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Recurrent eumycetoma caused by novel species *Madurella pseudomycetomatis*: A case report

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ABSTRACT

Mycetoma is a chronic-granulomatous disease characterized by the inflammation, swollen organ, draining sinuses containing blood, pus, and grains. We present a case of madura foot with novel etiologic agent *Madurella pseudomycetomatis*. Diagnosis was based on morphologic, physiologic, histipathologic and molecular methods. *In vitro* antifungal susceptibility tests revealed that MIC values for itraconazole, amphotericin B, and posaconazole were 0.0313 μg/ml, 0.0313 μg/ml, and 0.004 μg/ml, respectively. The patient was treated and recovered by itraconazole(400 mg/day) after prolonged course.

1. Introduction

Mycetoma is a subcutaneous disease that appears as chronic and granulomatous disorder. It is characterized by the inflammation, swollen organ, multiple draining sinuses containing of blood, pus, and grains which can be caused by a variety of certain fungi (eumycetoma) or filamentous bacteria (actinomycetoma) [1–4]. The disease is endemic in tropical and subtropical arid climate areas, with a focus in Soudan, India, Mexico, and other country of South America and with sporadic encountering in Asia, North America, and Europe [5–7]. Traumatic inoculation of the subcutaneous tissue with the thorn or soil causative organism through minor skin trauma is a popular route of infection [1–4,8]. The disease etiologic agents are entered in the different organs of the body such as hand, leg, knee, arm, thigh, perineum, paranasal sinuses, mandible, intraspine, bladder, brain, lung, head and neckfrom soil or due to plants contact, through the injured skin [3–5,8]. Mycetoma usually affects adult in age group of 30–50 years old. However, male laborers in rural areas that work barefoot, are predominant affected group [5,9–11]. The control of this disease requires understanding by the clinicians, continuous supply of antifungal agents and high quality diagnostic facilities [12]. Unfortunately, for eumycetoma, recurrent infections are common and amputations are still needed in a large proportion of the patients. Here, we present the first successfully treated case of recurrent madura foot eumycetoma caused by *Madurella pseudomycetomatis* in Iran.

2. Case

A 47-year-old female presented with a history of felt swilling on her foot. The patient was a farmer who often walking as barefoot manner in North of Iran. According to the patient statements, primary clinical symptoms such as swelling and pussy sinus occurred approximately in April 2010 (day 0) (Fig. 1A). She had been hospitalized in Imam Khomeini Hospital Complex, Tehran, in 5 July 2010 (~day +80). Eumycetoma madura foot had been diagnosed base on histopathological examination in 13 July 2010 (~day +88), while, laboratory medical mycology examinations and antifungal susceptibility test were not carried out. The patient treatment was started on itraconazole(400 mg/day) and she leaved the hospital with her concent(~day +117). During the managing with partially recovery, because of less accessibility to itraconazole, treatment with oral voriconazole(600 mg/day) was added in continue. The patient resumed her life with defect consuming of abovmentioned antifungal drugs with slightly symptom of inflammation and swollen in foot. Since that time, the swelling and pussy sinus in the patient's foot progressively increased. For second time, she was hospitalized again in Imam Khomeini Hospital Complex, Tehran in 10 September 2017(~month +89). The patient was taken up for an excision biopsy (Fig. 1B), and samples were sent to histopathological and mycological examination in Medical Mycology Laboratory of Tehran University of Medical Sciences. The samples apparent were surveyed and black granules were detected.The microscopic examination of 15% KOH mount revealed irregular wide septate mycelium with

