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Clinicopathological study of pigmented fungal lesions with special reference to subcutaneous phaeohyphomycosis: A 3-year study in a tertiary care hospital of Eastern India

Rajashree Pradhan[1], Suman Chatterjee[2], Bidisha Chakraborty[3], Sajeeb Mondal[4]

[1]Associate Professor, Department of Pathology, College of Medicine and Sagore Dutta Hospital, Kolkata, [2]Assistant Professor, Department of Biochemistry, [4]Associate Professor, Department of Pathology, Rampurhat Government Medical College, Rampurhat, [3]Consultant Pathologist, Suraksha Diagnostics, Kolkata, West Bengal, India

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ABSTRACT

Background: Fungal infections are predominantly opportunistic infections in immunocompromised patients and in immunocompetent adults with a history of trauma. Recently, there is an increase in trends of fungal infections in immunocompetent individuals also without any history of trauma. Pigmented fungi are a group of fungi that have pigment in their cell wall and also known as dematiaceous fungi. These pigmented fungi include phaeohyphomycosis with a differential diagnosis of chromoblastomycosis, cryptococcosis, mycetoma, and aspergillus. Phaeohyphomycosis is a subcutaneous or systemic infection characterized histologically by dark-colored fungal hyphae and yeast forms (black yeast). **Aims and Objectives:** The aim of the present study is to analyze the clinicopathological features of pigmented fungal infections in biopsy specimen based on their histomorphology. **Materials and Methods:** This was a prospective study in which we have analyzed the dematiaceous fungal species by their histomorphological characteristics in biopsy specimens. **Results:** A total of 21 cases of biopsy specimens (with the clinical presentation of cutaneous/subcutaneous lesions) were studied histologically which showed 13 cases of phaeohyp homycosis, seven cases of eumycotic mycetoma, and one case of chromoblastomycosis. There was male predominance (17, 80.95%) and the majority of the patients were >60 years (16, 76.19%). Most common site of infection was foot with predominant clinical presentation as subcutaneous mass lesion. **Conclusion:** All the cases of dematiaceous fungal infections were diagnosed by histopathological examination. Melanin in the cell wall of dematiaceous fungi is the pathogenic factor. Phaeohyphomycosis is no longer considered as a rare infection rather there is an increasingly incidence of recognized infection. Although culture and histopathological study remain important tools for final diagnosis, various molecular studies might be helpful for accurate diagnosis.

Key words: Pigmented fungi; Melanin; Phaeohyphomycosis; Histomorphology

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INTRODUCTION

Fungal infection also known as mycosis is a disease caused by fungi.

Fungal infections may be superficial, subcutaneous, or systemic.[1] Superficial fungal infections include tinea, subcutaneous type includes eumycetoma, phaehyphomycosis, and chromoblastomycosis,[2] and systemic fungal infections include mucor mycosis, cryptococcosis, histoplasmosis, and aspergillosis.[1]

Fungal infections more commonly affect immune compromised persons such as people having HIV/AIDS

Address for Correspondence:
Dr. Sajeeb Mondal, Associate Professor, Department of Pathology, Rampurhat Government. Medical College, Rampurhat, West Bengal, India. **Mobile:** +91-8777561521. **E-mail:** sjbmondal@gmail.com

infection and in people receiving steroids or cancers chemotherapy.[3]

An estimated 1.7 million deaths from fungal diseases were reported in 2020.[4]

Pigmented fungi include a diverse group of fungal infections which include Phaeohyphomycosis with a differential diagnosis of chromoblastomycosis, cryptococcosis, mycetomas, and aspergillosis.

Phaeohyphomycosis is caused by dematiaceous fungi whose morphological characteristics in tissue include hyphae, yeast-like cells, or a combination of these.[5]

The "term phaeohyphomycosis" was introduced to determine infections caused by dematiaceous (pigmented) fungi that contain melanin in their cell walls.[6] Fungal melanin is thought to be a virulence factor. The outcome of antifungal treatment is poor with mortality rate almost up to 80%.[7] Phaeohyphomycosis can be associated with an assay of melanistic filamentous fungi which include, alternaria species, rhinocladiella species, and exophiala species.[8] Although uncommon the number of cases of phaeohyphomycosis reported is increasing in recent years.

