MYCETOMAS CAUSED BY CURVULARIA LUNATA, MADURELLA GRISEA, ASPERGILLUS NIDULANS, AND NOCARDIA BRASILIENSIS IN SUDAN

EL SHEIKH MAHGOUB

Department of Microbiology & Parasitology, Faculty of Medicine, University of Khartoum

Four cases of mycetoma are described from the Sudan. The first is an authentic case due to Curvularia lunata. The others were due to Madurella grisea, Aspergillus nidulans and Nocardia brasiliensis.

Mycetoma in Sudan is mainly caused by Madurella mycetomi, Streptomyces somaliensis, Actinomadura madurae and A. pelletieri (Abbott 1956; Lynch & Moghraby 1961). Two rare agents of mycetoma, Corynespora cassicola and Aspergillus nidulans were reported by Mahgoub (1969, 1971). This paper confirms the pathogenicity of Curvularia lunata reported by Baylet, Camain & Segretain (1959) from Senegal, describes a second case of mycetoma due to A. nidulans, and reports 2 mycetomas due to M. grisea and Nocardia brasiliensis for the first time from the Sudan.

CLINICAL ASPECTS

Case 1

E.H., a 50-year-old cultivator presented at the Khartoum North Civil Hospital in 1970 with a painless swelling on the dorsal and medial part of the right foot. The lesion had started 10 years previously as a small swelling on the dorsum gradually increasing in size. It was excised 8 years before but recurred 1 year later. Sinuses opened from time to time and discharged black grains. The circumference of the foot, measured 12 cm from the tip of the first toe, was 29.5 cm; that of the left foot was 24.5 cm. Three papules and 3 healed sinuses were seen on the dorsal and medial aspects of the foot (fig. 1).

Radiology showed involvement of all tarsal bones and the proximal ends of the metatarsals. Osteoporosis, large and small multiple cavities as well as periosteal erosion were seen (fig. 2).

Case 2

B.S., a 65-year-old cultivator was admitted to the Khartoum North Civil Hospital in 1971 because of a left mycetoma pedis of 40 years duration. The lesion started with a painful swelling of the foot following a fall. It remained painful for the first 5 years at the end of which it was operated on in his district hospital. Almost immediately after operation the pain recurred and 3 years later a swelling appeared on the dorsum of foot, slowly increasing in size. He gave no history of grains being discharged.

A hard indurated dorsal swelling extending from the base of toes to the ankle was seen. The site of 3 healed sinuses was visible (fig. 3). The circumference of the foot, 15 cm from tip of first toe, measured 26 cm and increased to 28 cm during hospitalization.

A radiograph showed marked sclerosis around multiple cavities in all tarsal and metatarsal bones (fig. 4).
Case 3

A.E., a cultivator aged 43 years was admitted to Khartoum Civil Hospital as a case of fibrosarcoma of knee and thigh in 1971. He complained of pain and swelling of the right knee, for 3 years, following trauma. The swelling gradually increased and spread to the thigh. Sinuses opened from time to time discharging pus and white grains.

The patient looked ill, was anaemic and in great pain. Both knee and thigh were grossly swollen with limited painful movement of the joints. Active and healed sinuses were seen (fig. 5).

Radiology revealed involvement of the heads of the tibia and fibula. The patella and whole length of femur showed cavitation, osteoporosis and periosteal bone deposition in addition to the soft tissue tumour (fig. 6).

Case 4

A.K.H., a 30-years-old cultivator, reported to Khartoum Civil Hospital in January 1972. He complained of a painful swelling of the right foot particularly around the ankle, discharging pus and blood from various sinuses but no grains were seen. His condition started about 6 years before, without a history of trauma. A small swelling appeared on the lateral side of the right foot. He was given 20 injections of what seems to have been streptomycin. Swelling disappeared but recurred 4 years later; it increased gradually and extended over the whole foot.

On examination, a hard painful swelling with multiple papillomata and sinuses involving the lower third of leg, ankle, posterior half of foot and sole was seen (fig. 7). A radiograph showed multiple cavities in the calcaneum and tarsal bones, but the talus was not involved (fig. 8).

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Colour &amp; Size of grain</th>
<th>Growth Temperature</th>
<th>Rate of Growth</th>
<th>Colonial Morphology on primary isolation</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Black 1-2 mm</td>
<td>26°C &amp; 37°C Better at 26°C</td>
<td>Rapid</td>
<td>Dematiaceous fluffy Colony; no pigment; Sterile mycelia</td>
<td>Curvularia lunata</td>
</tr>
<tr>
<td>2</td>
<td>Black 0.5-1 mm</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>Dematiaceous velvety colony; no pigment; mycelia sterile.</td>
<td>Madurella grisea</td>
</tr>
<tr>
<td>3</td>
<td>White 1-2 mm</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>Velvety green colony; Aspergillus heads &amp; spores; no perithecia</td>
<td>Aspergillus nidulans</td>
</tr>
</tbody>
</table>

Mycology

Biopsy specimens were received from the first 3 cases. Grains were dissected out and cultured on Sabouraud’s dextrose agar with chloramphenicol. Mycological details of the organisms isolated are shown in the Table. Cultures from case 1 and 2 were sent to Dr. Phyllis Stockdale of the Commonwealth Mycological Institute, England who identified the first as Curvularia lunata (Wakker) Boedijn var. aeria (Batista, Lima & Vasconcelos) and thought the second seemed in some respects to be closest to Madurella grisea.