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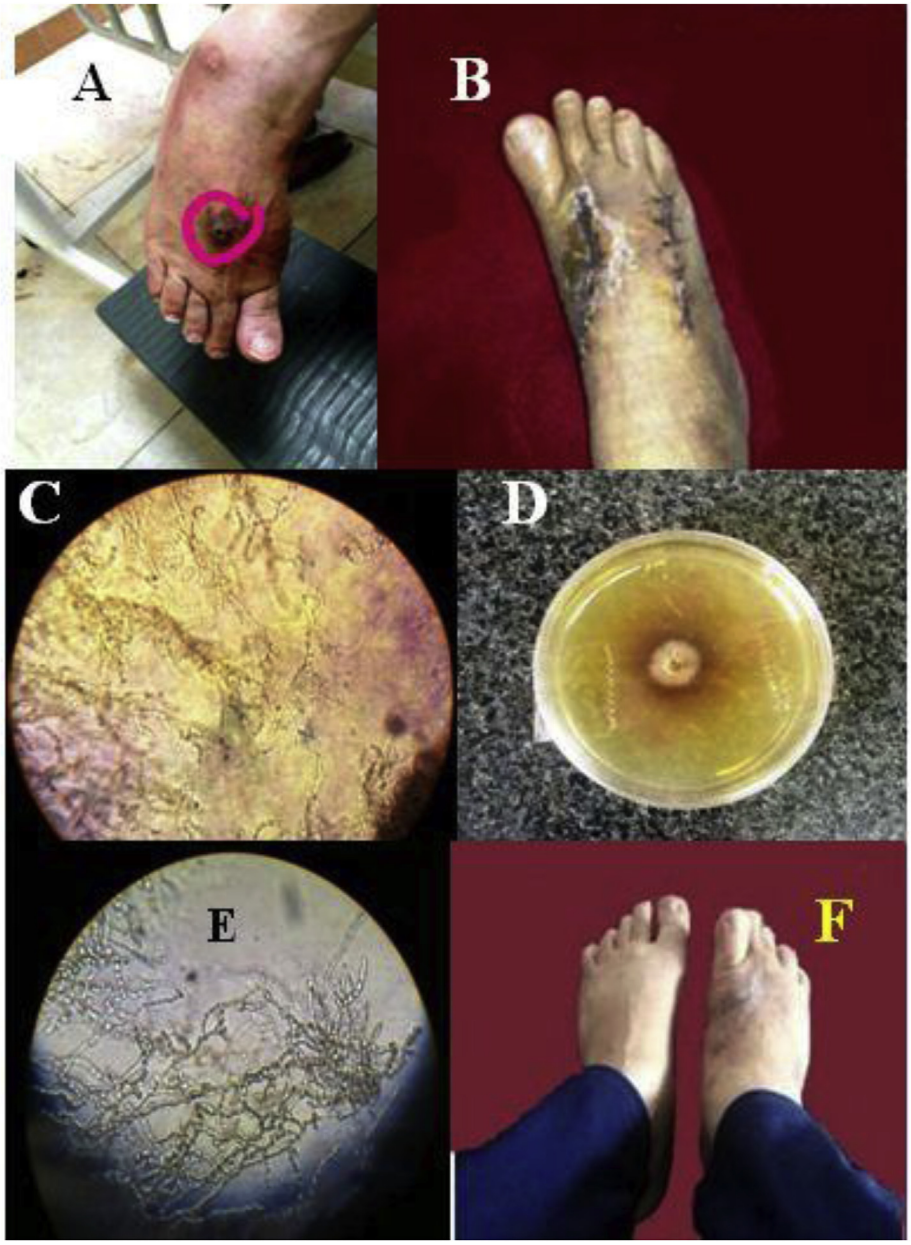

Fig. 1. (A) Clinical appearance of foot before treatment. Swollen foot with pusy sinus. (B) Biopsy and draining for sampling. (C) Direct microscopic examination of crushed granuls with KOH. Wide mycelium with chlamydoconidia. (D) Colonies on BHIA after a 3-week culture. Yellow –to- Brownish color. (E) Microscopic examination of the 3-week-old slide culture. Numerous chlamydospores and wide septated mycelium. (F) Recovered foot after prolong treatment with itraconazole.

Table 1
Comparison of clinical characteristics and antifungal susceptibility tests of previously case reported of eumycetoma caused by *M. pseudomycetomatis* with current our report.

Species	Sex	Age	Infectious sites	antifungal susceptibility (μg/ml)	Antifungal therapy	Final outcome	Reference
M. pseudomycetomatis	Female	47	foot	ITC: MIC-0 = 0.0313 AMB: MIC-0 = 0.0313 POS: MIC-0 = 0.004 VOR: MIC-0 = 0.125 RAVC: MIC-0 = 0.0125 Resistant: CAS	itraconazole	Recovery	Current study
M. pseudomycetomatis	Male	27	lower jaw	ITC: MIC-0 = 0.0625 VOR: MIC-0 = 0.0313 AMB: MIC-0 = 0.0313 TRB: MIC-0 = 2.0 Resistant: FLU, FLC	amphotericin B itraconazole	Recovery	[6]

Abrevation: ITC: itraconazole; AMB: amphotericin B; POS: posaconazole; VOR: voriconazole; RAVC: ravuconazole; CAS: caspofungin; TRB: terbinafine; FLU: fluconazole; FLC: 5- flucytosine.

