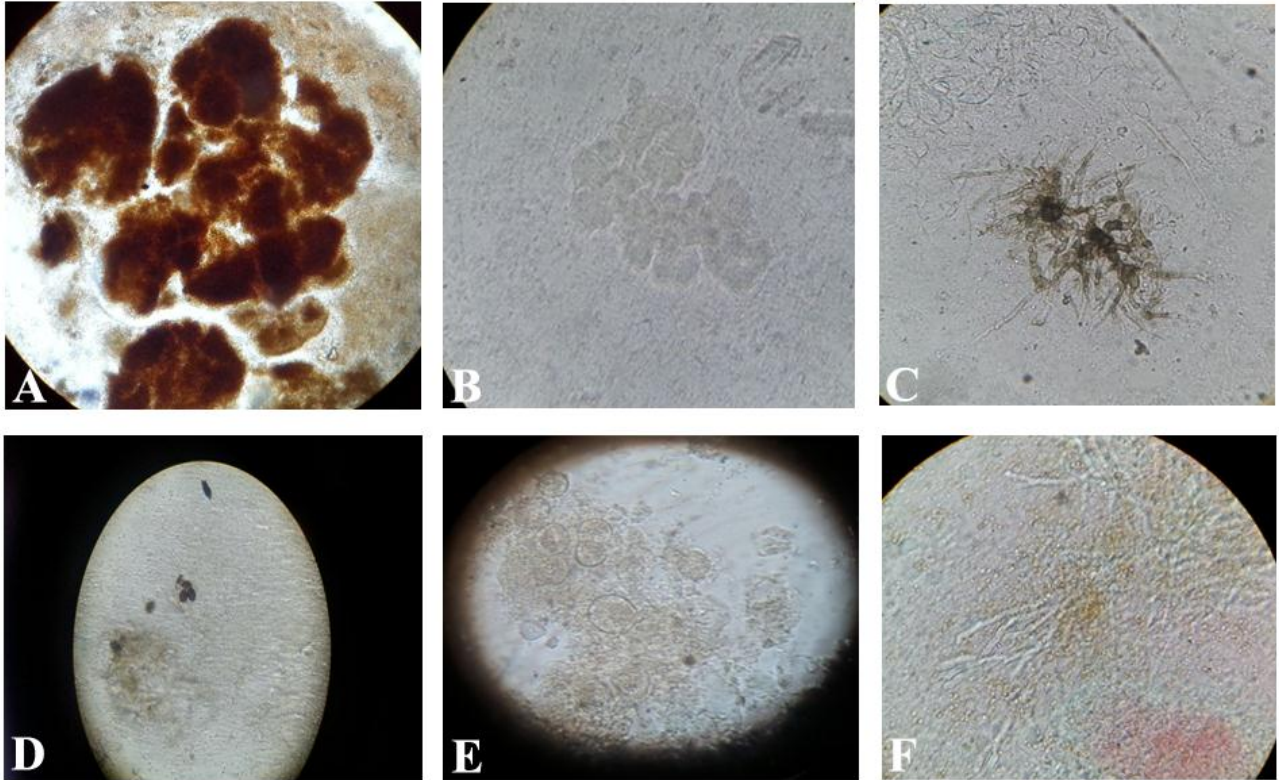


Figure 3: (A) Eumycotic grain– KOH wet mount in 40X showing multiple black grain with hyphae in the periphery; (B) Actinomycotic grain- KOH wet mount in 40X showing multi lobulated pale grain with scalloped margins; (C) KOH wet mount of phaeohyphomycosis- pigmented and hyaline moniliform septate hyphae; (D) KOH wet mount of chromoblastomycosis-sclerotic bodies; (E) KOH wet mount of rhinosporidiosis- multiple sporangia with endospores; (F) KOH wet mount of mucormycosis- broad ribbon like non septate hyphae with wide angled branching.

