Research Article

Cytopathological study of cutaneous and subcutaneous mycosis presenting as soft-tissue swellings: A 5-year retrospective study from a tertiary care center in South India

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INTRODUCTION

Cutaneous and subcutaneous mycosis can mimic skin and soft-tissue neoplasms clinically\textsuperscript{[1]}(Since fine-needle aspiration cytology (FNAC) is one of the diagnostic tools used in initial evaluation of these lesions, they can be easily missed unless the pathologist suspects and does appropriate special stains for fungus. The causative fungus varies with the geographic region and there are a limited number of studies on cytology of subcutaneous and cutaneous fungal infections from South India and etiological agents vary with geographic region, in this paper, we present clinical and cytological features of the same the objective of this study is to review and evaluate clinical and cytological features of subcutaneous and cutaneous mycosis with histopathological correlation wherever available.)

ABSTRACT

**Objectives:** Cutaneous and subcutaneous mycosis can mimic skin and soft-tissue neoplasms clinically and pose diagnostic challenge to pathologists on cytology. Since there are a limited number of studies on this topic from South India and etiological agents vary with geographic region, in this paper, we present clinical and cytological features of the same the objective of this study is to review and evaluate clinical and cytological features of subcutaneous and cutaneous mycosis with histopathological correlation wherever available.

**Materials and Methods:** This was an observational and retrospective study of 5-year duration. All cases diagnosed as mycosis on cytology were retrieved from pathology records. Cytology slides along with special stains for fungus were reviewed. Review of histopathology slides and culture correlation was done whenever available. Statistical analysis was done using frequencies and percentages.

**Results:** There were 39 cases during the study period (male – 34 and female – 5). On aspiration, all cases yielded pus; microscopy revealed necrotic debris in 39 cases, inflammatory infiltrate in 39 cases, epithelioid granulomas with multinucleated giant cells in 25 cases, and negative staining hyphal forms in 37 cases. Special stains for fungus showed septate hyphal forms suggestive of *Aspergillus* species in 34 cases, and yeast and pseudo hyphal forms of candida species in one case. A broad category as fungal infection without subtyping was given in four cases. Culture did not yield growth but fungus was identified on KOH mounts. Histopathology showed fungus in 13 of 14 cases done.

**Conclusion:** Subcutaneous mycosis should be suspected when aspiration yields pus and appropriate special stains must be done. *Aspergillus* species was the most common etiological agent in our study.

**Keywords:** Subcutaneous mycosis, *Aspergillus*, Soft-tissue swellings, Cytology
South India. In this paper, we present clinical and cytological features of subcutaneous and cutaneous mycosis from this part of the country with histopathological correlation wherever available.

MATERIAL AND METHODS

A descriptive, observational, and retrospective study of 5-year duration was done between June 2015 and May 2020 after obtaining approval from the Institutional Ethics Committee (IEC number GMC/IEC/366/2020). All cases diagnosed as mycosis on FNAC were retrieved from pathology records and slides were reviewed. The clinical details were obtained from the cytology requisition form. After FNA, alcohol-fixed smears were stained with hematoxylin and eosin (H&E) stain and air-dried smears were stained with Leishman stain. When fungal infection was suspected during the procedure or after seeing the cytology smears, additional smears were prepared and special stains for fungus such as periodic acid–Schiff stain (PAS) and Gomori methenamine silver (GMS) were done. In addition, Ziehl–Neelsen and Fite-Faraco stains were done to exclude tuberculosis and leprosy, respectively, if fungal stains were negative. Culture correlation was done whenever available. The specimens submitted for histopathology were received in formalin, and after routine processing, paraffin-embedded sections were stained with H&E stain. Special stains for fungus (PAS and GMS) were done whenever required.

Statistical analysis was done using frequencies and percentages.

RESULTS

There were a total of 39 cases during the study period of 5 years, of which 34 were male and five were female. The age range was between 35 and 75 years, with a mean age of 59 years.

