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Case Report

Defining the best time to initiate therapy and treatment goals in negative culture mycetoma: A case from a non-endemic country

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Abstract Mycetoma is a chronic granulomatous suppurative disease of the skin and underlying tissue caused by saprophytic fungus (eumycetoma) or actinomycetes (actinomycetoma). Despite having a low prevalence and low mortality rate, mycetoma may cause serious complications such as deformity and disability due to destruction of skin and adjacent structures. Various therapeutic options call for a thorough diagnostic approach before initiating therapy. Prompt treatment is imperative to limit disabilities. Furthermore, mycetoma is prone to recurrences, hence rigorous assessment of therapeutic goal is essential. We reported a case of actinomycetoma confirmed by histopathology examination and successfully treated with combination of rifampicin and cotrimoxazole.

Key words
Mycetoma; Negative culture; Non-endemic country.

Introduction

Mycetoma is a chronic granulomatous suppurative disease of the skin and underlying tissue caused by saprophytic fungus (eumycetoma) or actinomycetes (actinomycetoma). It is characterized by a triad of indolent subcutaneous nodules, numerous sinuses, and the discovery of granules in the discharge.[1,2] Mycetoma is classified among neglected tropical disease by the World Health Organization.[3] Although linked to low mortality rate, prompt diagnosis and appropriate treatment, it is essential to avoid complications such as deformity and disability due to destruction of skin and adjacent structures.[1,4,5]

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Indonesia is listed as a tropical country, yet the prevalence of mycetoma is low.[6] Yahya *et al*.[7] had reported 10 cases of mycetoma in Jakarta, Indonesia during 1989-2013. The lack of physician's awareness leads to misdiagnosis and delayed treatment initiation. Moreover, the lack of experience in identifying causative agent through microbiological examination in non-endemic countries which is crucial in determining the proper regimen and treatment completion aggravates the difficulties in mycetoma management.[1,2,4,8]

Herein, we attempt to formulate the best time to initiate therapy and the goals of treatment through a case of actinomycetoma with negative culture result. The final diagnosis was concluded by histopathological examinations and the treatment completion was assessed clinically.

Case report

We report a case of a 55-year-old male with

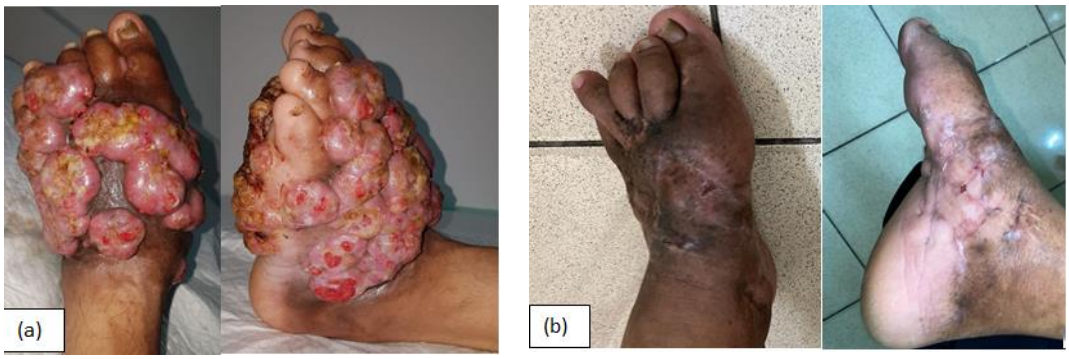

Figure 1 Clinical photograph of lesion on the left foot (a) before the initiation of therapy (b) after 12 months of therapy

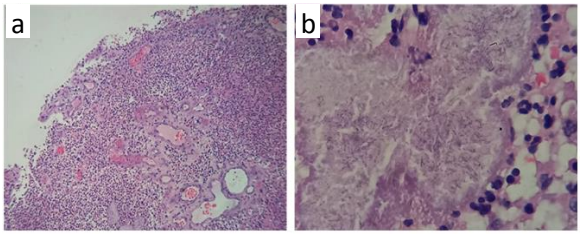

Figure 2 Histopathological examination shows (a) ulcer with dense inflammatory cells (HE, x400) and (b) Splendore-Hoeppli phenomenon surrounding fine granules and filament (HE, x1000).

multiple nodules on the left foot since one and a half year ago with history of penetrating trauma during a flood. The lesion started as a single nodule on the plantar side of the left foot which multiplied and grew in size. The nodules hardened, eroded, and exudied copious malodorous discharge. The patient then became chair ridden. Physical examination revealed multiple solid erythematous nodules with tumefaction on the left foot. There was also erosion, excoriations, with formations of yellow-to-black crust on top of the nodules **(Figure 1a)**. The adjacent skin was hyperpigmented and swollen and the affected leg became smaller in comparison to opposite leg. Radiological examination of the left foot showed osteomyelitis.