The aim of the present study to analyze the clinicopathological features of pigmented fungal infections in biopsy specimen based on their histomorphology.

Aims and objectives

To analyze the clinicopathological features of pigmented fungal infections in biopsy specimen basing on their histomorphology.

MATERIALS AND METHODS

Study design, duration and place of study

This was a retrospective study conducted in the Department of Pathology from January 2020 to June 2023.

Inclusion criteria

All patients having infection ranging from superficial skin infection, cutaneous mass lesions, subcutaneous cysts, and disseminated infection were included in the study.

Exclusion criteria

Fungal infections detected in autopsy specimens and in COVID-19 patients were excluded from the study.

Specimen handling

The surgical specimens (biopsy from the lesion) received were grossly examined and sections were submitted for routine histopathological processing. All the slides were stained with hematoxylin and eosin (H and E) and Periodic Acid-Schiff stain. Those cases in which organisms could not be identified optimally in the initial sections, multiple deeper sections were examined.

Data collection and presentation

A detailed case history examination and other relevant clinical details such as occupation and history of trauma were collected from the clinical records.

All the surgical specimens that were diagnosed with pigmented fungal infections were retrospectively and categorized according to age, sex, site of involvement, clinical presentation and histomorphological diagnosis.

Statistical analysis

IBM software version SPSS 20.0 was used for data analysis. All the findings were represented as numbers (n) and percentages (%).

Ethical clearance

The study was approved by the Institutional Ethics Committee (IEC).

RESULTS

Out of 21 cases studied 16 (76.19%) were >60 years of age with a male predominance (17, 80.95%) (Table 1).

History of trauma was present in 7 cases (33.33%) with diabetes as the most common associated comorbid condition (10, 47.61%) (Table 2).

Clinically, the most common site involved was foot (13, 61.9%), with the presentation of subcutaneous mass lesion (10, 47.61%) (Table 3).

Final diagnosis was given as phaeohypomycosis (13, 61.9%) and eumycotic mycetoma (8, 38.09%) (Table 4).

DISCUSSION

Fungal infections have a worldwide distribution affecting more than one billion people every year.[5] Despite its associated mortality, several fungal infections including mycetoma, sporotrichosis, and chromoblastomycosis are neglected.[9]

Pigmented fungi also known as dematiceous fungi are characterized by the presence of melanin in their wall.[7]

Dematiceous fungi are associated with a variety of clinical syndromes. Due to the diversity of infections, it has been a

great challenge to develop useful and consistent guidelines for management.[10]

Based on the histomorphological features in biopsy specimens, pigmented fungi are grouped into three broad categories:

1. Phaeohypomycosis,
2. Eumycotic mycetoma,
3. Chromoblastomycosis.

Table 1: Age and sex of the patients (n=21)

Parameters	Number	Percentage
Age		
>60 years	16	76.19
<60 years	5	23.8
Sex		
Male	17	80.95
Female	4	19.04

Table 2: History of trauma and comorbid conditions

	Parameters	Number	Percentage
A)	H/O trauma		
	Yes	7	33.33
	No	14	66.66
B)	Associated comorbid conditions		
	DM	10	47.61
	On steroid R_x	6	28.57
	Organ transplantation	1	4.76
	On anticancer therapy	1	4.76
	None	3	14.28

Table 3: Clinical date of the patients

Parameters	Number	Percentage
Site of involvement		
Foot	13	61.9
Ankle	3	14.28
Hand	3	14.28
Forearm	2	9.52
Clinical presentation		
Subcutaneous mall lesion	10	47.61
Cystic lesion	3	14.28
Abscess	5	23.8
Lesion with draining sinuses	3	14.27
Provisional clinical diagnosis		
Soft tissue mass	10	47.61
Lipoma	3	14.28
Ganglion cyst	3	14.28
Mycetoma	3	14.28
Abscess	2	9.52

In histosections, Eumycotic mycetoma is characterized by black grains and splender hopellei phenomenon (Figure 1).

