A study of cyto-histological correlation in diagnosis of mycetoma cases

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Abstract

Context: Mycetoma is a chronic, specific, granulomatous, progressive subcutaneous disease, characterised by sinuses discharging pus with granules. Actinomycetes and eumycetes are the causative agents. In the absence of discharging sinuses, a diagnosis may not be suspected. In such cases histopathology remains the only diagnostic modality, as often cultures are not done. Fine needle aspiration can aid in rapid diagnosis of mycetomas, however experience and literature regarding cytology of mycetomas is limited.

Aims: To delineate the cytological features of mycetomas and correlate with histopathology.

Materials and Method: Cytological smears of histologically proven cases of mycetoma were included in the study. Fine needle aspiration was done in 5 cases, scrape smears were available in 7 and squash cytology in 1 case. Cell block preparation was available in one case. Cytology smears were stained with H&E, Papanicoloau and Giemsa stains in all the cases. Gram stain, Periodic acid Schiff and/or Grocott methenamine silver stains were done wherever required.

Results: Of the 9 cases of actinomycetoma and 4 cases of eumycetoma including 1 case of pale grain mycetoma on histology, all 13 could be diagnosed accurately on cytology.

Conclusions: Differentiation between actinomycetoma and eumycetoma is as accurate on cytology as histopathology. Special stains can be reliably applied on cytological specimens for further confirmation. Cytology remains an accurate, inexpensive and a tolerated procedure that allows for rapid diagnosis of mycetoma.

Keywords: Actinomycetoma, Eumycetoma, Mycetoma

Key Messages: Differentiation between actinomycetoma and eumycetoma is as accurate on cytology as histopathology.

Introduction

Mycetoma is a chronic, specific, granulomatous, progressive subcutaneous disease. Mycetoma caused by filamentous bacteria actinomycetales is called actinomycetoma, while mycetoma produced by infection with true fungi is referred to as eumycotic mycetoma.[1]

Mycetoma that produces discharging sinuses is easily identifiable, however some mycetoma lesions present as subcutaneous masses without discharging sinuses. Their differential diagnoses ranges from epidermoid cysts, a variety of benign soft tissue tumors to osteogenic sarcoma and bone tuberculosis, owing to their eventual involvement of underlying bones.[1,2]

Actinomycetomas account for 60% of the cases of mycetomas, while eumycetomas constitute the rest. Pseudomycetomas are a disease similar to mycetomas in clinical presentation, but caused by aggregation of dermatophyte hyphae.[3,4]

Diagnosis of actinomycosis by fine needle aspiartaion cytology at various sites has been reported extensively.[5-9] Few studies have pointed at the utility of cytology in diagnosis of mycetomas.[10-12] However experience on the utility of cytology for diagnosis of mycetomas is limited in literature. In Indian context, there have only been few case reports describing cytology of mycetomas.[13-18]

We present 13 cases of histopathologically proven mycetomas with available cytology. The present study was aimed at delineating cytological features of mycetomas using fine needle aspiration, scrape and squash cytology.

Materials and Method

Cytological smears of histologically proven cases of mycetoma were included in the study. Fine needle aspirates were obtained with a 22-23 gauge needle attached to 10 ml syringe mounted on a syringe holder under aseptic precautions in 5 cases, with average of two punctures being done in each patient. Scrape smears were obtained in 7 cases by using a scalpel blade to pick the grains from freshly cut postoperative specimens and grains smeared on to the glass slides. Squash cytology was done in 1 case. Cell block preparation was done in one case as the grains could be isolated from the discharge obtained from the sinuses. The study was approved by the institutional ethics committee.

Smears obtained were wet fixed in alcohol and stained with hematoxylin & eosin (H&E) and Papanicoloau (Pap) stains. Air dried smears were stained with Giemsa stain. Gram stain, periodic acid Schiff (PAS) and Grocott methenamine silver (GMS) stains were done on smears wherever required.