Excisional biopsies have been performed several times during the past by surgeons and dermatologists. The histopathological interpretations from previous biopsies were verruca vulgaris and pyogenic granuloma, yet the lesion recurred. Meanwhile, we have performed two incisional biopsies during visit to this hospital. Samples of exudate and tissue were taken for direct examination and histopathological examination. No granules were found on our first histopathological examination; therefore, the result was inconclusive. Subsequent culture and resistance examination for bacterial and fungal organism were also performed. The last histopathological examination showed numerous polymorphonuclear cells, lymphocytes, histiocytes, plasma cells, and Splendore-Hoeppli phenomenon with fine granules composed of rod-shaped structures on the centre **(Figure 2)**. Gram staining was positive. Fungal and aerobic bacterial culture examinations showed no growth of microorganisms from histopathological specimen. However, anaerobic bacterial culture from exudate showed growth of *Staphylococcus aureus*.

Considering the clinical progression and the fine granules observed on the histopathological examination, we decided to initiate the regimen for actinomycetoma before we obtained Gram staining result. The patient was given 600 mg of Rifampicin and 1600/320 mg of Cotrimoxazole in divided dose. After 12 months of treatment, the patient only has minimal difficulties in mobilization and the lesion resolved leaving hyperpigmentation and scarring **(Figure 1b)**. X-ray examination still shows signs of

osteomyelitis, yet we decided to discontinue the treatment and monitor the patient closely every three months for clinical signs of relapsing actinomycetoma.

Discussion

Mycetoma has been classified by WHO as neglected tropical disease since 2013. It is common in "mycetoma belt" countries such as Mexico, India, and Sudan, but can still sporadically occur in other countries. Physicians may not be able to recognize this disease in the early stage. Moreover, mycetoma is usually misdiagnosed and mismanaged especially in non-endemic countries.[2,3,8] In fact, no more than 10 cases of mycetoma have been reported during in Jakarta, Indonesia during 1989 until 2013.[7] On top of that, our patient wasn't considered as a high at-risk individual aggravating the difficulties in diagnosis of mycetoma. The risk of acquiring mycetoma increases in productive men, working in agricultural field who are more prone to exposure of contaminated soil.[1,4,9] Nonetheless, our patient presented with some features from triad of mycetoma e.g. painless firm subcutaneous mass and multiple sinus as well as discharge-producing fistula, which raised suspicion towards mycetoma. About 90% of mycetoma lesions were found on lower extremity. In addition, the inoculation most likely occurs through penetrating trauma on the foot during rainy season in our country. It has been reported that increase in rainfall rate rises the incidence of actinomycetoma.[10]

Treatment in early stage is essential in preventing mycetoma progression. Without treatment, complications may develop. Furthermore, the therapeutic options in late stage are limited and may burden patient with disabling comorbidities.[2,8] Considering the size of the lesion (over 10 cm) and the onset of the disease (over 1 year), our patient was classified to have advanced or late stage mycetoma. Although prognostic factors for actinomycetoma have not yet been determined, it has been proposed that these factors increase the likelihood of amputation in eumycetoma cases. In addition, other complications including secondary infection, osteomyelitis, and muscle wasting, had emerged in this case. Therefore, management need to be initiated immediately in this case before amputation becomes the only therapeutic option available.

Identification of causative organism in mycetoma is the foundation of mycetoma treatment. Eumycetoma is treated with antifungal, while actinomycetoma is treated with antibiotics. The identification can be determined by examining tissue cultures from excisional biopsies, which is considered as the gold standard examination in determining the etiology of mycetoma. Unfortunately the culture of grain specimens is often not helpful because the causative organism is often invisible, and there is a risk of bacterial contamination.[11] It is reported that positive culture rates are as low as 35%. Negative culture can result from previous antibiotic treatment, overgrowth of concominant microorganism or inadequate methodology.[1,8,12,13] As it is shown, in this case, fungal culture from the second biopsy yielded negative result. Bacterial culture from the base of the ulcer showed *Staphylococcus aureus* growth which is not indicative of actinomycetoma. The recommended method for fungal culture in mycetoma is using Saboraud dextrose agar incubated at room temperature for about 6 weeks, while for bacterial culture, various media such as brain-heart infusion, blood agar, thioglycolate broth, or Lowenstein-Jensen can be incubated at 35-37^{0}C for about 10 days. Other points taken into consideration are previous medications and duration of disease, as those might affect the number of viable organisms in the lesion. Meticulous

requirements, lack of experience especially in non-endemic countries, might yield inaccurate result and lead to misdiagnosis.[1,2,12,13]

