Case Report

An approach to histology-based diagnosis and treatment of Madura foot

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Abstract

Madura foot is a deep mycosis commonly seen in tropical and subtropical countries such as India. Its incidence is likely to rise in temperate regions as well, due to the increase in worldwide travel.

The cases presented here are all agricultural workers from a rural part of northern India who had induration, fibrosis and minimal discharge from sinuses over the foot. Although culture remains the gold standard diagnostic test, this case report highlights the importance of histopathology in the early diagnosis and differentiation of common causative agents in Madura foot as repeated cultures are sometimes negative. Thus for mycetomas in which causative infectious agents cannot be isolated, histology may prove beneficial by avoiding inadvertent use of combined antifungal and antimicrobial agents so that a correct therapeutic modality can be decided, prognostic outcome be explained to the patient, and a preventable cause of deformity and disability can be identified and treated at an early stage.

Key words: mycetoma; Actinomycetoma; eumycetoma

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Introduction

Madura foot is a deep mycosis commonly seen in agricultural workers or in individuals who walk barefoot. It is caused by two groups of organisms, bacteria belonging to the group of Actinomycetes and the true mycetes named eumycetes.

The cases presented here are from the rural part of northern India; all were agricultural workers presenting with induration, minimal discharge from sinuses, and fibrosis over the foot for the last 4 to 5 years. Cultures sent on two occasions from all the cases were negative. Biopsy was sent simultaneously for histopathological examination and a diagnosis of actinomycetoma was given in three cases and eumycetoma in two cases. Differentiation between the two mycetomas is important as the two etiologic agents have a different course of disease progression and treatment [1]. As even multiple cultures can provide no growth at times [2], culture-negative cases can be diagnosed and common species can be identified on histopathology if a careful stepwise approach is followed. Actinomycetomas were treated with a favourable outcome without any relapse whereas eumycetomas had frequent relapses.

Case report

All five patients were male in the age group of 30 to 50 years presenting in the outpatient department with indurated swelling and fibrosis along with minimal discharge from sinuses over the foot for the last 4 to 5 years. Two patients had a history of antitubercular treatment to which they did not respond. A previous history of scant yellowish discharge was given by the patient without any grains in all cases. A deeper biopsy from the lesion was sent for histopathological examination; simultaneously discharge was sent for culture and sensitivity testing.

Microscopy in three cases on hematoxylin and eosin (H and E) stained sections showed small and large colonies, some round, some multilobated, surrounded by neutrophils along with epithelioid cells, plasma cells, and multinucleated giant cells at places forming granulomas. Macrophages were also seen at the periphery along with minimal fibrosis. In two cases the colonies which had multilobated appearance showed slightly pale central eosinophilia and a deeply basophilic outer border with ill-defined branching filaments. The basophilia was demarcated by eosinophilic hyaline-like material known as Splendore-Hoeppli Phenomenon (Figure 1a, 1b). In one case multilobated colonies showed fractures (Figure 1b), while in the third case colonies were

rounded homogenous eosinophilic with transverse fractures (Figure 1c). Special stains such as periodic acid-Schiff (PAS) and Ziehl-Neelsen's (ZN) stains were negative; however, Gram's stain showed branching filaments, 1 micron thick, not breaking into bacillary or coccoid forms, in all three cases (Figure 1d). Cultures were found to be negative on two occasions. Thus a histological diagnosis of actinomycetoma probably due to *Actinomadura* was given in the first two cases and *Streptomyces* in the third case. Large multilobated colonies of *Actinomadura* which had knobby projections gave the probability of *A. madurella* in one case while, in the other case, colonies showed fractures and the possibility of *A. pelletieri*. Treatment with a combination of drugs including amikacin, cotrimoxazole, and rifampicin along with regular surgical debridement of the lesion gave a good clinical response. The patient recovered fully within one to two years of treatment. Antimicrobial treatment was continued for six months even after full recovery.

In the remaining two cases microscopic sections showed eosinophilic rounded colonies surrounded by a similar type of inflammatory infiltrate as seen in actinomycetomas; however, most of the colonies were seen in between fibrotic tissues (Figure 2a). The colonies had ill-defined intricate filaments in an amorphous matrix. The matrix was highlighted by Gram's stain imparting a grainy aspect to the colony (Figure 2b); however, PAS stained sections highlighted interlacing septate hyphae 2 to 3 microns thick with rounded polygonal chlamydospores (Figure 2c, 2d). In both cases the diagnosis of eumycetoma, possibly due to *Madurella mycetomatis*, was given. Various antifungal agents along with surgical debridement of the lesion gave variable clinical response with frequent relapses over a period of two to three years.