The duration of the lesion ranged between 15 days and 4 years, with some (two cases) reporting sudden increase in size.

The common location of lesions was extremities [Table 1]. One case had two lesions, one in the forearm and another in the finger.

The size of the lesion ranged between 1 and 3 cm.

Eleven cases were known cases of diabetes mellitus and two cases had a history of hypertension. One case was an old case of tuberculosis with the left upper lobe necrotizing pneumonia diagnosed on X-ray. One case was a known case of pancreatitis with renal calculi. Three cases were farmers by occupation and another three cases were carpenters. Prior history of trauma or thorn prick could be elicited in five cases.

| Study                        | Location    | Frequency (percentage) | Percentage |
|------------------------------|-------------|------------------------|------------|
| Present study (total – 39 cases) | Extremities |                        |            |
| Hand                         | 6 (14)      | 90                     |            |
| Fingers                      | 7 (14)      |                        |            |
| Forearm                      | 9 (28)      |                        |            |
| Foot                         | 8 (19)      |                        |            |
| Leg                          | 5 (14)      |                        |            |
| Toes                         | 2 (4)       |                        |            |
| Knee                         | 1 (4)       |                        |            |
| Back                         | 1           | 5                      |            |
| Loin                         | 1           | 5                      |            |
| Jinkala et al. study[1] (total – 29 cases) | Extremities |                        | 94         |
| Verma et al. study[2] (total – 70 cases) | Extremities |                        | 82         |

The clinical differential diagnoses entertained were lipoma, fibrolipoma, neurofibroma, ganglion cyst, epidermoid cyst, and tuberous xanthoma.

On ultrasound, differential diagnoses entertained were ganglion cyst and epidermoid cyst.

On aspiration, all the cases yielded pus, and on microscopy, the smears revealed necrotic debris in 39 cases and inflammatory infiltrate comprising degenerate neutrophils, eosinophils, and macrophages lymphocytes in 39 cases [Figure 1] and epithelioid granulomas and multinucleated giant cells in 25 cases [Figure 1a]. Aspiration of pus in soft-tissue swellings which appeared as cyst clinically [Figure 2a], especially in extremities, and presence of eosinophils and foreign body giant cells raised a suspicion of fungus, and on careful evaluation, negative staining hyphal forms were seen against an inflammatory, necrotic background [Figure 1b], and in within foreign body giant cells [Figure 1c] in 38 cases which were better appreciated when condenser of the microscope was lowered and diaphragm closed. Although May–Grunwald–Giemsa could not be done due to financial constraints, Leishman stain was good enough to identify or rather raise suspicion of fungal elements when negative staining structures were seen against an inflammatory, necrotic background [Figure 1f]. The special stains for fungus (PAS and GMS) showed septate hyphal forms with acute angle branching in 38 cases [Figure 1e], of which 34 cases, the fungi were suggestive of Aspergillus species, and in four cases, a broad classification as fungal infection was given. These four cases were reviewed again and few
of the hyphae showed pigment focally [Figure 1d] raising a suspicion of phaeohyphomycosis; however, these cases did not have culture or histopathological correlation. One case showed yeast and pseudohyphal forms of *Candida* species. Hence, the final diagnosis on cytology was given as acute suppurative inflammation of fungal etiology. Culture correlation was available for seven cases; however, the culture did not yield growth in any case though fungus was identified on KOH mounts in two cases.

The lesion appeared clinically as a tense cyst [Figure 2a]. Fourteen of these cases had surgical excision and the specimens were submitted for histopathological examination. Grossly, the lesions were cystic with necrotic material in the cyst lumen. Microscopy of H&E sections revealed fibrocollagenous cyst walls with acute inflammatory infiltrate, palisading epithelioid cells, and few foreign body giant cells [Figure 2b and c]. Septate fungal hyphae with acute angle branching were seen on PAS and GMS stain [Figure 2d] in 13 cases and were reported as acute suppurative inflammation with *Aspergillus* species and one case showed only foreign body giant cell reaction.