Many methods have been developed to aid organism identification, such as histopathology, cytology, skin test, serology, and molecular methods. In fact, only molecular methods, such as polymerase chain reaction, can identify organism at species levels. In addition, none of them have been extensively studied to determine the sensitivity and specificity of each method in comparison to culture. In the current center, the only available alternative methods are histopathology and cytology. Another study stated that histopathology has over 75% diagnostic accuracy compared to culture. Moreover, inflammation and fibrosis in advanced stage might lower the success rate of the fine needle aspiration technique to obtain the required specimen.[12] Several studies reported the differences between both clinical entities. Considering the clinical progression and the fine granules observed on the histopathological examination, it was decided to initiate the regimen for actinomycetoma immediately. When the Gram staining showed a positive result and the last histopathological examination showed numerous polymorphonuclear cells, lymphocytes, histiocytes, plasma cells, and Splendore-Hoeppli phenomenon, the diagnosis was concluded to be actinomycetoma and continued the treatment was continued.

Treatment of actinomycetoma is based on complications found in the patient, such as being resistant to standard therapy, bone involvement, location of the lesion (head, neck, thorax, clan abdomen), and dissemination to other organs. In this case, there was bone involvement, therefore Welsh regiment was recommended. Welsh regiment consists of Amikacin 15 mg/kg/day divided into 2 doses administered for 21 days and Cotrimoxazole 8/40 mg/kg/day for 5 weeks. The cycle can be repeated 4 times at most.[1,12,13] Nevertheless, in this patient, we gave treatment of Rifampicin 600 mg and Cotrimoxazole 1600/320 mg divided into 2 doses per day, which is more indicated in patients with no complication. There was discrepancy in treatment because patient refused to be hospitalized for Amikacin treatment that was given intravenously. Thus, it was decided to give oral treatment of Rifampicin and Cotrimoxazole to the patient. A 12-month treatment to this patient showed improvement of the lesion, leaving only hyperpigmentation and scarring. The patient experienced minimal difficulties in mobilization and no osteomyelitis was found in the last radiography and ultrasound examination.

Mycetoma treatment is challenging and requires long periods of drug therapy with or without surgical procedures (excision of the lesion, bone curettage, amputation).[14] Furthermore, majority of the cases have negative culture, therefore provide inadequate information regarding the disease, treatment, and evaluation.[15] In endemic countries, the diagnosis of mycetoma can be made based on the clinical triad symptoms including: (1) painless subcutaneous tumor-like swelling, (2) multiple draining sinuses and fistulas, and (3) grainy discharges, which are the colonies of bacteria or fungi, with different colors, sizes, and textures, depending on the species of the causative microorganisms. However, using this method in non-endemic countries is challenging due to the lack of typical clinical presentation.[16,17] Histopathologic examination that shows suppurative granuloma may increase suspicion towards mycetoma, but it is still hard to determine the causative organism. Thus, culture is required to identify the organism properly, as morphological features of grains can be similar in many agents causing mycetoma. Unfortunately, despite having 96% accuracy for diagnosis, various culture medias are required and this method is

time-consuming as it needs to be incubated for 4-6 weeks before reporting it as negative.[16]

A more effective algorithm for mycetoma management is needed to facilitate early treatment initiation and decrease the overall morbidity of the disease, especially in nonendemic countries.[16] In this case and several studies by Efared *et al;*[8] Patra el al,[15] Liu *et al.*[12] the cases were similar, with a high suspicion of mycetoma with negative culture result but the patient was in advanced conditions. In this kind of setting, a thorough clinical examination is the first reliable diagnosis method. Triad symptoms of mycetoma are the main findings that raise suspicion towards mycetoma. Afterwards, we can use histopathology as another diagnostic tool. The main merit of histopathology is to differentiate eumycetoma from actinomycetoma, although identification at species level is not reliable and almost impossible.[8] In histopathology, eumycetoma will show hyphae, thick club-shaped structures, embedded in cement like matrix, whereas actinomycetoma will show multilobated colonies, pale central eosinophilia, basophilic outer border with ill-defined branching filaments, and Splendore-Hoeplii phenomenon.[1,8] Culture method as the gold standard will still be done to identify the causative agents, nevertheless the therapy should be started if the patient has complications. The therapy regimen is chosen according to histopathological result, eumycetoma is treated with antifungal and actinomycetoma is treated with antibiotics.[8,12,15] When clinical suspicion is high, the therapy should be initiated during waiting for the result of culture or other diagnostic tools. The treatment evaluation should rely on the clinical improvement. Despite the long-term treatment and recurrences observed in medical treatment, aggressive surgery is not the first line of treatment. Amputations are generally due to misdiagnosis or a long-standing disease that spreads to deeper structures of the body. Mycetoma should be treated medically rather than surgically.[8,18,19]

Conclusion

An ideal and effective diagnostic tool for mycetoma is still lacking. A combination of techniques is required to reach a diagnosis. Early and accurate diagnosis is beneficial in optimizing treatment and avoiding complications.

