

CASE REPORT

Fungal and Bacterial Mycetoma Diagnosis from a Histological Perspective: A Local Report of Two Cases Involving Foreign Labourers

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ABSTRACT

Mycetoma is a chronic subcutaneous granulomatous inflammation due to infection by fungus (Eumycetes) and bacteria (Actinomycetes) by penetrating inoculation of the microorganism into an injured skin. Late presentation is common, which leads to various deformities. In a standard setting, mycetoma diagnosis requires a combination of clinical assessment, histopathological features, and culture. However, microbiological culture takes time to be ready. Therefore, the classical histomorphological features supported by histochemical stains are the options to provide a fast diagnosis for the commencement of appropriate treatment. We described two cases of eumycetoma and actinomycetoma, emphasising the differences in their histomorphological features and histochemical stains that may facilitate the diagnosis.

Keywords: Mycetoma, Actinomycetoma, Eumycetoma, Madura Foot

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INTRODUCTION

Mycetoma is caused by fungal (eumycetoma) or bacterial (actinomycetoma). In 2016, the World Health Organization had declared mycetoma as a neglected tropical disease with an undetermined global burden (1). Nevertheless, the disease is endemic in various tropics and subtropics regions of the world, notably known as the 'mycetoma belt', where reported cases are extensively seen in countries like Sudan, India and Mexico.

Mycetoma cases are rarely observed in Malaysia. The first two cases were reported way back in 1969, and in 1982, the first case of Mycetoma caused by *Streptomyces Somaliensis* was reported (2). There have been no more reported cases since then in Malaysia. This might be due to improvement in socioeconomic status, leading to an increase in healthcare awareness. Mycetoma is more prevalent in men (4:1), reflecting their occupational exposure. Nowadays, the affected population in Malaysia is mainly foreign labourers with poor living conditions and inadequate access to medical treatment.

In the pathogenesis of mycetoma, pathogen, host,

and ecosystem interact. When a person encounters dirt, thorns, or other etiologic agents during manual tasks, the etiologic agents are inoculated. The affected individuals usually presented late with painless swelling mass, fistulas, sinuses, and discharges, which are mutilating and deforming. It usually affects the lower limb, especially the foot, but other body areas could also be affected. Although mycetoma infection can be confirmed by direct microscopy of the granules taken from the discharge, a biopsy is still essential for the diagnosis to initiate the specific treatment. Mycetoma is a chronic disease, but with a correct diagnosis, the causative agents are sensitive to commonly used antimicrobial agents though it requires long-term therapy. Due to the rarity of the disease, misdiagnosis or low index of suspicion may delay the diagnosis and causing treatment failure (3).

We present two cases with late presentations. However, due to financial constraints, the patients requested early hospital discharge with treatment and clinic follow up. In these cases, simple, rapid and sensitive histological diagnosis plays an important diagnostic tool.

CASE REPORT

Case 1

A 37-year-old Burmese gentleman, a labourer, presented with pus discharge from his right foot sinuses. He has been having right foot swelling for nine months. The swelling measured 2x2cm, firm, non-tender with

pus discharged from sinuses. There was no history of trauma to the foot. Ultrasound revealed a multiloculated superficial and deep-seated abscess at the medial foot. The clinical diagnosis at that point was an abscess. Excisional biopsy was performed, and microscopic findings showed multiple granulomas with prominent multinucleated giant cells admixed with lymphocytes and neutrophils. At the centre of granulomas, fungal hyphae surrounded by neutrophils were observed positively stained with Grocott-Gomori Methenamine Silver (GMS) and Periodic Acid Schiff (PAS) stains (Fig.1), which confirmed eumycetoma. As the patient insisted on early hospital discharge due to financial constraints and other personal problems, he was discharged on day three with Itraconazole 200 mg twice daily for four weeks. During follow-up at the clinic after four weeks, there was no more pus discharge, and the foot swelling was subsided. Itraconazole was continued for another four weeks.

