



## WHITE GRAIN EUMYCETOMA CAUSED BY *Aspergillus nidulans*, A RARE REPORT FROM DISTRICT OF MAHARASHTRA INDIA

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**Abstract-** Mycetomas are chronic subcutaneous infections caused by fungi and bacteria. When the etiology is fungal they are known as Eumycetomas. We describe a case of white grain eumycetoma by *Aspergillus nidulans* (*A. nidulans*) of the foot in a 60 yrs. old male patient agriculturist by occupation from Maharashtra, India. *A. nidulans* mycetoma shows more bleeding than usually seen and it is more painful than other fungal agents. South and North part of India are endemic for Mycetoma. Maharashtra is western part of India where Mycetoma is not endemic. In present case lesion was painful probably due to *A. nidulans* infection and /or by secondary bacterial infection by *MRSA* and *M. morgani*.

**Conclusion-** High index clinical suspicion, accurate categorization of lesion into eumycotic or actinomycotic along with culture correlation, is vital for prognosis for positive clinical outcome.

**Keywords-** *Aspergillus nidulans*, White grain mycetoma, Diabetes mellitus

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### Introduction

Mycetomas are chronic subcutaneous infections caused by fungi and bacteria. When the etiology is fungal they are known as Eumycetomas. The organisms are traumatically implanted into the deep dermis or subcutaneous tissue from the natural environment and cause a subcutaneous infection characterized by the formation of large, macroscopic aggregates of fungal or actinomycetes filaments known as grains [1,2]. A dense infiltrate of Polymorphonuclear leukocytes and other inflammatory cells accumulates around these structures. It does not spread beyond the locality of the initial site of infection and is seldom fatal. As there are pronounced differences in the treatment and prognosis of mycetomas resulting from different organisms, in particular between the eumycetomas and actinomycetomas, identification of causative agent plays a key part in their management [2-4]. Mycetoma is endemic in Africa, from Sudan to Somalia through Mauritania and Senegal. Other endemic countries include Mexico and South and North parts of India. Mycetoma is most often seen in men, the male -female ratio of infection being 4:1. It is mainly a disease of rural workers aged between 16 to 45 yrs. Herewith we describe a case of white grain eumycetoma by *Aspergillus nidulans* (*A. nidulans*) of the foot in an agriculturist from district of Maharashtra India [4,5].

### Case Report

A 60 yrs. old male patient agriculturist by occupation from Maharashtra, India, was admitted on 15/12/2011 in Pad. Dr. D.Y. Patil Medical College and Hospital Pimpri, Pune-18. He had ulcer on the sole of right foot from last 5 months. During the admission he was afebrile and general conditions were fair. The foot was hard and woody with no sign of acute inflammation. On examination, ulcer size was 3x2 cm on sole of right foot [Fig-1]. No history of pus discharge, but also presented ulcer on right buttock. Patient gave history of trauma by thorn 1 year earlier. Initially ulcer was small in size but gradually increased. Ulcer was non-healing. He was treated with voveran, rantac, Augmentin (IV) and vitamin. Patient was advised regular dressing. Ulcer gradually increased in size with no history of fever, no lymphadenopathy. Patient was suffering from diabetes mellitus (DM). Initially patient was on regular treatment but later discontinued treatment by himself.

Routine clinical biochemistry was carried out - showed liver function test, urea creatinine, protein within normal limits. Hematological investigations revealed Hemoglobin (Hb) 11.6, TLC-6800, DLC-N-64%, L-31%, E-2%, M-1%. Serology for human immunodeficiency virus was non reactive.

Purulent discharge was collected and sent for microbiological culture investigations. Routine bacteriological culture showed isolation of *MRSA* and *Morganella morganii*. Same treatment was continued. Ten days later he complained of pain with serous non-foul smelling discharge. On examination there was a sinus opening and tract could be traced up to calcaneum and patient had difficulty in walking. The X-ray examination of foot showed calcaneal spur with osteosclerotic lesion on B/L aspect of calcaneum with suspected cavity on posterior aspect of body of calcaneum. MRI of calcaneum was done-Impression "The calcaneum does not primarily appear to be the source of infection since architecture and trabecular pattern is intact. Probable soft tissue involvement with periosteal reaction over the plantar aspect of calcaneum as response to local inflammation". Further the lesion increased in size and the whole area became hard, swollen.



Fig. 1- Sinus on sole of foot

Patient was diagnosed as chronic osteomyelitis of calcaneum-inflammatory, probably fungal etiology. Patient was advised for microscopic examination for discharge of sulfur granules.

#### Microbiological Methods

After a thorough cleaning of the lesion with alcohol, a pasture pipette was introduced into sinus and the fluid was aspirated. After washing with saline, fluid showed presence of white granules measuring 0.5 to 1.2 mm in diameter. Microscopic examination of granules in KOH mount, showed hyaline, septate hyphae. White granules were cultured on Sabourauds dextrose agar with chloramphenicol incubated at 25-30 °C [4-6]. Fungal growth appeared after three days of incubation. Identification of the growth was done by colony morphology, pattern of sporulation, pigmentation and growth rates. Colonies were white coloured with granular surface with abundant rugae which gradually developed a green centre. After a period of seven days it turned wrinkled, developed deep green colour at centre with white brim [Fig-2]. Microscopy of the fungal growth by (LPCB) Lactophenol Cotton Blue. It showed several cleistothecia including smaller sac like asci that contained ascospores [Fig-3].