References

1. Kusmarinah Bramono. Aktinomisetoma. In: Bramono K, Suyoso S, Widaty S, Ramali LM, Siswati AS, Ervianti AV, editors. Mikosis Profunda. 1st ed. Surabaya: Airlangga University Press; 2019. p. 53–65.
2. Reis CMS, Reis-Filho EG de M. Mycetomas: an epidemiological, etiological, clinical, laboratory and therapeutic review. *Anais brasileiros de dermatologia*. 2018;**93**:8–18.
3. World Health Organization. Neglected tropical diseases [Internet]. WHO. [cited 2023 Feb 19]. Available from: https://www.who.int/health-topics/neglected-tropical-diseases
4. Eliza Miranda. Eumisetoma. In: Bramono K, Suyoso S, Widaty S, Ramali LM, Siswati AS, Ervianti AV, editors. Mikosis Profunda. 1st ed. Surabaya: Airlangga University Press; 2019. p. 41–52.
5. Relhan V, Mahajan K, Agarwal P, Garg VK. Mycetoma: an update. *Indian J Dermatol*. 2017;**62(4)**:332.
6. Suyoso S, Ervianti AV, Astari L, Anggraeni S, Widia Y. Pendahuluan: mikosis profunda. In: Bramono K, Widaty S, Ramali LM, Siswati AS, Ervianti AV, editors. Mikosis Profunda. 1st ed. Surabaya: Airlangga University Press; 2019. p. 53–65.
7. Yahya S, Widaty S, Miranda E, Bramono K, Islami AW. Subcutaneous mycosis at the department of dermatology and venereology dr. Cipto Mangunkusumo National Hospital, Jakarta, 1989-2013. *J Gen Proc Dermatol Venereol Indonesia*. 2016;**1(2)**:1.
8. Efared B, Tahiri L, Boubacar MS, Atsam-Ebang G, Hammas N, Hinde EF, *et al*. Mycetoma in a non-endemic area: a

diagnostic challenge. BMC Clin Pathol. 2017;**17(1)**:1–6.
9. Zeeshan M, Fatima S, Farooqi J, Jabeen K, Ahmed A, Haq A, *et al*. Reporting of mycetoma cases from skin and soft tissue biopsies over a period of ten years: A single center report and literature review from Pakistan. *PLoS Neg Trop Dis*. 2022;**16(7)**:e0010607.
10. Bakshi R, Mathur DR. Incidence and changing pattern of mycetoma in western Rajasthan. *Indian J Pathol Microbiol*. 2008;**51(1)**:154.
11. Argentina F, Karim PL. Extensive Actinomycetoma Clinical Manifestations and Histopathology: A Case Report. Bioscientia Medicina: *J Biomed Transl Res*. 2022;**6(15)**:2705–12.
12. Liu A, Maender JL, Coleman N, Hsu S, Rosen T. Actinomycetoma with negative culture: a therapeutic challenge. *Dermatol Online J*. 2008;14(4). Available from: https://escholarship.org/uc/item/19m6r34k
13. Bravo FG, Arenas R, Sigall DA. Actinomycosis, nocardiosis, and actinomycetoma. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, *et al.* editors. Fitzpatrik's Dermatology. 9th ed. New York: McGraw-Hill Education; 2019. p. 2876–91.
14. Sampaio FMS, Wanke B, Freitas DFS, Coelho JMC de O, Galhardo MCG, Lyra MR, *et al*. Review of 21 cases of mycetoma from 1991 to 2014 in Rio de Janeiro, Brazil. *PLoS Neg Trop Dis*. 2017;**11(2)**:1–18.
15. Patra S, Senthilnathan G, Ramam M, Arava S, Bhari N. Linezolid: A novel treatment option for the treatment of a non-responsive case of actinomycotic mycetoma. *Indian J Dermatol Venereol Leprol*. 2021;**87(3)**:473–5.
16. Husain U, Verma P, Suvirya S, Priyadarshi K, Gupta P. An overview of mycetoma and its diagnostic dilemma: time to move on to advanced techniques. *Indian J Dermatol Venereol Leprol*. 2023;**89(1)**:12–7.
17. Hao X, Cognetti M, Burch-Smith R, Mejia EO, Mirkin G. Mycetoma: Development of Diagnosis and Treatment. *J Fungi*. 2022;**8(7)**:743.
18. van de Sande WW. Global burden of human mycetoma: a systematic review and meta-analysis. *PLoS Neg Trop Dis*. 2013;**7(11)**:e2550.
19. Fahal A, Mahgoub ES, Hassan AME, Abdel-Rahman ME. Mycetoma in the Sudan: an update from the mycetoma research centre, University of Khartoum, Sudan. *PLoS Neg Top Dis*. 2015;**9(3)**:e0003679.