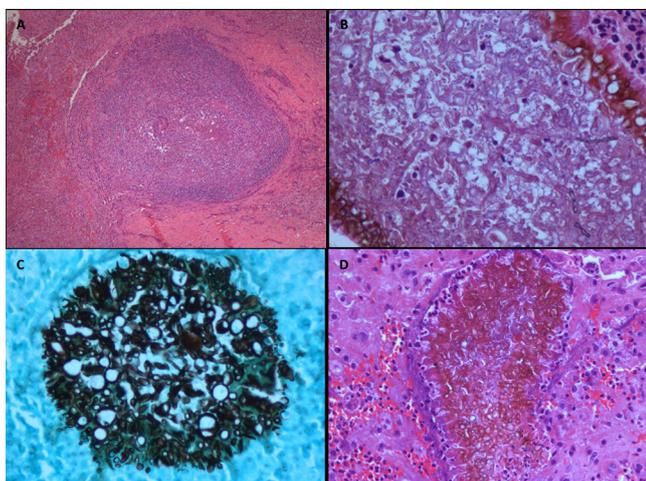


Figure 1: Granuloma with central neutrophils and fungal hyphae with occasional multinucleated giant cells (A) (H&E, 200x). Septate hyphae of mycetoma (B) (H&E 1000x) positively stained with GMS stain (C) (1000x) and PAS stain (D) (400x)

Case 2

A 21-year-old Vietnamese lady who works at a construction site presented with a gradual increase in the size of left foot swelling for one year, associated with pain upon walking. She had a history of wound laceration over the site secondary to a motor vehicle accident. Intraoperatively, there were multiple skin nodules at the foot dorsum with minimal pus discharge. The lesion was excised, and microscopically, there were epidermal ulceration and multiple granulomas with microabscesses in the deep dermis and subcutaneous tissue. In some of the microabscesses, the Splendore Hoeffli phenomenon characterised by intense eosinophilic material with central radiating thin filaments, positively stained with Gram stain and GMS were observed (Fig. 2). The morphological features and stains confirmed Actinomycetoma. She requested hospital discharge on the fourth-day admission. Despite the discharge, she was started on intramuscular amikacin 15 mg/kg/day twice

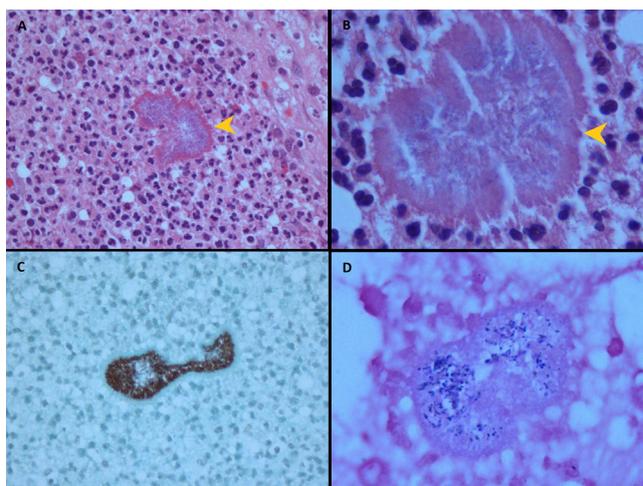


Figure 2: Microscopic features of Actinomycetoma exhibiting fine filaments surrounded by eosinophilic hyaline-like material called Splendore-Hoeffli Phenomenon (arrow) in (A) (H&E 400x) and (B) (H&E 1000x). The fine filaments are GMS stain positive (C) (1000x) and Gram stain positive (D) (400x)

daily for three weeks as an outpatient, concurrently with oral trimethoprim-sulfamethoxazole (Co-trimoxazole) 8/40 mg/kg/day for five weeks. The patient's condition improved by 80% at the end of this period.

DISCUSSION

Both mycetoma patients described in this report were young male and female adults, following many previous studies that have shown a greater prevalence of mycetoma between the ages of 20 and 40. It was, however, more often observed in males (4). As the number of foreign workers increases in Malaysia, including female labourers, they are also at risk of getting this infection depending on the job nature and living conditions. Due to the rarity of mycetoma cases in Malaysia, these reported cases were not suspected clinically earlier and only confirmed by histopathological examination (HPE) from the biopsies. The mycological culture, including from the pus discharge, was also not performed before the treatments started. A fungal culture is a gold standard test for species identification and should be aimed to enable susceptibility testing to guide the treatment decisions. However, it is time-consuming, especially when it requires immediate treatment, is prone to contamination, and requires a high level of expertise (4).

