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HISTOPATHOLOGICAL IDENTIFICATION OF VARIOUS CAUSAL SPECIES OF MYCETOMA PREVALENT IN NORTH-WEST RAJASTHAN (Bikaner region)

M C R Vyas, H L Arora and K R Joshi

Two hundred and seventeen histopathologically proved cases of mycetoma collected from the records of the Pathology Department over a period of 13½ years (from January 1971 to May 1984) were studied using haematoxylin and eosin stained paraffin sections, and wherever necessary Gomori's silver methanamine technique, PAS stain, Gram's stain and Zeihl Neelson's stain to outline the morphological characteristics of the causative agents in the tissue and the surrounding tissue reaction.

Maduramycotic mycetoma (Eumycotic mycetoma) was diagnosed in 179 (82.5%) cases and actinomycetoma (Schizomycetes) was seen in 38 (17.6%) cases. The commonest causal agent in the maduramycetoma group was *Madurella mycetomi*, encountered in 170 cases. In the actinomycetoma group, *Actinomadura somaliensis* was observed in 17 (44.2%) cases, *Actinomadura madurae* in 14 (36.1%) and *Nocardia* species in 2 (5.2%) cases.

Key words : Mycetoma, Maduramycetoma, Actinomycetoma.

Mycetoma is a chronic progressive fungal granulomatous disease usually affecting the soft tissues and the bones of the extremities and characterized by a swelling and deformity of the involved part with development of sinuses through which pus and granules are discharged.[1,2] It is caused by a variety of fungal and bacterial agents. The causal agent can be identified either by the culture of the organism or by histopathology of the involved tissue. Histopathologically, it is now possible to differentiate between actinomycotic and true fungal mycetoma. Moreover, precision of the histopathological method is considered almost as good as that of cultures because most of the grains seen in the tissue in mycetoma are highly characteristic.[3-10] The routine haematoxylin and eosin stain is by and large the most useful for identifying the causal agent.[1]

From the Department of Pathology, S. P. Medical College, Bikaner and the Department of Microbiology, Dr. S. N. Medical College, Jodhpur.

Address correspondence to : Dr. H. L. Arora, Department of Pathology, S. P. Medical College, Bikaner-334 001, Rajasthan, India.

The present study was undertaken to evaluate the prevalence of various causative fungal species of mycetoma in this north-western part of Rajasthan, employing simple H and E stained paraffin sections, and to describe the histopathologic criteria for the specific diagnosis of the causative agents of mycetoma.

Materials and Methods

Haematoxylin and eosin stained sections of 217 histopathologically confirmed cases of mycetoma recorded in the Department of Pathology, S. P. Medical College, Bikaner over a period of 13½ years (from January 1971 to May 1984), were reviewed for the grain morphology and its surrounding tissue reaction. Whenever necessary, Gram's stain, PAS stain and Gomori's silver methanamine stain were also employed to demonstrate the fungal elements in the grain.

Results

Various causal species observed were categorized on the basis of classification of Winslow and Steen[3] and Verghese and Klokke.[4] On this basis, out of 217 mycetoma cases, 179 (82.5%) cases were caused by maduramycotic

species and the remaining 38 (17.6%) cases by actinomycotic species. None of the cases was diagnosed as botryomycosis. *Madurella mycetomi* was the commonest causal agent amongst the maduramycotic group, it was seen in 170 (94.08%) cases. *Madurella grisea* was seen in 5 (2.8%) cases, *Phialophora jeanselmei* and *Allescheria boydii*/*Cephalosporium* group and *Leptosphaeria senegalensis* in 1 case each. In 2 cases, the species could not be identified.

In the actinomycotic group, out of the 38 cases, *Actinomadura somaliensis* was seen in 17 (44.7%) cases, *Actinomadura madurae* in 14 (36.5%), *Actinomadura pelletierii* in 2 (5.2%)and *Nocardia* species in 2 (5.2%). Three species could not be identified as the fungal grains were too scant for a proper study.

Histopathological Features of the Fungal Grains :

Maduramycotic (Eumycotic) mycetoma was characterised by grains containing broad branching hyphae, often with chlamydospores in the periphery along with brown to black pigment in the matrix. In doubtful cases or when the fungal colonies were few, PAS stain distinctly brought out the morphological details (Fig.1A).

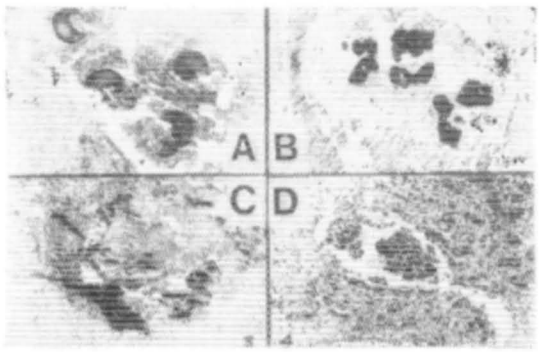

Fig. 1. A. *M. mycetomi*.
B. *Leptospheria senegalensis*.
C. *M. grisea*.
D. *Phialophora* (H & E × 200).

