A case of onychomycosis caused by *Aspergillus terreus* in India

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Abstract: A case of onychomycosiss caused by *Aspergillus terreus* (a non-dermatophyte) has been reported for the first time from India. The person was 78 years old housewife having a history of paronychia and she was suffering from diabetes. Advanced age, diabetes and paronychia were the predisposing factors of her onychomycosis.
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Introduction.

Onychomycosis is caused by dermatophytes (90%) and also by nondermatophytes (10%)[1]. Of the nondermato molds (NDMs) *Aspergillus* species are responsible for up to 3% of all cases of onychomycosis[3] In India onychomycosis caused by *Aspergillus* has been accounted to be 22-35.33%[4] and it has been reported from various parts of this country, caused by *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*. *Aspergillus* terreus has been found to cause onychomycosis in Egypt[5], Iran[6], Italy[7], Sri Lanka[8] and U.K.[9]. The present paper deals with a case of onychomycosis caused by *Aspergillus terreus* in India. On the 5th september, 2024 a 78-years old housewife having a history of paronychia with suspected onychomycosis in fingernail of middle finger of right hand was referred by a physician (dermatologist) to the Department of Microbiology of Burdwan University, West Bengal, India for duly detection of the pathogen involved. The patient told that she was suffering from diabetes for the last 8 years since 2017.

Methods.

The affected nail was cleaned with 70% alcohol. Then sample from nail plate was scraped by a surgical blade no.5. Nail sample was also taken from nail edge by cutting with a sterilized nail cutter. Nail samples were collected three more times in the similar way at an interval of ten days. Nail samples were collected in self-sealing sterilized bags for direct microscopic examination as well as for isolation of the pathogen from them in culture media. For direct microscopic studies nail samples were stained with cotton blue and mounted in lactophenol. Nail samples were also embedded in a drop of 20% potassium hydroxide (KOH) and incubated at 37°C for 10-15 min. for direct microscopic examination. Nail samples were collected repeatedly (4 times) and inoculated on Malt extract agar (MEA) medium, Potato dextrose agar (PDA) medium and Sabouraud's dextrose agar (SDA) medium to see whether the pathogen observed in direct microscopy appear in culture media as well. The cultures were incubated at 25°C aerobically for up to 3 weeks and examined regularly for growth characters.

Results

Physical examination of the affected nail showed that the nail plate was slightly thicker than her all other normal nails and the white colour of the nail plate changed to reddish-brown and free edge of the nail was yellowish brown (Figure 1). Direct microscopy showed some simple-septate, up to 5µm wide, hyaline, thin-walled hyphae (Figure 2 and 3) as well as some slightly thick-walled hyphae (Figure 4) within the nail fragments. Abundant conidiophores were also observed with globose conidial heads (vesicles) bearing metulae and phialides in every nail segment giving the diagnosis of *Aspergillus*. The conidiophores (Figure 5) were very long, up to 250 µm, smooth-walled, slightly thick-walled and up to 5µm wide, some conidiophores arose from foot cell of hyphae (Figure 6). Conidiophore heads were up to 15 µm in diam, compact, columnar and biseriate (Figure 7); conidia produced in basipetal chain, hyaline, globose to slightly angular (Figure 8), slightly thick-walled, smooth, 2.0-3.7 µm in diam.

Unique feature of this pathogen was the production of aleuroconidia (Figure 9) directly on the hyphae which were larger than the phialoconidia, 6-7 µm in diam.Colony appeared in culture was cinnamon-brown in colour (Figure 10) and was floccose. Septate hyphae (Figure 11) and aspergillary heads (Figure 12) were produced in culture isolated from every nail sample. Some arthrospores (Figure 13) were also produced but these were very rare in their occurrence.

Discussion

Bongomin *et al*[2]. stated that a positive direct microscopy and repeated culture of *Aspergillus* spp. provided no dermatophyte isolated is sufficient to diagnose *Aspergillus* onychomycosis. English[10] suggested six criteria to diagnose NDM causing any onychomycosis, which are: (i) identification of the NDM in the nail by microscopy, ii) isolation in culture, (iii) repeated isolation in culture, (iv) failure to isolate any dermatophyte in culture, (v) histology, and(vi)inoculum counting, but later Gupta *et al*[11] recommended only 3 of these six criteria to rule out the microbe to be a contaminant. Some workers [12, 13] suggested that NDM found in the onychomycosis should be cultured and the patient should be reexamined and three separate nail samples should be taken from the affected nail and if NDM is confirmed in all the three cultures, the diagnosis of NDM is established. From all these studies it is concluded that if mold is initially detected in a nail sample from a suspected patient three additional nail samples have to be cultured and if all the three samples show identical mold growth then the mold is confirmed as the true onychomycosis pathogen.[23,25]. Accordingly, in the present study nail samples were collected repeatedly and inoculated on different culture media (MEA, PDA and SDA) and the same *Aspergillus* species with identical mold growth were observed every time.

Hence, the present study satisfied all of the required criteria mentioned by different workers[2, 11, 13] to identify the causal organism of the NDM onychomycosis and the pathogen was found to be a species of *Aspergillus*. Identification of the pathogen was more accurately confirmed as *Aspergillus* terreus due to: (i) cinnamon-brown colour of its colony; (ii) presence of hyaline, slightly thick-walled, smooth, long conidiophore (250x5 µm); (iii) vesicle globose, up to 15 µm in diam., (iv) biseriate conidial heads;(v) conidia small, smooth walled, hyaline, up to 3.75 µm in diam.; (vi) presence of unique aleuroconidia of 67 µm diam.; and (vii) production of arthrospores in culture. Therefore, it is the first report of a case of onychomycosis in India caused by *Aspergillus* terreus. In the present case of onychomycosis diabetes, advanced age (Noguchi et al.2016) and paronychia of the patient were the predisposing factors of the disease.

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EXPLANATION OF FIGURES

Figures 1-9. Infected nail and its pathogen: 1. Infected nail; 2. & 3. Thin-walled hyphae from infected nail; 4. Thick- walled hypha from infected nail; 5. Conidiophore arising from hypha; 6. Conidiophore arising from foot cell of hypha; 7. Coidial head; 8. Conidia; 9. Aleuroconidia arising from hypha.

Figures 10-13. Cultural characters:10. Culture of pathogen isolated from nail; 11. Thin-walled hypha from culture; 12. Conidiophore produced in culture; 13. Arthrospores produced in culture.