Results

9 actinomycetomas, 4 eumycetomas including 1 case of pale grain mycetoma were studied.10 lesions were in the lower limb, 1 in the upper limb and 2 in the head and neck region (Tables 1 & 2).

All smears showed inflammatory infiltrate consisting mainly of neutrophils, with many lymphocytes, plasma cells and eosinophils. Macrophages and multinucleate giant cells were present in 12 of our cases. Macrophages often contained pigments of fungal organisms and were a vital clue to examine more closely when organisms were not obviously seen.

Actinomycetes were seen as homogeneously blue fragments showing fine radiating filaments akin to rabbit tail or dust bunnies on Giemsa stain (Fig. 1a, b & c). On H&E they showed fine basophilic filaments in pinkish background (Fig. 1d). Bacterial colonies were seen intimately surrounded by inflammatory cells. Fine gram positive filaments were seen on Gram staining (Fig. 1e). Cell blocks prepared from isolated grains in one of the actinomycetomas showed all the features of histopathology including splendore Hoeppli material around the grains and the inflammatory cells surrounding the colony. (Fig. 1f, g & h)

Eumycetomas showed dark brown fungal filaments in close association with inflammatory cells. Presence of similar pigmented material within macrophages was helpful. Two of the cases showed features of *Madurella mycetomatis* on histopathology (Fig. 2 a-d), while one case showed colonies akin to *Curvularia geniculata*. In one case presenting as ankle swelling, fungal elements could not be easily identified on routine staining and GMS was applied. However presence of strange debris within macrophages which were GMS positive alerted us to the presence of fungal elements. On excision this case turned out to be a pale grain mycetoma with no pigmentation (Fig. 2 e-h).

Table 1: Cytology specimen and final histopathology diagnosis of mycetoma (n=13)

S. No	Age/ Sex	Location	Cytology specimen	Final diagnosis
1	45/M	Foot	Scrape	Actinomycetoma
2	44/M	Foot	Scrape	Actinomycetoma
3	40/M	Foot	FNAC	Actinomycetoma
4	25/F	Neck	FNAC	Actinomycetoma
5	38/M	Foot	Scrape	Actinomycetoma
6	45/M	Foot	Scrape	Actinomycetoma
7	38/M	Jaw	FNAC	Actinomycetoma
8	46/F	Foot	Squash	Actinomycetoma
9	38/M	Foot	FNAC	Actinomycetoma
10	26/M	Palm	Scrape	Eumycetoma
11	54/F	Ankle	FNAC	Eumycetoma
12	43/M	Foot	Scrape	Eumycetoma
13	32/F	Foot	Scrape	Eumycetoma

Table 2: Cyto-histological correlation (n=13)

Cytology	Cases	Histopathology	
		Actinomycetoma	Eumycetoma
Actinomycetoma	9	9	
Eumycetoma	4		4