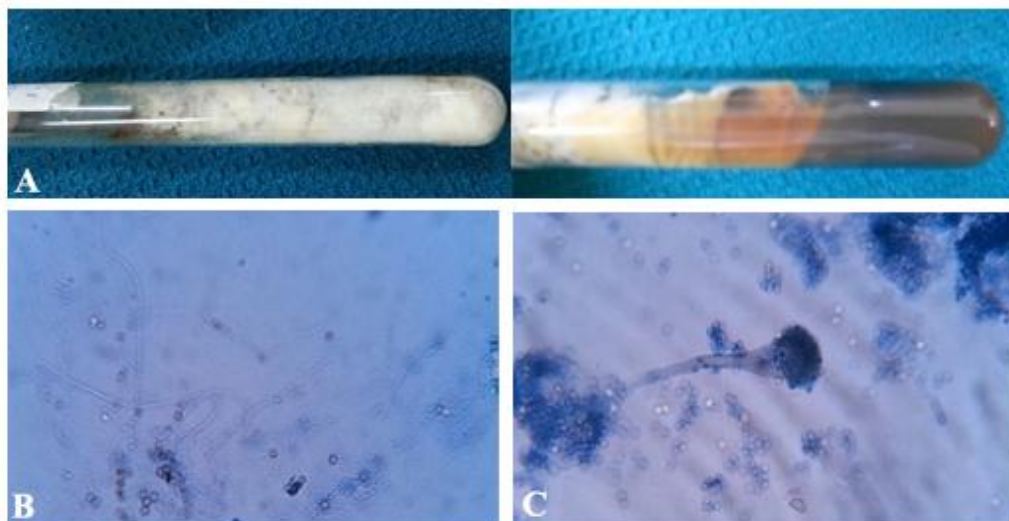


Figure 4: (A) Macroscopic appearance of *M. mycetomatis*- white powdery colony with reverse black pigmentation; (B) Lacto phenol cotton blue mount of *M. mycetomatis*- septate hyphae with numerous chlamydo spores; (C) Lacto phenol cotton blue mount of *A. fumigatus*– smooth conidiophores with single row of phialides covering the upper half of the vesicle.