chlamydoconidia(Fig. 1C). The H&E-stained sections of the biopsy showed an admixture of chronic inflammatory cells and grain tissue similar to eumycetoma (Madura foot). The granules were inoculated onto Sabouraud's dextrose agar with chloramphenicol (SDA; Difco) and brain heart infusion agar (BHI; Merck, Germany) media and incubated at 28 °C and 37 °C, respectively. After three weeks, the orange-to-brownish colonies were observed on culture media(Fig. 1D). Microscopically, lactophenol cotton blue wet mount of the colony showed pale brown, branched wide mycelium with aboundant chlamydiospore (Fig. 1E). Their identity was confirmed by internal transcribed spacer 1 (ITS1), 18S and ITS2 regions sequencing. Briefly, DNA was extracted from fresh cultures with DNA Extraction Kit DNP (SinaClon, Iran) according to manufacturer's protocol and stored at −20 °C. Amplifying of rDNA was done using ITS1 and ITS4 primers. Sequence data obtained compared with Gen Bank database (http://blast.ncbi.nlm.nih.gov) and was identified as *Madurella pseudomycetomatis* having 98% sequence identity with the ex-type isolate of that species(strain CBS 248.48, ID: JX280868.1; strain TMMU 3956, ID: EU815933.1 and strain CBS 124574, ID: MK876784.1). Additionally, *in vitro* antifungal susceptibility testing, involving determination of minimum inhibitory concentrations (MIC) and minimum effective concentrations (MEC, for caspofungin only), of six antifungal agents was performed according to recommendations stated in the Clinical and Laboratory Standards Institute(CLSI) M38-A2 document [13]. Table 1 summarized the MIC results. MIC values for itraconazole, amphotericin B, and posaconazole, voriconazole, and ravuconazole were 0.0313 μg/ml, 0.0313 μg/ml, 0.004 μg/ml,0.125 μg/ml, and 0.0156 μg/ml, respectively. While it was resistant to caspofungin (growth in 64 μg/ml, as a first well). According to antifungal susceptibility results, availability and reduction of antifungal side effects, the patient – after second hospitalization-was treated with itraconazole (400 mg/day) unceasing course to 18 months in continue. During antifungal treatment, the renal and liver function had been evaluated. Also, appearance and reducing of foot infelammation and its tumefaction had been surveying during treatment. Finally, the patient was cured and her foot was recovered (Fig. 1F) after prolonged course of itraconazole treatment(ceasing and nuceasing course ~ years +9).

3. Discussion

The presented case is first recurrent eumycetoma caused by *Madurella pseudomycetomatis* in Iran. In order to make decision for appropriate plan of treatment, identification of causative agent is necessary because of classification of mycetoma into two groups of eumycetoma and actinomycetoma [1,3,10,14]. To date, its true incidence, prevalence, and route of infection are not well understood, due to the fact that mycetoma is not a reportable disease [15]. Due to the lack of proper prevalence data, currently the true burden of this disease is not known. The clinical symptoms of two types are not significantly different from together, eumycetoma has a slow course in comparison to actinomycetoma [5,7,10,14]. In most reports, history of trauma has been existed in the past [3,5,9,14]. The current patient had history of barefoot walking on the farm and this reason can be as a main risk factor of patient involvement. Primary laboratory diagnosis of mycetoma is based on direct microscopic examination, histopathology, and culture [1,3–5]. There are several causative fungal agents induce eumycetoma, and identification of these fungi by standard mycological procedures is a challenge for the mycological laboratory. Species identification level is important because of different susceptibilities to antifungal agents by different species. However, molecular approaches have proven the sterile agents of human mycetoma comprise numerous, phylogenetically distinct species. De Hoog et al. introduce a novel *Madurella* species differing from *M. mycetomatis*, *M. pseudomycetomatis* and *M. grisea* based on their ITS, beta-tubulin (BT2) and RNA polymerase II subunit (RPB2) sequences and some phenotypic characters [16]. In presented case, rDNA partial sequencing of ITS1, 5.8S and ITS2 regins in alignment had 98% identity with registered gene sequences of *M. pseudomycetomatis* strain(CBS 248.48, ID: JX280868.1), *M. pseudomycetomatis* strain(TMMU 3956, ID: EU815933.1) and *M. pseudomycetomatis*(CBS 124574, ID: MK876784.1). A few reports are about *M. pseudomycetomatisin vitro* susceptibility that indicate the antifungal activities of amphotericin B, itraconazole and voriconazole were more than fluconazole, flucytosine and terbinafine [5]. In our case, amphotericin B and itraconazol MICs were in agreement with earlier studies [5]. However, voriconazole was less effective on *M. pseudomycetomatis*, that different from their findings. In addition, the susceptibility of new antifungal drugs such as ravuconazole and posaconazole on isolated strain was assessed that was more susceptible to them. In conclusion, we reported a case of madura foot with the specific epithets of its etologic agent *M. pseudomycetomatis* which refers to the close relationship of the species to *Madurella mycetomatis*. Moreover, antifungal treatment leads to a considerable response to itraconazole, posaconazole and amphotericin B. The molecular sequencing method is capable to differentiate between *M. mycetomatis* and novel species *M. pseudomycetomatis*. Since correct species identification is important for the optimal choice of antifungal therapy.

Conflict of interest

The authors have no conflicts of interest to declare for this study.

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