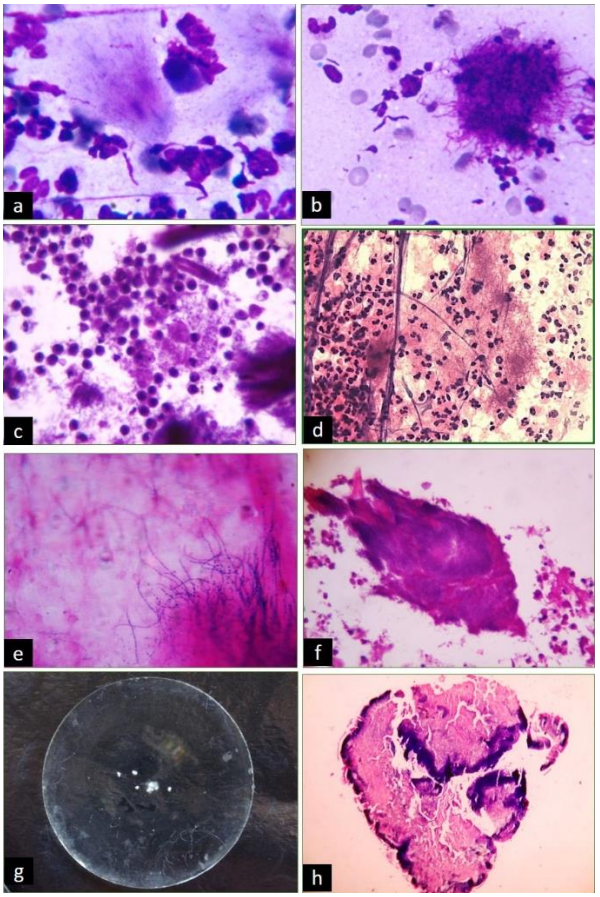

Fig. 1: Actinomycetoma: (a-c) Filamentous bacteria surrounded by inflammatory cells (Giemsa x200) (d) Colonies on H&E (x200) (e) Fine gram positive filaments on Gram stain (x400) (f) Grains on H & E (X200) (g) Grains on gross

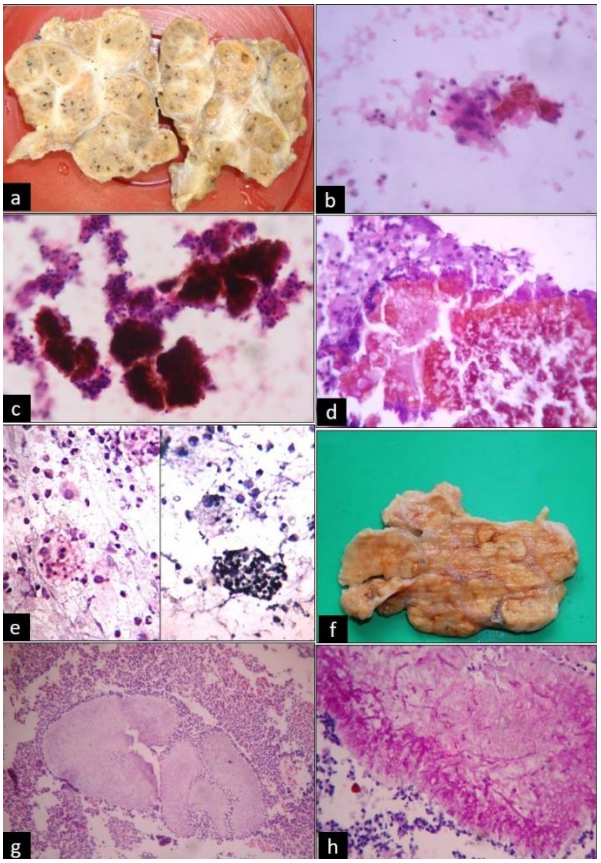

Fig. 2: Eumycetoma: (a) Gross (b,c) Scrape cytology (d) Histopathology of Madurella mycetomatis (e) Cytology smear of pale grain mycetoma on H&E (left) and GMS positive debris in macrophages (right) (x200) (f) Gross (g) H&E morphology (x100) (h) PAS stain on tissue section (x400)

Discussion

The diagnosis of mycetoma is made tentatively on clinical examination when clinical triad of subcutaneous mass, sinuses and discharge (with grains) are present.[19] Clinical examination alone will not identify the causative organism, nor does it accurately delineate the extent of spread of disease.[12] The extent of disease can be confirmed by multiple imaging tools like radiography, ultrasound imaging, CT scan and magnetic resonance imaging (MRI). Identification upto species level is possible on histopathology using various stains, including 20% potassium hydroxide, Brown and Brenn, Periodic acid Schiff and/or Grocott methenamine silver stains. Other techniques of utility include serology, culture, skin tests and molecular techniques.[12,19-21] Fine needle aspiration cytology of soft tissue masses is an established technique for diagnosis.[22] It is a simple, safe, effective and rapid diagnostic technique that is well tolerated by patients. It avoids unnecessary resections and also avoids difficulties in the management of mycetomas.[10,11]