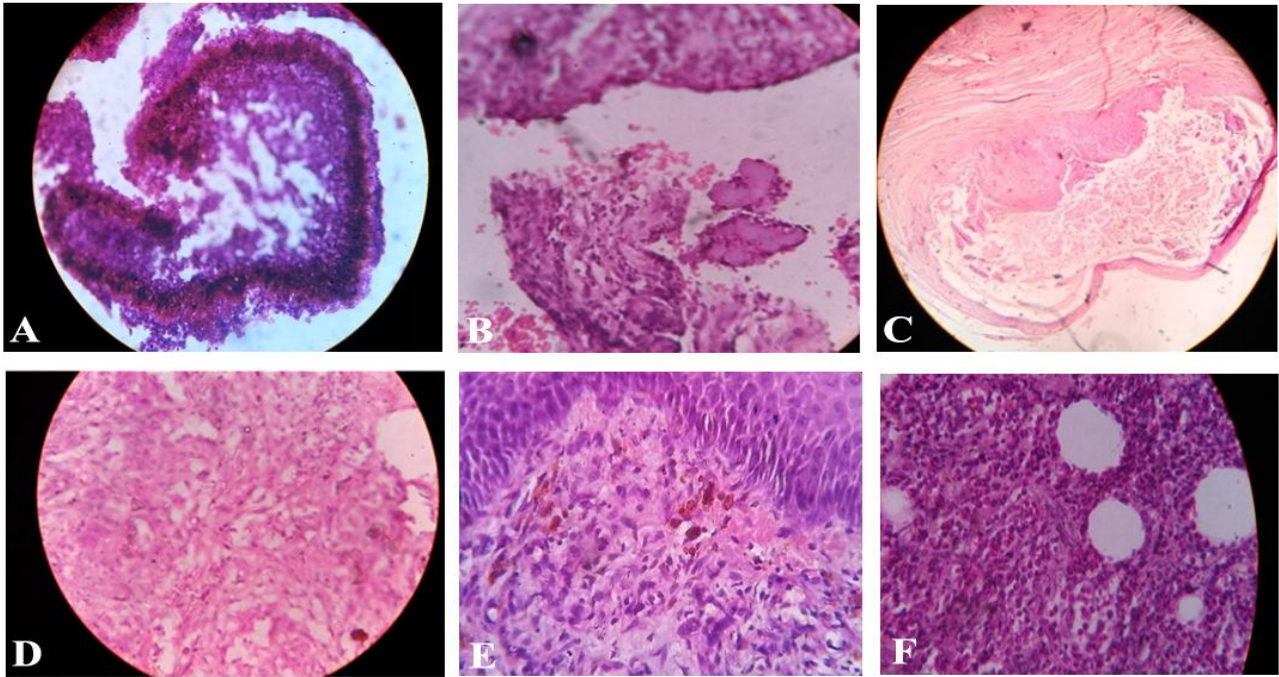


Figure 5: (A) H & E section of eumycotic mycetoma showing eumycotic black large grain of *M. mycetomatis* with radial, parallel hyphae and chlamydospores in the periphery *M. mycetomatis* and irregularly arranged hyphae in the centre; (B) H & E section of actinomycetoma showing pale grain of *A. madurae*- multilobulated grain with dark basophilic mantle in the periphery and pale basophilic homogenous centre surrounded by eosinophilic fringe of Splendore Hoeppli phenomenon; (C) H & E section of phaeohyphomycosis- cyst- cyst wall with central necrosis and surrounding fibrosis; (D) H & E section of phaeohyphomycosis –multiple irregular septate hyphae and yeast like cells; (E) H & E section of chromoblastomycosis showing the sclerotic bodies; (F) H & E section of basidiobolomycosis- septal panniculitis with eosinophilic infiltrates.

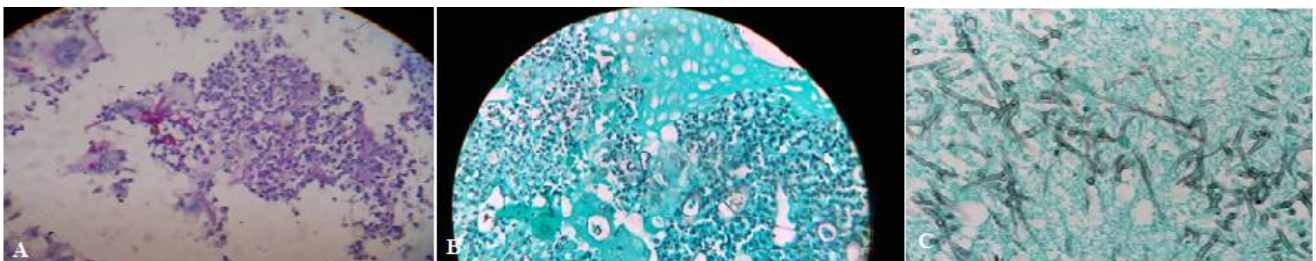


Figure 6: (A) HPE in PAS of phaeohyphomycosis showing pink colored septate moniliform hyphae; (B) HPE in GMS of basidiobolomycosis – fungal hyphae seen as black hollow tubes against green background; (C) HPE in GMS of aspergillosis- black colored thin septate hyphae with acute angled branching against green background.

Most common site affected was the lower extremity (28 patients- 68.3%) followed by upper extremity (5 cases – 12.2%), nasal cavity (3 cases- 7.3%), lumbar region (2 cases- 4.9%). Face, hard palate and genitals was involved in 1 patient each (2.4%). Table 1 depicts the site affected in the various mycoses.

Subcutaneous mycoses

Mycetoma: All the 16 patients had the characteristic presentation of swelling, nodules and draining sinuses (Figure 2A). Duration of infection varied from 3 months to 20 years. Out of the 15 patients with eumycetoma, 11

(73.3%) gave history of discharge of black grains. The discharge was serosanguinous with black grains (Figure 2 B) in patients with eumycetoma while there was purulent discharge in the patient with actinomycetoma. Pedal involvement was seen in 12 (75%) patients while the remaining 4 patients had extra pedal involvement of gluteal region (13.3%), hand (6.6%) and face (6.6%). Radiological investigations like X-ray, CT and MRI of the involved sites revealed bony involvement in 10 (62.5%) patients. Of the 15 patients with eumycetoma, 9 (60%) patients had osteolysis, osteoporosis and fusion of small bones of foot while one patient with lesions over the face had osteolysis of temporal bone with invasion of

underlying dura and brain matter. Osteosclerosis of 3rd and 4th metacarpal bones was seen in the patient with actinomycotic mycetoma (Figure 2C).

Phaeohyphomycosis: Duration of infection ranged from 2 months to 3 years. Among the 12 patients, 8 (66.7%) were immunocompromised and 4 (33.3%) were immunocompetent. Of the 8 patients with immunosuppression, 3 (37.5%) had diabetes mellitus, 3 (37.5%) were post renal transplant and 2 (25%) were post renal transplant with diabetes mellitus. Incidence of phaeohyphomycosis among the post renal transplant patients was 7% (5 out of 72). Average duration between renal transplant and onset of phaeohyphomycosis was 2.5 years. Cystic swelling was the most common clinical presentation seen in 11 (91.7%) patients (Figure 2D) while one patient had cyst and multiple nodules. Out of the 11 patients with cyst, 9 (81.8%) had single cyst while the remaining 2 (18.2%) had 2 cysts. Two (16.6%) patients presented with recurrence of lesions after surgical excision.