Serology

Sera from these patients were tested against antigens from M. mycetomi, S. somaliensis, A. pelletieri, A. madurae, N. brasiliensis, N. caviae and N. asteroides using
agar-gel diffusion. All tests were negative except sera from case 4 which reacted with *N. brasiliensis* antigen alone.

Following the cultural diagnosis, antigens were made from the respective organisms by the method described by Murray & Mahgoub 1968. A strain of *Curvularia lunata* No. B-450 was provided by Dr. L. Ajello of Atlanta, Georgia, U.S.A. Positive lines of precipitation developed with the respective fungi.

**Histopathology**

Histopathological studies were performed on specimens from the first 3 cases but grains were only found in Nos. 1 and 3 i.e. *C. lunata* and *A. nidulans*.

*C. lunata*

In sections stained with H & E the grain was in the middle of an abscess, the predominant cells being polymorphonuclear leucocytes and few giant cells. The grain was elongated, measuring 500 x 225 μ and vesicular in appearance (fig. 9). Vesicles were more numerous in periphery. Their cell walls stained deep brown but pigment was not seen. The grain stained strongly positive with PAS but no more details were visible.

*Aspergillus nidulans*

The histological picture of *A. nidulans* grains was described previously (Mahgoub 1971). In H & E stained sections, the grains were round and about 350 μ in diameter. They were pale and consisted of closely packed irregular, highly segmented hyphae and spores. Sections stained with PAS revealed greater detail (fig. 10).

**Discussion**

These organisms, isolated from cases of mycetoma, confirm the wide variety of causative organisms and their wide geographical distribution.

*Curvularia lunata*

This dermatiaceous fungus, not known to cause mycetoma was first suspected by Baylet et al. (1959) when they isolated it from a black grain mycetoma in Senegal. However, other species of *Curvularia* were reported from mycetomas in animals. Bridges & Beasley (1960) isolated *C. geniculata* from mycetomas in a cat and a horse, and Brodey, Schryver, Deubler, Kaplan & Ajello (1967) obtained it from a dog. Because *Curvularia* spp. are common saprophytes, Baylet et al. were in doubt about its pathogenicity. Here the causative nature of the fungus was not only demonstrated by isolation from single grains but also by demonstration of precipitating antibodies in 3 sera, taken from the patient at different times, against a known strain of *C. lunata*. Like the grains of the Senegalese case, these were black and in H & E sections resembled those of *Leptosphaeria senegalensis*.

*Aspergillus nidulans*

Nicolle & Pinoy (1906) first isolated *A. nidulans* from a mycetoma in Tunisia. The second case was reported 60 years later by Baylet, Lacoste, Camain, Basset, Chabal & Izarn (1968) from Senegal. A third case was reported by Mahgoub (1971) from Sudan and this present one is the fourth authentic case. It is felt that it should be considered a genuine causative organism of mycetoma and not be discarded as a contaminant from mycetoma cultures. Also, it is necessary to confirm cultural and histopathological identification by serological diagnosis.
Nocardia brasiliensis

This is the cause of the majority of mycetomas in Mexico (Gonzalez Ochoa 1962). Although by no means limited to South America, the survey by Mariat (1963) showed a relationship of mycetoma agents to rainfall. Nocardia spp. were reported from areas with high precipitation and relatively temperate climate. My patient came from a village in Jebel Mara (Jebel=mountain) with a similar climate. In the drier parts of Sudan, M. mycetomi and S. somaliensis are commonly isolated from mycetoma lesions.

Résumé

Quatre cas de mycétome en République Démocratique du Soudan sont décrits. Le premier d'entre eux est un cas authentique causé par Curvularia lunata, le second est causé par Madurella grisea, le troisième par Aspergillus nidulans et enfin le quatrième par Nocardia brasiliensis.

Acknowledgements

I would like to acknowledge the help of Dr. Phyllis Stockdale of the Commonwealth Mycological Institute for identifying 2 of the cultures and Dr. L. Ajello of Atlanta, U.S.A. for providing a strain of Curvularia lunata for serological study.

References

Figure 1.—Case 1.—Mycetoma pedis of 10 years duration due to *Curvularia lunata*.
Figure 2.—Case 1.—showing cavities and periosteal erosion.
Figure 3.—Case 2.—Mycetoma pedis due to *Madurella grisea*.
Figure 4.—Case 2.—showing multiple small cavities.
Figure 5.—Case 3.—Mycetoma due to *Aspergillus nidulans* involving the knee and thigh.
Figure 6.—Case 3.—showing large cavities and new periosteal bone formation.

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Figure 7.—Case 4.—Mycetoma of the ankle due to *Nocardia brasiliensis*.
Figure 8.—Case 4.—showing multiple cavities in calcaneum and tarsal bones.
Figure 9.—Grain of *Curvularia lunata*; note vesicular nature. H&E × 400.
Figure 10.—Grain of *A. nidulans*. Compact hyphae and vesicles are seen. PAS × 400.