Chromoblastomycosis is seen as pigmented (golden brown) yeasts resembling "copper pennies".

Phaeohypomycosis is characterized by the presence of pigmented (dark brown) septate hyphae, Pseudohyphae, and yeasts (Figure 2).[11]

The term Phaeohypomycosis was introduced by Ajello et al., in 1974.[6] According to the modified classification by Rippon,[12] phaeohypomycosis can be divided into five types:

i) Superficial (black piedra and tinea nigra),
ii) Cutaneous (dermatomycosis and onychomycosis), mycotic keratitis,
iii) Subcutaneous (phaeohypomycotic cyst),
iv) Invasive, systemic and
v) Cerebral.

One of the most common types of lesions in phaeohypomycosis is subcutaneous cysts on abscess which presents clinically as a discrete, asymptomatic, and well-encapsulated subcutaneous mass in contrast to the eumycotic mycetoma in which draining sinus tracts with granules seen (Figure 3 a and b).

The differential diagnoses include ganglion cyst, epidermal inclusion cyst, baker cyst, foreign body granuloma, erythema nodosum, and benign neoplasms like lipoma and neurofibroma.[13]

In our study out of the total 21 cases, 13 cases were phaeohypomycosis and eight cases were diagnosed as eumycotic mycetoma basing on their histomorphological characteristics in tissue sections.

In our study, most of the patients were in 50–70 years age group which was similar to the study by Revankar.[14] Out of 21 cases, 15 were male and six were female, which was in contrast to the study by Ritter et al.,[15] which should female predominance.

In our study, the patients presented clinically as subcutaneous mass lesions in 16 cases and superficial skin infection in five cases. The most common site was the foot, followed by forearm and hand.

Table 4: Microscopic findings of the patients (n=21)

	Microcscopic findings	Final diagnosis	Number	Percentage
A)	Darkly pigmented yeast like cells, Hyphae, and pseudohyphae	Phaehyphonmycosis	13	61.9
B)	Suppurative granuloma, with characteristic dark grains and thick club shaped structures.	Eumycotic Mycetoma	8	38.09

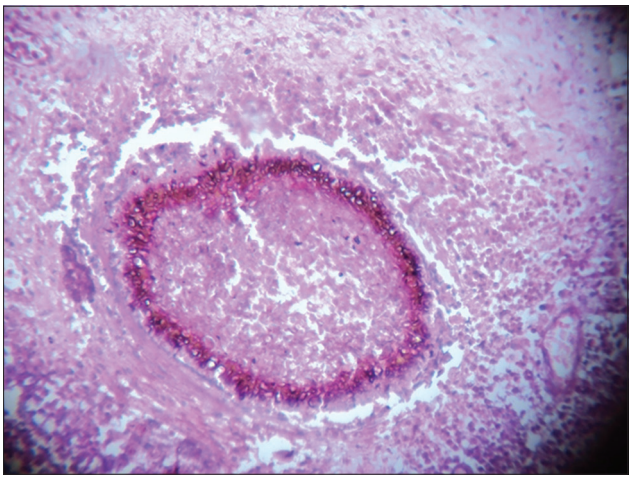

Figure 1: Tissue section showing histopathology of black grain eumycotic mycetoma (H and E, ×100)

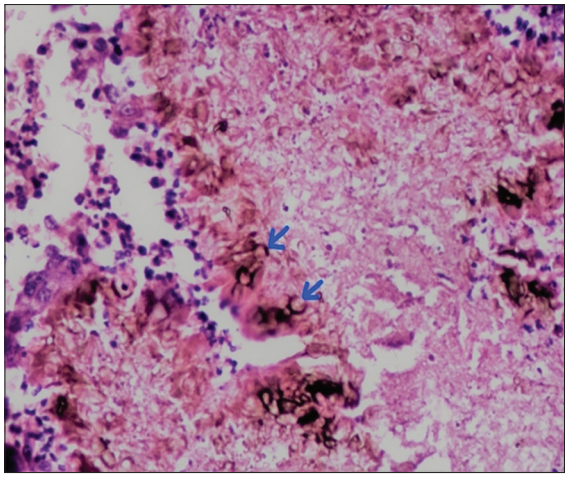

Figure 2: Histopathology of phaehyphomycosis, black yeast form (arrow) (H and E, ×100)