Chromoblastomycosis: Duration of infection ranged from 3 months to 3 years. Among the 3 patients, 2 (66.6%) presented with hypertrophic plaques with verrucous surface. Both had multiple lesions with moderate severity (Figure 2E). The third patient had been referred after surgical excision of a single ulcerative growth over the right leg.

Basidiobolomycosis: All the 5 patients were children in the age group between 3-6 years. They presented with well-defined swelling with rounded margins, with insinuation of fingers beneath the margins being possible and hyperpigmentation of skin surface (Figure 2F). Duration varied from 2 to 8 months. They were all referred after biopsy with the diagnosis of granulomatous inflammation. Average delay between the onset of infection and correct diagnosis was 5 months.

Rhinosporidiosis: The single patient with rhinosporidiosis was a 61 year old male patient from an endemic area, with features of erythematous polypoidal plaque over back, both lower legs, left upper palpebral conjunctiva, laryngeal and nasal mucosa (Figure 2G).

Opportunistic mycoses

Mucormycosis was present in three patients, of which 2 were diabetic and one had hepatitis C infection. Rhino orbito cerebral mucormycosis was the most common type (66.6%) seen in 2 patients (Figure 2H), with mortality rate of 33.3%. Maxillary sinus was involved in 2 patients, while the third patient had palatal perforation with necrosis. Aspergillosis was present in a 13 year old boy who presented with bilateral nasal obstruction and nasal discharge of 1 month duration. Initial diagnosis was allergic fungal sinusitis which on investigation was proven to be aspergillosis (Figure 2I).

Potassium hydroxide (KOH) wet mount

Of the 41 patients, 5 children with basidiobolomycosis were referred after biopsy and hence both KOH and culture could not be done. KOH wet mount direct microscopic examination, done in the remaining 36 patients was 100% positive (Table 2) (Figures 3A–F). Gram stain, Leishman stain and acid fast bacilli staining were the other stains done in patients with mycetoma. Gram positive filaments of high bacterial dimension was observed in the patient with actinomycetoma while the AFB stain was negative.

Culture

Among the 36 adult patients, 1 patient with chromoblastomycosis was referred after surgery. As *Rhinosporidium seeberi* is not cultivable, culture was done in the remaining 34 patients. Out of 34 patients, 23 (67.6%) had positive culture (Table 3) (Figures 4A–C).

Histopathology

Histopathological examination in H&E confirmed the clinical diagnosis in 27 (77.1%) patients. Biopsy was not done in six patients (4 patients with phaeohyphomycosis did not turn up for excision and 2 others were on medical management). Rest of the patients (8) required special stains– PAS and GMS for the confirmation of the diagnosis. Histopathology of eumycotic mycetoma revealed the presence of black grains either within the micro abscess or as fragmented grains. *Madurella mycetomatis* grains were seen as brown to black grains with radially arranged brown septate hyphae with or without chlamydozoospores in the periphery (cortex) and irregularly arranged hyphae in the medulla (Figure 5A). In actinomycetoma, multi lobulated grains with homogenous deeply stained basophilic periphery and slightly pale basophilic centre and fringe of radiating eosinophilic clubs (Splendore Hoeppli phenomenon) at the periphery. The grains were surrounded by inflammatory infiltrate consisting of predominately neutrophils, eosinophils and mononuclear cells (Figure 5B). In phaeohyphomycosis, cystic lesions were found to have a fibrous capsule with granulomatous reaction showing vascular proliferation, fibrosis, and inflammatory infiltrates (Figure 5C and D). The fungal elements were seen as pigmented septate moniliform hyphae, hyaline hyphae and yeast cells. Mycopathology of chromoblastomycosis in all the 3 patients showed hyperkeratosis, massive acanthosis, pseudoepitheliomatous hyperplasia, fibrosis, characteristic sclerotic bodies and inflammatory infiltrate consisting of neutrophils, mononuclear cells, epithelioid cells, plasma cells and multi-nucleated giant cells (Figure 5E). In all the 5 children with basidiobolomycosis, eosinophilic granuloma was present with fungal hyphae being seen as tubes and hollows surrounded by Splendore Hoeppli phenomenon. Dermal fibrosis and septal panniculitis were seen in all five patients (Figure 5F). Histopathology

of rhinosporidiosis showed epidermal hyperplasia, papillomatosis with numerous thick walled sporangia in varying stages of maturation. Inflammatory infiltrate consisting of lymphocytes, plasma cells and giant cells were seen. In the 3 patients with mucormycosis, necrosis with acute and chronic inflammatory infiltrates around the blood vessels and numerous broad, branching coenocytic hyphae were found in dermis. In the child with aspergillosis, mycopathology revealed hyaline, thin septate hyphae with plenty of eosinophilic infiltrates. Special stains like PAS and GMS were done to diagnose the infection in which mycopathological findings in H & E were not contributory, and to confirm the diagnosis in patients who had positive findings in H & E. (Figure 6 A-C).