Results and discussion

Madura foot is a deep mycosis caused by eumycetes (fungi) and actinomycetes (filamentous bacteria). It is seen in tropical and subtropical regions. The disease was first recognized by Dr Gill in 1842 from South India [3]. Its incidence is likely to rise in temperate regions as well due to increase in worldwide travel [4]. Eumycotic mycetomas were more common in northern India; however, the recent trend shows an increase in incidence of Actinomycetomas [5]. Besides mycetomas, clinical differential diagnosis in patients presenting with chronic discharging sinus in an extremity includes chromomycocysis, blastomycosis, coccidiomycosis, sporotrichosis, botryomycosis, syphillis, yaws and neoplasia [6]. Out of these, tuberculosis and mycetoma are the commonest. Common Actinomycotic agents are *Actinomadura*, *Streptomyces* and *Nocardia*, whereas common eumycotic agents are *Madurella*, *Pseudallescheria*, *Acremonium* and *Leptosphaeria*. Cultures of mycetoma are usually problematic due to stringent growth requirements, contamination by other bacterial organisms and because patients usually present late when the fibrosis predominates over the purulent discharge. Thus repeated attempts to culture the microorganism can fail [2,7].

In our case all patients presented late as they belonged to a rural area in northern India from where transportation of culture samples is problematic. The colour of the discharged grains is not helpful as pale yellow grains or discharge can be seen in both actinomycetomas and eumycetomas. Yellowish discharge observed by our patients could be due to secondary infections. A deeper biopsy from the lesion was attempted to provide a more substantial contribution in identifying the causal microorganism [8]. Histological sections from actinomycotic mycetomas showed variably sized grains (large and small) round and multilobated with a deeply stained basophilic outer border and slightly paler centre, as has been described for *Actinomadura madurae.* However, multilobated colonies of *Actinomadura pelleteri* showed fractures while grains or colonies of *Streptomyces* were rounded with homogenous eosinophilic appearance, showing longitudinal cracks [9,10,11] (Figure 1a, 1b, 1c). The basophilic outer border showed ill-defined branching filaments (Figure 1 d). Special stain (PAS) was negative and ruled out eumycetes as the causal organism, as they are composed of thick septate hyphae [4]. These filamentous bacteria were negative in ZN stain, ruling out *Nocardia,* which are partially acid fast [4]. Thus, in all three cases of actinomycetoma, the morphological appearance of the colonies combined with the use of special stains confirmed Actinomycetes as the causal organism with a probable diagnosis of *Actinomadura* in two cases and *Streptomyces* in one case. Cultures done on two occasions were negative. Treatment for Actinomycetoma was given with a combination of drugs (amikacin, trimethoprim-sulfamethoxazole and rifampicin) along with surgical debridement producing good results with full recovery in one to two years. Antimicrobial treatment was continued even after full recovery for six months to prevent relapse [12]. In the

