INTRODUCTION

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

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

Fungal infections more commonly affect immune compromised persons such as people having HIV/AIDS.
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.⁹ 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.\textsuperscript{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)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>16</td>
<td>76.19</td>
</tr>
<tr>
<td>&lt;60 years</td>
<td>5</td>
<td>23.8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>80.95</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>19.04</td>
</tr>
</tbody>
</table>

### Table 2: History of trauma and comorbid conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) H/O trauma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>33.33</td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>66.66</td>
</tr>
<tr>
<td>B) Associated comorbid conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>10</td>
<td>47.61</td>
</tr>
<tr>
<td>On steroid Rx</td>
<td>6</td>
<td>28.57</td>
</tr>
<tr>
<td>Organ transplantation</td>
<td>1</td>
<td>4.76</td>
</tr>
<tr>
<td>On anticancer therapy</td>
<td>1</td>
<td>4.76</td>
</tr>
<tr>
<td>None</td>
<td>3</td>
<td>14.28</td>
</tr>
</tbody>
</table>

### Table 3: Clinical date of the patients

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

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

<table>
<thead>
<tr>
<th>Microscopic findings</th>
<th>Final diagnosis</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Darkly pigmented yeast like cells, Hyphae, and pseudohyphae</td>
<td>Phaeohyphomycosis</td>
<td>13</td>
<td>61.9</td>
</tr>
<tr>
<td>B) Suppurative granuloma, with characteristic dark grains and thick club shaped structures.</td>
<td>Eumycotic Mycetoma</td>
<td>8</td>
<td>38.09</td>
</tr>
</tbody>
</table>

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”\textsuperscript{9}.

Phaeohypomycosis is characterized by the presence of pigmented (dark brown) septate hyphae, Pseudohyphae, and yeasts (Figure 2).\textsuperscript{11}

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

i) Superficial (black piedra and tinea nigra),
ii) Cutaneous (dermatomycosis and onychomykosis), mycotic keratitis,
iii) Subcutaneous (phaeohyphomycotic 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.\textsuperscript{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.\textsuperscript{14} Out of 21 cases, 15 were male and six were female, which was in contrast to the study by Ritter et al.,\textsuperscript{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.
This was similar to the study by Abraham et al., 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., out of Cases 3 were diabetic.

The diagnosis of subcutaneous phaeohyphomycosis depends on Campos-Takaki and Jardim's 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.
2. Melanin may act as a virulence factor by scavenging free radicals and hypochlorite produced by phagocytic cells in the oxidative burst and these prevent killing of the organisms.

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. 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, along with. It is and D1/D2 analysis is also helpful for the identification of this diverse group of fungi.
2. Matrix-assisted Laser Desorption/Ionization-Time of Flight (MALDI-ToF) mass spectroscopy may also become an accurate method for dematiceous mold identification.

CONCLUSION

Although phaeohyphomycosis 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
acknowledgments

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REFERENCES


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

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