DISCUSSION

Subcutaneous mycoses are more prevalent in the tropical and subtropical regions with hot and humid climate. These infections are more common in the adult males from the rural areas who are predisposed to trauma and contact with soil and decaying vegetation. Among the 41 patients, 73.2% were males and 26.8% were females. The ratio of male to female was 2.7:1 which was in accordance to the earlier studies by Kindo et al and Yahya et al who reported a ratio of 3:1.^{5,6} The frequency of subcutaneous fungal infections was more in males, which is due to the fact that they were more exposed to an environment, conducive to the spread of organisms. The most common age group affected was 41-50 years (29.2%). The youngest age affected in this study was a 3-year-old boy with subcutaneous zygomycosis, whereas the youngest age group in Kindo et al study was 9 year old child with candidiasis.⁵ In the study by Bhat et al, the youngest age was 14 years and the mean age of patients with subcutaneous infection was 49.4 years but in this study the mean age was 38.4 years.⁷ The differences in the youngest age can be explained by the fact that, there were no children with basidiobolomycosis reported in those studies. The most common occupation associated with deep mycoses in this study was agriculture (50%) in concordance with 52.8% reported by Yun et al.⁸ Bhat et al also observed agriculture to be the main occupation.⁷ In this study, 63.4% of patients were from rural areas while 19.5% were from semi urban areas, similar to the observation by Bhat et al that subcutaneous mycoses were more prevalent in rural areas.⁷ This is explained by the fact that subcutaneous mycoses result due to the inoculation of saprophytic fungi present in the soil, decaying vegetation and the rural population, especially the adult males are more exposed to such an environment.⁹ History of trauma was present in 48.8% of patients in this study, close to 41.2% reported by Yun et al.⁸ Most often, patients with subcutaneous mycoses which are characterised by long incubation periods, do not recall the trivial trauma. The duration of illness in subcutaneous infections ranged from 2 months to 20 years in this study which was consistent with the study by Bhat et al in which duration of illness was about 4

months to 20 years.⁷ Systemic comorbidities associated in this study were diabetes mellitus (63.6%) followed by renal transplantation (45%). Kindo et al too reported diabetes mellitus to be the most common co-morbidity.⁵ The lower extremities were the most common site affected in 68.3% of patients in this study similar to the observation of 64% lower limb involvement by Bhat et al.⁷

Among the subcutaneous mycoses, mycetoma (43.2%) was the most common infection similar to the study done at Jakarta (50%).⁶ However, it is in contrast to the studies from Mangalore (Bhat et al) and Assam (Bordoloi et al) in which chromoblastomycosis was the commonest subcutaneous fungal infection seen in 64% and 40% of patients respectively.^{7,10} Sporotrichosis was the most common subcutaneous mycosis encountered in Tanda at Himachal Pradesh.¹¹ Phaeohyphomycosis was the second most common infection (32.4%) in this study, whereas actinomycotic mycetoma and sporotrichosis were the second common infections seen in 16% of patients each, in the Mangalore study.⁷ These findings show that there is varied prevalence of subcutaneous mycoses in different parts of India. This could be attributed to varying factors like environment, climate, rainfall, occupation and even habit of bare foot walking which is more common among the agricultural workers in Tamil Nadu.

Mycetoma

In India, actinomycotic mycetoma is more common (54.3%-83.3%) in most of the states, except in Rajasthan where eumycetoma is the most common (62.5%) type of mycetoma.¹² However, eumycotic mycetoma was the most common mycetoma (93.8%) observed in this study which is in contrast to the literature and the observation in the previous study from the same institution by Kaliswaran et al who reported actinomycotic mycetoma as the most common mycetoma (72%) followed by eumycetoma (28%).¹³ This could be explained by the fact that with the advent of broad spectrum antibiotics and effective therapeutic regimens, majority of the patients with actinomycetoma get treated in the periphery and only the patients with the difficult to treat eumycetoma get referred to tertiary institutions like this study centre. In this study, one patient with eumycotic mycetoma over face had invasion of temporal bone and brain parenchyma. Beeram et al also reported a case of eumycotic mycetoma with parietal lobe involvement.¹⁴ *Madurella mycetomatis* was the most common organism isolated similar to the study by Bakshi et al.¹⁵