Figure 1. Histological features of Actinomycetoma

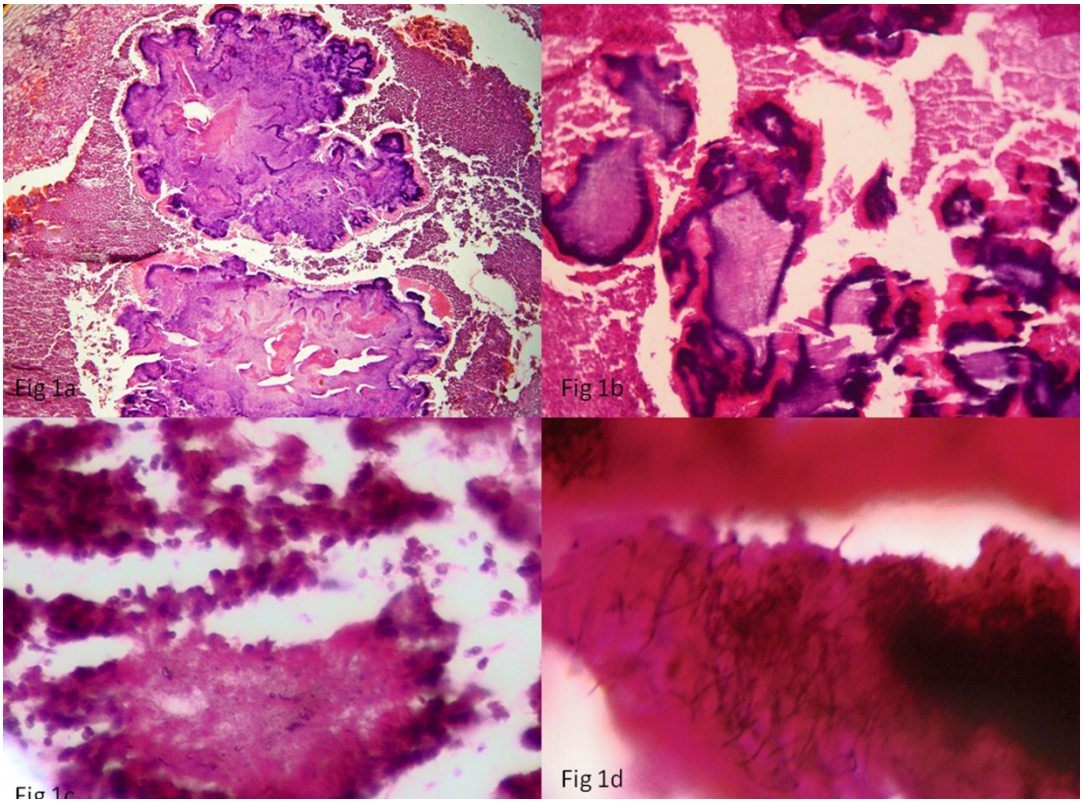

Figure 1a. Histological sections showing multilobated colonies of actinomycetoma with peripheral basophilia, knobby projections and Splendore-Hoeppli Phenomenon (H and E 100x)
Figure 1b. Histological sections showing multilobated colonies of actinomycetoma with fractures (H andE100x)
Figure 1c. Histological sections showing rounded homogenous eosinophilic colonies of actinomycetoma with ill-defined

eumycotic cases in which histological sections showed PAS-positive septate hyphae (Figure 2c, 2d), the morphological appearance of the colonies were in favour of *Madurella mycetomatis* in both cases [10,13]. Various antifungal agents were tried along with surgery over a period of two to three years with frequent relapses and remissions. Such relapses have been commonly observed even in culture-positive cases in some studies [10] as treatment of eumycetomas is often unsatisfactory and is based on efficacy of surgical debridement and excision along with long-term use of antifungal agents [13].

Histology may prove useful in differentiating actinomycetoma from eumycetoma. In some cases of actinomycetoma, a particular etiologic agent can be identified if a stepwise approach is followed concentrating on the details of the colonies and the use of special stains. In cases of Madura foot, biopsy material stained with H and E shows grains or colonies within abscess cavities with or without surrounding granulomatous reaction. There was no difference between the granulomas of actinomycetomas and eumycetomas. However, eumycotic colonies were more frequently surrounded by fibrotic tissue (Figure 2a) [13,14].Our approach to the diagnosis starts with three special stains, *i.e.*, PAS, Gram's stain, and ZN stain. If the colonies are Gram positive and show filamentous bacteria less than 1 micron thick (Figure 1d), actinomycetes should be considered, ruling out botryomycosis which are Gram-positive cocci or bacilli. If the colonies show PAS-positive hyphae 2 to 6 microns thick, eumycetes should be considered (Figure 2c, 2d).

In cases of Gram-positive actinomycotic colonies, with filaments breaking into bacillary or coccoid forms with partial positivity on ZN staining, *Nocardia* should be the primary diagnosis. On the other hand, if the filaments do not break, morphology of the colonies on H and E stain should be carefully analysed. In cases of large multilobated colonies having dense peripheral basophilia with club-shaped knobby projections, *Actinomadura madurae* (Figure 1a) should be considered unless such large multilobated colonies show fractures, in which case *A. pelletieri* should be considered (Figure 1b). If the colonies are rounded with homogenous eosinophilic staining, a provisional