**DISCUSSION**

Fungal infections of subcutis and dermis are very rare and are known as subcutaneous mycosis. They are caused by heterogeneous groups of fungi that are usually inoculated into dermis or subcutis after local trauma.[2] That is why these lesions are more common in male gender, certain occupations...
such as farmers and carpenters and in extremities of the body since they are more prone to trauma due to the outdoor nature of their work. Similar observations were made in our study and several other studies.[1-4] The clinical presentation of mycosis probably varies with the causative fungus. In Jinkala et al. study, where predominant causative agent was phaeohyphomycosis, the presentation was like a subcutaneous cyst.[1] In Verma et al. study, where the predominant causative organism was chromoblastomycosis and sporotrichosis, the presentation was as verrucous plaques and nodules.[2] In our study, none of the cases had verrucous plaques, but all cases presented as tense nodules of subcutaneous abscess, and most of the cases (87.1%) were caused by Aspergillus. The clinical presentation of Aspergillus depends on host immunity and can have varied presentations ranging from violaceous macules, to papules, hemorrhagic bullae, pustules, and subcutaneous abscess to ulcerative lesions.[5]

| Geographic region          | Author       | Most common causative organism |
|----------------------------|--------------|--------------------------------|
| Northeast India            | Verma et al. | Chromoblastomycosis            |
| South India-Puducherry     | Jinkala et al. | Phaeohyphomycosis             |
| South India-coastal Karnataka | Bhat et al. | Chromoblastomycosis            |
| South India-Andhra Pradesh | Present study | Aspergillus species            |
Subcutaneous mycosis can occur both in immunocompetent and immunocompromised hosts. Haridasan et al. have reported seven cases of subcutaneous phaeohyphomycosis in immunocompromised hosts who were kidney transplant recipients.[6] Eleven cases in our study were diabetic. For fungus to gain access into the host, the protective barriers should be disrupted, this could happen either with immunosuppression like in organ transplantation and diabetes mellitus or with trauma. Although the host immunity does not determine the risk of infection, it affects the severity of infection and risk of systemic spread.[1]

Queiroz-Telles et al. have listed the common causes for subcutaneous mycosis as sporotrichosis, chromoblastomycosis, mycetoma, lobomycosis, rhinosporidiosis, subcutaneous zygomycosis, and subcutaneous phaeohyphomycosis in their article.[7] There are studies and case reports of cutaneous mycosis caused by Aspergillus species.[8-11] The possible causative organisms might vary with different geographic regions [Table 2]. Verma et al. have reported the most common cause of subcutaneous mycosis in their study from Northeast India as chromoblastomycosis.[3] We have not come across any case of chromoblastomycosis in our study probably because it is very uncommon in our region. The most common cause of subcutaneous mycosis in our study which is done from Andhra Pradesh state in South India is Aspergillus species. Aspergillus spores are widely disseminated in the soil and decomposed vegetation, posing a high chance of accidental inoculation. The cutaneous aspergillosis is commonly caused by Aspergillus flavus and Aspergillus fumigatus. Gochhait et al. have studied spectrum of 66 fungal infections on FNAC and the common fungi reported in the study were aspergillosis (36), followed by mucormycosis (6), histoplasmosis (3), cryptococcus (4), and candidiasis (2) and 15 cases were broadly classified as fungal infection only.[12] However, most of the aspergillosis and mucormycosis cases in their study were of head-and-neck region. Cases presenting as subcutaneous mycosis in their study were only two, which were caused by Candida species. Candida mimicking as a soft-tissue mass lesion is rather unusual. Amita et al. have reported one such case. It can occur either by hematogenous route or traumatic inoculation.[5] Jinkala et al. in their 9-year study from South India have identified fungal elements on cytology in 24 out of 29 cases, of them 16 were reported as phaeohyphomycosis and four cases were reported as Aspergillus.[1] We have a single case of candida in our study. The rest of the cases were Aspergillus species. It can be seen from this data that the common causative fungi vary in different studies and probably depend on the geographic location, because these fungi are commensals whose existence depends on environmental factors and they cause infection only when they are accidentally inoculated into the human body. Unfortunately, culture did not yield growth for confirmation in our study. Although culture is a gold standard, it may not always yield positive results. The rate of positivity in fungal culture varies widely in different studies and is around 50%.[2,9] Molecular tests like PCR can also be used for confirmation, but these tests are not widely available at all centers.