Phaeohyphomycosis

The overall incidence of phaeohyphomycosis among the solid organ transplant recipients in this study was 7% which was higher than that reported by Schieffelin et al (3.6%) and George et al (1.5%) (Table 4).^{16,17} This could be due to the increase in the number of solid organ transplantations in the recent years and the

immunosuppressants used in these patients, which predisposes them to develop phaeohyphomycosis.¹⁸ Severo et al from Brazil also reported an increase in the incidence of phaeohyphomycosis in the last decade and the increase was attributed to iatrogenic immunodeficiency.¹⁹ Drugs like tacrolimus used as immunosuppressant in post renal transplant (PRT) patients is said to provide protection against fungal infection, but the antifungal activity of this drug is decreased in cooler sites like hands and feet. This explains why phaeohyphomycosis was more common in feet in PRT patients in this study.¹⁶ *Phialophora verrucosa* was the most common organism isolated in this study whereas *Exophiala jeanselmei* was the commonest organism reported in the studies by Schieffelin et al and Severo et al.^{16,19} This could be due to the geographical variation in the prevalence of organisms in different parts of the World. Unlike the study by Sheikh et al, foreign bodies like splinter were not observed in this study.²⁰

Chromoblastomycosis

Hypertrophic plaques with verrucous surface was the common presentation in this study similar to the observation by Agarwal et al.²¹ Two out of 3 (66%) patients in this study had more than one lesion whereas 69% of patients presented with single lesion in the study by Pradhan et al.²² In this study, 2 (66.6%) patients had moderate disease and one patient had mild disease. Assessment of severity is important, as the most severe forms are either slow responsive or even become totally non-responsive to antifungal treatment. Complications like lymphedema, ankyloses are more common with more severe forms.²³ There was no culture positivity which could have been due to secondary bacterial infections.

Basidiobolomycosis

This most common subcutaneous mycoses in children, which is endemic in south India is most often misdiagnosed as chronic abscess by family physicians. Though this infection has a very characteristic clinical feature, high degree of clinical suspicion is required for an early diagnosis. Histopathological examination showed predominant eosinophilic infiltrates in dermis and subcutis. This could be explained by the Th2 immune response with release of IL-4 and IL-10 that recruit eosinophils to site infected with basidiobolomycosis.²⁴ The diagnosis of basidiobolomycosis in this study was made mainly on clinical grounds and confirmed by mycopathology, as all the patients had been referred after biopsy.

Rhinosporidiosis

Our lone patient diagnosed with disseminated cutaneous rhinosporidiosis with nasal and ocular involvement was an immunocompetent patient who hailed from an endemic area.

Mucormycosis and aspergillosis

There were only 4 systemic opportunistic mycoses reported in this study. This was because of the fact that specimens were also sent to the department of microbiology and only few cases get referred to mycology section for confirmation of diagnosis. Of the 3 patients with mucormycosis, one patient with rhino orbital cerebral involvement had uncontrolled diabetes mellitus as discussed by Chakrabarti et al.²⁵ In this study, the patient who had allergic fungal sinusitis due to *Aspergillus fumigatus* had past history of chronic rhinosinusitis. The study by Kim et al also showed that chronic rhinosinusitis was an important predisposing factor for allergic fungal sinusitis.²⁶ There were no endemic mycoses observed during the study period.

CONCLUSION

Mycetoma is the most common subcutaneous mycoses in this part of India. Incidence of deep mycoses is on the rise especially phaeohyphomycosis which has increased not only in post renal transplant patients, but also in immunocompetent individuals. This highlights the significance of proper disposal of dead and decaying vegetation, which is the main source for the saprophytic fungi causing deep mycoses. Simple potassium hydroxide examination will help to clinch the diagnosis, provided care is taken in the sample collection. It becomes imperative to be well versed with varied clinical presentations, mycological and histopathological features of deep mycoses so that an early diagnosis and prompt treatment will reduce the morbidity and improve the quality of life of these patients who require long term medication.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Sivayogana R, Madhu R, Ramesh A, Dhanalakshmi UR. A prospective clinico mycological study of deep mycoses in a tertiary centre in Tamil Nadu. *Int J Res Dermatol* 2018;4:126-35.