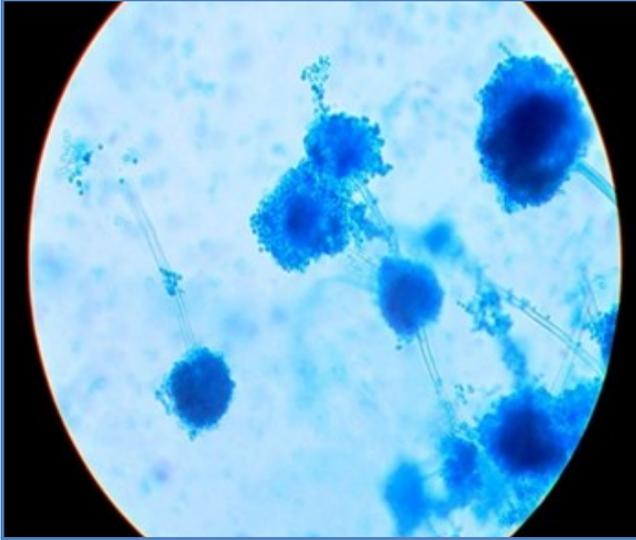


Fig. 2- *Aspergillus nidulans* growth on SDA agar. Characteristics wrinkled colonies having thick green coloured at centre with white brim



Fig. 3- LPCB shows several cleistothecia including smaller sac like asci that contain ascospores

In members of *A. nidulans* group, the colour of the ascospores varies from red-brown to purple or violet. *A. nidulans* are known to reproduce sexually, with sexual spores in the ascospores within asci. We found similar findings. Mature ascospores in the shape of double convex lens or two symmetrical valves were observed microscopically. Globose subglobose shaped cleistothecia were observed which favours *A. nidulans*. Slide culture techniques were set up for detail morphological study. In LPCB of slide culture vesicles hemispherical, 8-10µm diameter, biserate conidiogenous cell, spherical, rugose, sub-hyaline conidia were observed [Fig-4]. Hence on basis of these microscopic and culture finding the isolate was identified as *A. nidulans*. Patients was started on antifungal therapy.



**Fig. 4-** LPCB shows vesicles hemispherical, conidiogenous cell biseriate, conidia were spherical, rugulose, sub-hyaline.

### Discussion

Eumycetoma is a slowly progressing granulomatous infection developing after traumatic implantation of fungus-contaminated plant materials like thorns. About thirteen species of fungi are known to cause white grain eumycetoma such as *Acremonium falciforme*, *Acremonium killense*, *Acremonium recifei*, *Aspergillus nidulans*, *Aspergillus flavus*, *Fusarium moniliforme*, *Fusarium soloni*, *Scedosporium apiospreum*. The diagnosis of eumycetoma is confirmed by the demonstration of grains in lesion and their identification. This is important in order to provide information on prognosis and treatment. For instance, it is important to differentiate eumycetoma, which often respond poorly to chemotherapy [5-8]. According to Nicolle and Blanc (1920) the first report of isolating *A. nidulans* var. *Nicolle* from a patient with mycetoma with white granules was isolated in 1906 by Pinoy from Tanzania [5]. Mycetoma with lesions of the arm and head was reported by Puestow (1929) with *A. nidulans* which was the only sole source of organism recovered from culture [6]. No other report was made till Baylet *et.al* in 1968 who isolated *A. nudulans* from a patient with mycetoma of the knee in Senegal [8,9]. In 1971, a fully documented case of maduramycetoma (eumycetoma) of the foot caused by *A. nidulans* was reported from Sudan by Mahaoub ES [9]. Three more cases due to *A. nidulans* were also reported in Sudan since then. From India, the first case of *A. nidulans* mycetoma was reported by Joshi KR in 1985 from Rajasthan [10]. After 26 yrs., this is likely to be a case of mycetoma caused by *A. nidulans* from India and first case from state of Maharashtra. *A. nidulans* mycetoma shows more bleeding than usually seen and it is more painful than other fungal agents. South and North part of India are endemic for Mycetoma. Maharashtra is western part of India where Mycetoma is not endemic. In present case lesion was painful most probably due to *A. nidulans* infection and/or by secondary bacterial infection by *MRSA* and *M. morgani*. Patient with granulocytopenia, high dose corticosteroids treatment and immunocompromised status following marrow transplantation, chronic granulomatous diseases are various risk factors for invasive Aspergillosis. In present case diabetes mellitus (DM) and irregular treatment of DM was in favor for Eumycetoma as also

secondary bacterial infection leading to painful lesion.

### Conclusion

High index clinical suspicion, accurate categorization of lesion into eumycotic or actinomycotic along with culture correlation, is vital for prognosis for positive clinical outcome.

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