M. mycetomi grain was differentiated from *M. grisea* on the basis of distribution of the brown to black pigment in the grain. In the former, the pigment was distributed uniformly, while in the latter, it was at the periphery leaving a central non-pigmented area (Fig. 1C).

In the *Cephalosporium* species, the grain did not contain any pigment and it was made up of hyphae and small chlamydospores. This could be differentiated from *Allescheria boydii* which like *Cephalosporium* also had a non-pigmented grain made up of hyaline hyphae, but unlike it, its periphery had large chlamydospores.

Leptospheria senegalensis grains were found singly or in groups with a pale, poorly pigmented centre consisting of hyaline hyphae without interstitial cement, while the periphery had dense black pigment in which the vesicular structures were seen (Fig. 1B). *M. grisea* closely resembled *L. senegalensis*, but had less crenellated borders, and irregularly arranged hyphae at the periphery surrounded by black pigment.

Phialophora species grains appeared brown, but did not have brown cement-like material as seen in *Madurella* species. The periphery had a narrow eosinophilic band composed of chlamydospores and a few hyphae. The centre of the grain was devoid of fungal elements and contained inflammatory cells (Fig. 1D).

In actinomycetoma, the grains were composed of fine non-segmented filaments without distinction of the wall from its contents. Chlamydospores were absent.

Actinomadura somaliensis grains were rounded or multilobed and had a homogenous appearance showing linear cracks as a result of sectioning. The grains were basophilic with an eosinophilic band of radiating filaments at the periphery (Fig. 2C).

The grains of *Actinomadura madurae* were large, rounded, folded or vermiform, and had a central light eosinophilic area with loose filaments and a densely haematoxylin stained deriphery of fine filaments (Fig. 2B).

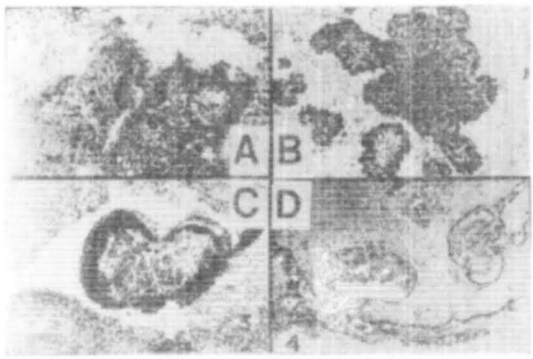

Fig. 2. A. *Actinomadura pelletierii*.
B. *Actinomadura madurae*.
C. *Actinomadura somaliensis*.
D. *Nocardia* (H & E × 200).

Actinomadura pelletierii grains were small compared to the other two *Actinomadura* species, and were rounded, oval or lobulated with irregular angulated borders. The mycelial elements were not seen. The whole grain stained dark violet with haematoxylin, with a very narrow eosinophilic band surrounding the grain (Fig. 2 A).

The *Nocardia* species had typical small grains in the sections, but differentiation between various species was difficult histopathologically. The grains were small, rounded, oval or irregular and stained eosinophilic, a few haematoxylin-stained blue dots were also seen, Gram stain revealed strongly Gram-positive branching filaments (Fig. 2 D).

The tissue reaction observed in these lesions was essentially of a granuloma, the causative agent was frequently seen lying in granulomatous tissue. The cellular zone composed of polymorphs, was narrow in maduramycetoma, while it was relatively wider in actinomycetoma. The other cellular components seen were lymphocytes, plasma cells, histiocytes, xanthomatous cells and foreign body giant cells in some cases of maduramycetoma.

Comments

The species-wise distribution has shown that *M. mycetomi* is the commonest mycetoma in Rajasthan. Chouhan and Agarwal[5] in contrast had observed 20 cases of actinomycetoma out of a total of 24 cases studied in Jabalpur.

Another significant observation in our study was that a relatively large percentage of cases were caused by *Actinomadura somaliensis* and *Actinomadura madurae* which have not been frequently identified in the earlier reports.[5,6]

It is our belief that even rare species like *Leptospheria senegalensis*, *Allescheria boydii*, *Cephalosporium* group and *Phialophora* species can also be identified by simple histological methods or by special stains wherever necessary.

A histopathological examination also eliminates the risk of misdiagnosis by the isolation of a contaminant fungus in the culture, because the organisms causing mycetoma grow slowly and can thus easily be over-grown by a saprophytic fungus.[1,10]

The major limitation of the histopathological examination is that it is not possible to differentiate the various species of Nocardia.[1,5] It is difficult to distinguish between *Allescheria boydii* and the white grains of *Cephalosporium* species. Similarly, a precise distinction was also not easy between *Madurella grisea* and *Leptospheria senegalensis*.

Apart from these, it is important to look for bacteria causing botryomycosis, in which Gram's stain would demonstrate the presence of cocci, cocco-bacilli or bacilli.[4]

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