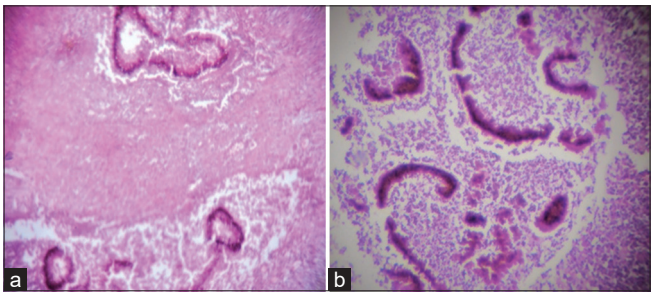

Figure 3: (a and b) Histopathology of phaeohyphomycosis – Exophiala (H and E, ×100)

This was similar to the study by Abraham et al.,[13] almost three-fourth of their cases were located on or near the hands on feet.

In our study, most of the patients (15%) had associated comorbid conditions such as diabetes, on corticosteroid therapy anti-cancer therapy, or post a history of organ transplantation. Out of this diabetes was seen in the majority of the patients. According to Abraham et al.,[14] out of Cases 3 were diabetic.

The diagnosis of subcutaneous phaeohypomycosis depends on Campos-Takaki and Jardim[16] histologic examination and culture. In our study, all the cases were diagnosed basing on the histomorphological features in tissue sections.

Now coming to the clinical significance of melanin in the cell wall of dematiceous fungi.

Fungal melanin is thought to be a virulence factor. The pathogenesis of the disease can be contributed by the following proposed mechanism.

1. In experimented animal models, disruption of specific genes involved in melanin production leads to markedly reduced virulence.[17]
2. Melanin may act as a virulence factor by scavenging free redicals and hypochlorite produced by phagocytic cells in the oxidative burst and these prevent killing of the organisms.[18]

Limitations of the study

1. Besides the routine hematoxylin and eosin stain, a special stain Fortana-Mallon stain which is specific for melanin should be used for diagnosis.[19] In our study, Fortana-Mallon stain was not done routinely in all cases.
2. No other genetic studies in the patients were done to assess the risk for the development of disseminated infection by these fungi.

Future perspective

1. Polymerase chain reaction of highly conserved regions of ribosomal DNA has the potential to be a useful technique for the identification of dematiceous fungi,[20] along with. It is and D_1/D_2 analysis is also helpful for the identification of this diverse group of fungi.[21]
2. Matrix-assisted Laser Desorption/Ionization-Time of Flight (MALDI-ToF) mass spectroscopy may also become an accurate method for dematiceous mold identification.[22,23]

CONCLUSION

Although phaeohypomycosis is an uncommon infection, there is increasing number of cases being reported in recent years.

Along with increase in incidence, there are diverse ranges of generic causing infections, when present as a subcutaneous swelling/mass lesion often mistaken for malignancy. In disseminated infection, prognosis is very poor. Since the pigment is not always evident and there are no specific

non-culture-based methods routinely available for diagnosis histopathological study of these fungi for morphology and special stains for melanin are extremely important for diagnosis.

With accurate diagnosis and prompt management, unfavorable outcomes could be negated.

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Authors Contribution:
RP- Conceptualization and writing original graft. **SM-** Project administration and Corresponding Author**. BC-** Supervision. **SC-** Writing - review and Editing.

Work attributed to:
College of Medicine and Sagore Dutta Hospital, Kolkata, West Bengal, India.

Orcid ID:
Rajashree Pradhan - https://orcid.org/0000-0001-6770-7367
Suman Chatterjee - https://orcid.org/0009-0005-4383-3365
Bidisha Chakraborty - https://orcid.org/0000-0002-1984-0793
Sajeeb Mondal - https://orcid.org/0000-0002-1597-8584

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