Sometimes, fungal elements might not be seen in subsequent histopathology further complicating the diagnosis.[2] Histopathology was available for 14 of our cases. On histopathology, granulomatous inflammation is commonly seen, which can be of two types either a suppurative granuloma or a tuberculoid granuloma and depends on host immune status. The 2017 ESCMID-ECMMERS guidelines strongly recommend direct microscopy (preferably using optical brighteners), histopathology, and culture for the diagnosis of Aspergillus and serum and
bronchial lavage fluid galactomannan levels for the diagnosis of invasion.[13]

The cytology of cutaneous and subcutaneous mycosis varies from case to case depending on the host immune response and can show inflammatory cells comprising eosinophils, neutrophils, foamy histiocytes, necrosis and/or epithelioid granulomas, and multinucleated giant cells. PAS and GMS stain help in confirmation by highlighting the fungal hyphae. In addition, Fontana-Masson stain can be used to demonstrate melanin pigment which will be positive in phaeohyphomycosis; but it should be interpreted carefully since several other fungi such as Zygomycetes, dermatophytes, and few species of *Aspergillus* can also stain positive with this stain. That is why morphology of fungus is important in arriving at a diagnosis; an algorithmic approach basing on the hyphal thickness, septations, and branching will help in further subtyping of the fungus.[14] As shown in the flowchart [Figure 3], the hyphae of phaeohyphomycosis are thick (5–15 µm), brown, septate with refractile pale walls, and bubbly cytoplasm, whereas the hyphae of *Aspergillus*, *Fusarium*, and dermatophytes are thin (approximately 5 µm) with delicate walls. In contrast, mucormycosis has thick aseptate hyphae with non-parallel uneven refractile walls which are intensely eosinophilic on H&E and have a hollow appearance in tissue sections.[14,15]

One of the drawbacks of FNAC is that we cannot rule out invasive fungal infection on cytology and the other being that we cannot confirm the species of fungus without the aid of culture though we can give a broad classification.

**CONCLUSION**

Subcutaneous mycosis is one of the rarer causes of soft-tissue swelling of extremities and should be born in mind as one of the differential diagnoses of soft-tissue swellings, especially when FNAC yields pus or necrosis and appropriate special stains for fungus should be done, otherwise, these cases are easily missed. The causative fungus varies with different geographic regions and *Aspergillus* was the most common etiological agent in our study.

**COMPETING INTEREST STATEMENT BY ALL AUTHORS**

The authors have no conflict of interest to declare.

**AUTHORSHIP STATEMENT BY ALL AUTHORS**

All authors have participated and contributed sufficiently in the preparation of the manuscript. All authors have read and approved the final version of the manuscript.

**ETHICS STATEMENT BY ALL AUTHORS**

The study was approved by Institutional Ethics Committee, Guntur medical college, Guntur, AndhraPradesh, India. (IEC number GMC/IEC/366/2020).

**LIST OF ABBREVIATIONS (IN ALPHABETIC ORDER)**

- FNAC - Fine needle aspiration cytology
- GMS - Gomori meyenamine silver
- H&E - Hematoxylin and Eosin
- PAS - Periodic acid Schiff stain.

**EDITORIAL/PEERREVIEW STATEMENT**

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a double-blind model (the authors are blinded for reviewers and vice versa) through automatic online system.

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