Mycetoma grains encountered during cytology may pose diagnostic difficulties and hence a need for cytopathologists to be familiar with cytological features of mycetomas. Actinomycetes are identified on cytology by their growth pattern in colonies made up of dense masses of hematoxylin stained tangled filaments that radiate outward and tend to be eosinophilic at the periphery.[23] Actinomycetes filaments appear blue in the centre and pinkish at the periphery on Romanowsky stains. Their appearance has been likened to dust bunnies/ dust balls/bales of wool-like on Pap smears, colonies with dense centre surrounded by delicate filaments radiating from the central condensation. These balls appear a characteristic grayish blue on Pap stain. Similar appearance is seen on Pap stained smears obtained from fine needle aspiration.[6,7,13-15] Smears from eumycetomas are characterised by brownish, branching, septate hyphae embedded in a matrix which stains positively with Periodic acid Schiff or Gomori Methenamine silver stains, both demonstrating large sized hyphae of eumycetomas.[10,11,14]

Mycetoma lesions are characterised by presence of polymorphous inflammatory cells. This kind of inflammatory response helps differentiate mycetomas from other soft tissue masses containing histiocytes and histiocyte like giant cells. Fragments of grains are found in intimate relationship with inflammatory cells, this feature allows for differentiating fungal elements from artifacts.[10,21] Three types of reaction patterns have been described in mycetomas, ranging from type I consisting of predominantly polymorphonuclear cells, with no granuloma formation to Type III, where granuloma formation is seen with little polymorphonuclear infiltrate.[11,21] These patterns are evident in our cases as well, one of our cases of eumycetoma in the palm showed Type III reaction with well-formed granulomas identified on cytology and confirmed on histopathology. *Das et al* have suggested that in all cases of superficial swellings and deep seated masses, especially those of long standing duration, when aspirate smears show rich acute inflammatory infiltrate, actinomycetes must be looked for.[5]

Grains are a distinctive feature of mycetomas, they can be differentiated from artifacts on cytology owing to intimate association with inflammatory cells. This feature is evident even on cell block preparation as observed in one of our cases.[11]

Although most mycetomas present as sinuses that discharge grains, some present as subcutaneous masses, it is in such cases that fine needle aspiration cytology is particularly useful. Furthermore, discharged grains from sinuses are often unviable. Grains obtained from fine needle aspiration are viable and highly suitable for culture, they are even free from bacterial contamination.[10]

Differentiation between actinomycetomas and eumycetomas is as accurate on cytology as histopathology. Special stains can be reliably applied on cytological specimens for further confirmation and material can be sent for culture studies.[11,13-15] Material

obtained from fine needle aspirates can also be utilised for preparation of cell blocks as demonstrated in one of our cases. Many of the obscuring elements and confusing artefacts seen on smears such as skeletal muscle fibres, melanin pigment and foreign body giant cell reaction are less of a hindrance on cell block preparation. These artefacts have been observed to affect diagnostic sensitivity and accuracy of cytological diagnosis.[11]

Some pitfalls of fine needle aspiration are poor collection, sampling errors, presence of too few grains and pain following repeated needle insertions. Ultrasound guidance for fine needle aspiration has been shown to allow for targeted aspiration of grains and prevent discomfort of repeated painful aspirations.[11,20,21] Interpretation of smears requires considerable experience and more importantly awareness regarding possibility of mycetoma as a differential diagnosis for subcutaneous mass lesions, on the part of cytologist.[10,12-15]

Conclusion

Histopathology techniques for diagnosis of mycetoma require a deep surgical incision, hospital stay and anaesthesia besides the time taken to render a diagnosis. In comparison cytology remains an accurate, rapid, inexpensive and minimally invasive well tolerated procedure with high diagnostic accuracy.

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