

Case report

Madurella mycetomatis causing multiple mycetoma lesions: A rare clinical presentation

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Case report:

In this communication, we report on a 32-year-old unemployed male who presented to the Mycetoma Research Centre (MRC), Soba University Hospital, University of Khartoum, Khartoum, Sudan on Monday 3rd of September 2018 with long-standing eumycetoma. The condition started 20 years prior to presentation with small right foot painless swelling between the 4th and 5th toes. It gradually increased in size over the years. It used to discharge sero-purulent discharge that contained black grains through multiple sinuses. The swelling then extended to involve the sole of the foot. The patient recalls no history of local trauma. Ten years ago he developed another painless swelling located between the left big and second toe with multiple sinuses discharging black grains, four months following local trauma. The swelling gradually increased in size to involve the dorsum, the lateral and the medial aspects of the left foot. During all this time he was on irregular medical care at a local dispensary.

In 2013, the swellings became massive in size and severely infected which urged the patient to seek medical help at a district hospital, but he did not comply with the medical advice and he eventually had left below knee amputation under general anaesthesia. Due to his poor medical compliance,

he ended with right below knee amputation under spinal anaesthesia with uneventful post-operative recovery.

In 2017, the patient noticed a painless swelling in the lateral aspect of his left little finger; it increased rapidly in size and in one-year time it involved all the fingers, the palm, and the dorsum aspect of the left hand with multiple sinuses and black grains discharge. The patient had no constitutional symptoms. The patient has a family history of mycetoma, of low socioeconomic status, known smoker and is jobless due to his illness.

At presentation he was ill, depressed, walking with bilateral below knee silicone sleeve prosthesis. Haemodynamically he was stable. Systemic examinations were within normal apart from bilateral below knee amputation. Local examination of the left hand revealed huge multiple subcutaneous masses encompassing the whole left hand with multiple active sinuses discharging black grains, Fig. 1. His liver functions test showed serum bilirubin of 0.24 mg/dL, direct bilirubin of 0.10 mg/dL, total protein of 8.69 g/dL, serum albumin of 4.52 g/dL, alkaline phosphatase of 160 U/L, AST of 13 U/L and ALT of 11 U/L. His renal function test showed normal blood urea of 14.8 mg/dL, serum creatinine of 0.88 mg/dL, serum

sodium of 138.6 mmol/l and serum potassium of 4.04 mmol/l. His random blood sugar was 84.9 mg/dl. His complete blood count examination showed normal leucocytes; total white blood cells counts of $5.3 \times 10^3/\text{mm}^3$, red blood cells count of $5.38 \times 10^6/\mu\text{l}$, haemoglobin of 14.9 g/dl, platelets count of $283 \times 10^3/\mu\text{l}$ and packed cell volume (PCV) of 43.7%. His immune profile was determined, and it showed normal CD3, CD16 and low CD 19, Table 1, Fig. 2.

Hand lesion ultrasound examination findings were in line with massive eumycetoma; multiple thick cavities with multiple grains. Left hand conventional X-Ray showed massive soft tissue swelling with bone involvement. A tru-cut needle biopsy was done under local anaesthesia and grains were collected, they were partly persevered in normal saline for grains culture and molecular identification and partly in 10% formal-saline for histopathological examination. Paraffin processed tissue block was prepared from the surgical biopsy which measured 1.1 x 1.5 mm. The tissue block was cut using rotary microtome, and subsequently, 3-5- μm sections were obtained. The sections were stained with Haematoxylin and Eosin stain (H&E). Microscopical examination of the sections showed multiple black grains surrounded by granulation tissue and marked histiocytic, and mixed inflammatory cellular infiltrates, in line with *M. mycetomatis* eumycetoma.

For mycological identification, the black grains were washed three times in saline solution and then cultivated on Sabouraud dextrose agar (SDA) with gentamicin (0.1 g/L) at 37°C. After two

weeks incubation the culture showed flat, tough yellowish to brown colonies, these colonies tend to be folded, and with time it turned heaped, the aerial mycelia were observed, and the characteristic of brown diffusible pigmentation were noticed. This morphological appearance was in line with *M. mycetomatis*.

For molecular identification, grains isolated from the biopsy material and processed for PCR identification, and the ITS was positive for *M. mycetomatis* specific primers, Fig.3. The patient was started on itraconazole 400mg per day in two divided doses and 5mg folic acid once daily, and he was on regular follow-up at the MRC without much improvement of his condition.

Table 1. Lymphocytes subset report

Description	%	Normal range	Absolute count	Normal range
T cells (CD3)	81.3	49 - 81	1251.00	527 – 2846
T helper cells (CD3/CD4)	42.1	28 - 51	648.00	332 – 1642
T cytotoxic cells (CD3/CD8)	34.2	12 -38	526.00	170 – 811
B cells (CD19)	3.68	7 - 23	57.00	78 – 899
NK cells (CD16)	12.3	6 - 29	190.00	67 – 1134



Fig. 1A: Photography of the patient showing bilateral below knee amputations and massive left hand eumycetoma with multiple sinuses.



Fig.1B: Photography of the patient's left hand showing massive left hand eumycetoma with multiple sinuses.