Figure 2. Histological features of Eumycetoma

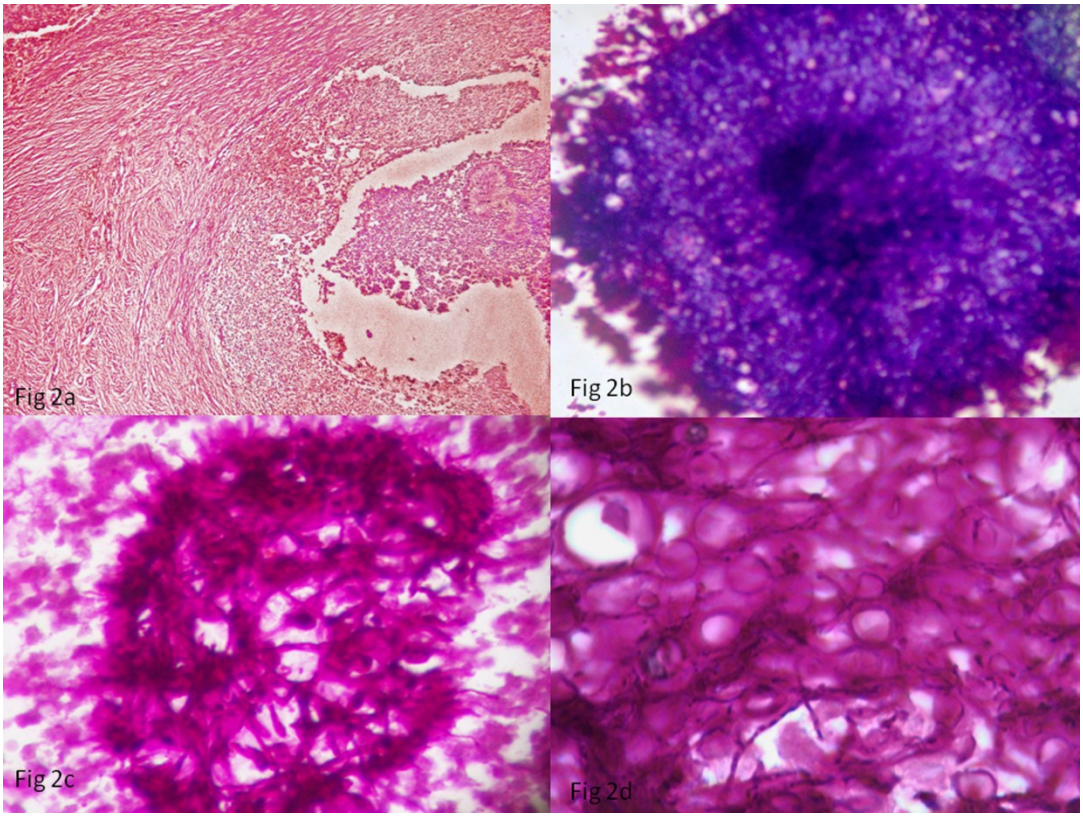

Figure 2a. Histological sections showing eumycotic colony within fibrocollagenous tissue (H and E 100x)
Figure 2b. Gram's stain highlighting amorphous matrix imparting grainy appearance to the colonies (Gram's stain 400x)
Figure 2c. Histological sections showing interlacing hyphae and oval club shaped ends, chlamydospores (PAS 400x).
Figure 2d.Histological sections showing septate hyphae. Note the PAS positive amorphous matrix in the background (PAS 1000x).

diagnosis of *Streptomyces* can be given (Figure 1c) [10,11].

In cases of PAS-positive eumycotic colonies showing hyphae, one should consider whether an amorphous matrix highlighted by Gram's stain or PAS stain is present or not (Figure 2b, 2c). The presence of an amorphous matrix narrows the diagnosis to consideration of only three eumycotic agents, *Madurella mycetomatis*, *Madurella grisea* and *Leptosphaeria*. If this amorphous matrix is present throughout the colony imparting a grainy appearance, a provisional diagnosis of *M. mycetomatis* can be given. If a peripheral amorphous matrix is present, the etiologic agent can be any one of the three. Absence of the amorphous matrix rules out the above three and is indicative of other eumycotic agents [12,14].

Differentiation between the two etiologic agents is important as the treatment and prognosis for them is different. With multidrug therapy, particularly the modified Welsh regimen in actinomycetoma, the therapeutic outcome is excellent [7]. For eumycotic mycetoma, surgery followed by antifungal therapy at an early stage is the best possible therapy at present if delayed outcome is poor even after aggressive treatment [1]. Progression to fibrosis, mutilation, and loss of function is rapid in Actinomycotic/Nocardial mycetoma cases [4], so an early diagnosis in cases of Madura foot is important because delay in diagnosis of 6 to 10 years is usual in a tropical country such as India [15].

Thus histology has a beneficial role and remains the only option in culture-negative cases. Biopsy in formalin is easier to transport and requires less time. Serodiagnosis with ELISA is not always diagnostic due to variable levels of humoral response to infection [16] and ancillary investigations such as PCR are not readily available at all centres. Histological diagnosis can be relied upon for the treatment of actinomycetomas avoiding the unnecessary use of

antifungals where a causative infectious agent for a mycetoma cannot be isolated [13].

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