Due to the potentially lethal nature of mycetoma, early diagnosis is crucial to commence on the specific treatment, and HPE is one of the most rapid and accurate diagnostic tools available before culture. Grains examinations such as morphological analysis of fresh grains, direct microscopy and cytological investigation can also be conducted before performing a skin biopsy. The grains are aggregates of pathogenic organisms within abscesses that slowly form sinuses. Morphological analysis of the grains may provide rapid

preliminary identification of the aetiological agent, although it can be misleading in some cases. Each causative organism has different grains characteristic and patterns, having different sizes and stained different colours on H&E stain. In these cases, the grains were not noticeable as the skin biopsy was too small, and the tissues had been preserved in the formalin. Direct microscopy is a rapid method but lacks specificity. The findings from the cytological smears are comparable to those observed in histological sections, provided that the grains are present.

Actinomycetes and eumycetes have distinctive histomorphological characteristics. Actinomycetes consist of tiny, branching filaments that are around 1 micron thick, whereas eumycetes are composed of 4–5 micron thick septate hyphae. Sometimes, the actinomycetoma colonies are surrounded by eosinophilic hyaline-like material called as Splendore-Hoepli Phenomenon. The histomorphological identification by H&E stain is incomplete without the aid of special histochemical stains to support the diagnosis of mycetoma. Gram stain is important in distinguishing between bacterial and fungal mycetoma agents as in these cases. The stain displays the fine filamentous agent within the grains of actinomycetoma as gram-positive and eumycetoma as gram-negative. Modified Ziehl-Neelsen stain is helpful in separating *Nocardia* spp. from other actinomycotic agents where the former stained positive. Other than that, the most useful stains for detecting hyphae and chlamydo spores in eumycetoma grains are Periodic acid-Schiff (PAS) and Grocott methenamine silver (GMS). Gram stain, GMS and PAS stain the polysaccharide component of the fungal cell wall, producing purplish-blue, black and magenta colours, respectively.

Other than HPE and mycology culture, PCR is another option for mycetoma diagnosis. The causative agent can be identified quickly, reliably, and easily using molecular-PCR. It is, however, costly and not readily accessible in endemic areas or even certain tertiary institutions, as in these cases. A new deparaffinization-PCR technique of the formalin fixed paraffin embedded (FFPE) tissue would allow to identify the aetiological agent as in these present cases where the fungal culture has been missed to perform on the fresh tissue biopsy. To date, there is no serological test available that can reliably diagnose mycetoma.

Mycetoma treatment in these case reports was in line with previously published reports. The combination of Co-trimoxazole and Amikacin Sulfate remains the recommended treatment for actinomycetoma cases, and several studies have shown a cure rate of about 90% (5). Amikacin has been shown to be beneficial in disseminating infections or those that have been resistant to other treatments. Eumycetoma is treated with ketoconazole or itraconazole. To establish a nice fibrous

capsule surrounding the lesion, itraconazole 200–400 mg is prescribed daily for six months, followed by wide local excision and itraconazole 200–400 mg daily until the lesion subsides. Treatment is stopped when the sinus is fully healed, the mass has disappeared clinically and radiologically by CT scan or MRI, and the organism is no longer present (5).

The physician's ability to make a presumptive diagnosis would be aided by knowledge of the disease's epidemiology and clinical presentations. Even though only HPE was performed in these cases, the diagnoses were not missed, the treatment could be started early, and the conditions improved.

CONCLUSION

The histopathological analysis is the fastest diagnostic method to discriminate eumycetoma or actinomycetoma aided by histochemical stains. This allows for the selection of the appropriate treatments to prevent the disease's progression and, as a result, improve the patient's health and quality of life. Nevertheless, a fungal culture is still a gold standard test for species identification as well as susceptibility testing to guide the treatment decision. Clinical knowledge and a high index of suspicion of mycetoma presentation are also crucial and cannot be missed.

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