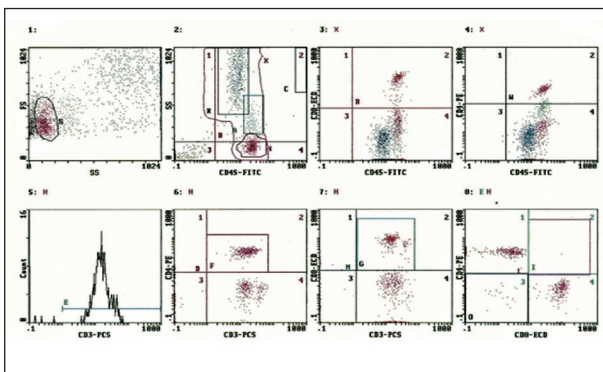


Fig.2: showing flowcytometry results indicating normal CD3, CD16 and low CD 19

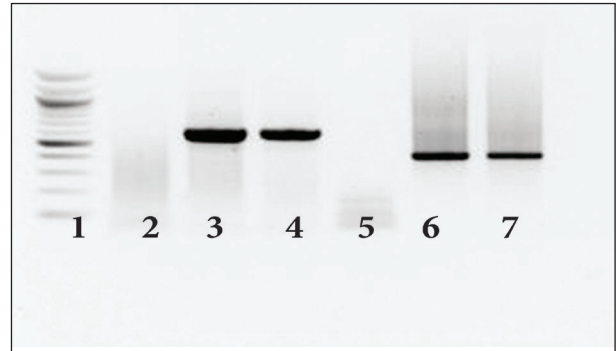


Fig.3: showing a PCR results from the patient sample;

Lane 1: 100 bp DNA ladder;

Lane 2 ITS negative control;

Lane 3 ITS positive control;

Lane 4 Patient grain DNA was positive for ITS;

Lane 5 *M. mycetomatis* negative control;

Lane 6 *M. mycetomatis* positive control;

Lane 7 Patient grain was positive for M.

mycetomatis specific primers

Discussion:

Mycetoma is a badly neglected medical and health problem, endemic in many tropical and subtropical regions. (1,2) The disease is caused by many micro-organisms of fungal and bacterial origin, and hence it is classified as eumycetoma and actinomycetoma respectively. (3,4) It is characterised by a chronic specific granulomatous inflammatory response forming granuloma initially at the subcutaneous tissue that then spread to involve the skin, deep structures and bones. (5,6)

Sudan is a mycetoma highly endemic country with the highest reported prevalence, and *M. mycetomatis* is the most frequently reported causative agent. (7,8) Mycetoma has many negative impacts on patients, family and community. Transmission is still questionable; however; the disease is believed to occur as a result of traumatic implantation of the causative organism into the subcutaneous tissue through minor trauma. (1,2) In the reported patient, history of local trauma was reported at only one site. That is not in line with this implantation theory, but minor unrecognized trauma may be overlooked. However, other significant genetic,

immunogenic or environmental factors should be considered.

Although the Mycetoma Research Centre, University of Khartoum is dealing with more than 8500 patients, such presentation is infrequent, and the explanation is unclear.⁽⁹⁾ His immune profile was within normal apart of B cells deficiency; that is not in line with a study conducted by Mahgoub and his associates that investigated the immunology status of mycetoma patients, and their results showed that there was a definite rise in the IgM and IgA classes of immunoglobulins and a decrease in IgG. The net results of their study is that patients with mycetoma had a deficiency in cell mediated immunity rather than humoral immunity⁽¹⁰⁾. Therefore, the deficiency of B cells reported in this patient may be an important contributory factor for this aggressive disease course. B cells play an important role in the fight against fungal infections by producing pathogen-specific host-protective antibodies during infection course. Furthermore, B cells can promote host-protective responses through antibodies-independent mechanisms and by the pro-inflammatory cytokines expression. In experimental models, B cell-deficient mice exhibit increased susceptibility to experimental systemic candidiasis and, in humans; several case reports have described unusual fungal infections after B cell depletion. ^(11,12,13)

In a study reported by Lin and his associates, on patients with diffuse large B cell lymphoma treated with rituximab, they observed that elderly patients tended to have a high rate of fungal infection. ⁽¹³⁾ Furthermore, another study conducted by Kamar and his colleagues compared the incidence of infections in 77 patients receiving B cell depletion therapy (BCDT) after kidney transplantation with 909 control patients without B cell depletion and they reported a significantly higher rate of fungal infections in the BCDT group, whereas the bacterial infection rate was similar between the two groups. ⁽¹⁴⁾

The patient is of low socioeconomic status, poor health education, from a deprived remote locality with the meagre medical facility and all these may have contributed to his poor medical compliance. Furthermore, the painless disease nature, scarcity of medication, the low cure rate and high drug side effects encountered with the present mycetoma treatment may also contribute to the poor compliance and, hence, the poor treatment outcome encountered in the reported patient. This patient had bilateral lower limbs amputations, and disused hand and these had caused massive disability, and that had affected his ability to earn his living. All of this had worsened the negative impacts of mycetoma on the patient, his family and the community at large.

In summary, in this communication, we reported on the first patient with multiple massive aggressive eumycetoma lesions caused by *M. mycetomatis* with B cells deficiency. The clinical presentation and aggressive mycetoma behaviour encountered in this patients is unique and unreported, and it is worth reporting it.

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