Case report

Malassezia furfur in a case of onychomycosis: colonizer or etiologic agent?

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The etiologic role of Malassezia furfur in onychomycosis is a contentious diagnostic problem because its keratinolytic ability has never been verified. This case report describes the isolation of M. furfur from the infected nails of a child clinically diagnosed with onychomycosis, and discusses the role of this organism as an etiologic agent/colonizer. The patient presented with subungual hyperkeratosis and onycholysis without associated paronychia. Budding yeast cells compatible with M. furfur were repeatedly demonstrated in KOH wet mounts of damaged nails, histopathology of hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) stained sections showed penetration of fungal elements between deeper layers of keratin, and numerous colonies of M. furfur were isolated on three consecutive occasions from nail specimens collected from different areas of hand and toenail lesions. No evidence of nail invasion by dermatophytic or nondermatophytic filamentous fungi were found by direct microscopy or culture. Microscopy and culture were negative following 12 weeks of ketoconazole treatment, which resulted in growth of healthy nail plates with normal beds. We can infer from these observations that M. furfur was an etiologic agent rather than a colonizer in the patient’s nails even though direct keratinolytic character of this fungus was not demonstrated.

Keywords etiologic agent/colonizer, ketoconazole therapy, Malassezia furfur, onychomycosis

Introduction

Malassezia furfur is a basidiomycetous lipophilic yeast that is frequently found as a constituent of the normal cutaneous mycobiota of humans. It is well known as an etiological agent of pityriasis versicolor (PV), a superficial mycotic infection of the skin particularly prevalent in the tropics and subtropics [1–3]. It has also been reported in association with several other diseases such as seborrhoeic dermatitis, folliculitis, atopic dermatitis, confluent and reticulate papillomatosis and psoriasis [4–7]. The etiologic role of M. furfur in onychomycosis is a contentious diagnostic problem because Malassezia yeasts have not been shown to degrade keratin, an ability that is generally possessed by fungal organisms able to invade skin. This communication deals with the isolation and etiologic significance of M. furfur in a child clinically diagnosed with onychomycosis.

Case report

A 13-year-old boy, resident in Delhi, India, with widespread infection for the past year involving all
the nails of his hands and feet clinically diagnosed as onychomycosis, was referred to the Department of Medical Mycology, Vallabhbhai Patel Chest Institute, in January 2002 for investigation of a possible mycotic etiology. Oral fluconazole 150 mg once per week had been administered for 12 weeks without tangible improvement. On initial examination, all of the nails of his hands and feet showed distal and lateral onycholysis with subungual hyperkeratosis and brownish discoloration (Fig. 1) but no associated skin lesions. Clinically, the nails did not show any pathognomic features of nail psoriasis. No predisposing local factors were apparent in the patient but he was not investigated for any immunodeficiency.

Multiple samples of nail clippings and scrapings, including some debris from a nail bed, were collected after cleaning the site with 70% ethanol. A part of this material was examined microscopically after digestion in 20% potassium hydroxide (KOH); the rest was cultured on routine mycological media at 28°C [1]. After 10 days incubation, two colonies of Candida albicans and numerous tiny colonies resembling bacterial growth appeared in culture on Sabouraud glucose agar supplemented with 0.05% chloramphenicol, as well as on Sabouraud glucose agar containing 0.05% cycloheximide. Microscopic examination of the suspected bacterial growth showed oval to spherical budding cells compatible with Malassezia species. The isolate was subcultured to glucose–peptone–yeast extract agar supplemented with olive oil and to Leeming and Notman Agar (LNA) [8] and incubated at 32°C. Observations on colonial and microscopic morphology of the isolate were made after 3, 5, 7 and 14 days incubation.

The yeast isolate was identified as Malassezia furfur. After 3 days incubation at 32°C, a streak culture on glucose–peptone–yeast–olive oil agar revealed cream-colored, yeast-like growth with irregular margins [9]. LNA medium incubated at 32°C after 7 days incubation showed cream-colored, convex colonies, 2–4 mm in diameter, with a dull surface and an entire margin. The isolate also grew at 40°C.

Microscopically, mostly oval cells were seen, 2–5 × 1.5–2 μm, occasionally subglobose cells, 2–4 μm in diameter, and elongated cells, 4–6 μm, with broad-based budding. The isolate was catalase positive, utilized Tween 20, 40, 60 and 80 in the agar diffusion test [10], and showed growth in the cremophor EL assimilation test [11]. The isolate was sent to the Centers for Disease Control and Prevention, Atlanta, GA, USA, for DNA sequencing to confirm the identification.

DNA was prepared using the DNeasy Tissue kit (Qiagen, Valencia, CA, USA). DNA was amplified using the ITS-1 and ITS-4 primers, which amplify the ITS region of the ribosomal subunit, and the NL-1 and NL-4 primers, which amplify the ~600-bp D1D2 region of the large ribosomal subunit [12]. Amplified DNA was sequenced in both strands on an ABI 310 instrument using the BigDye Terminator Kit (Applied Biosystems, Foster City, CA, USA). Sequences were aligned using the Sequencher program version 4 (Gene Codes Corporation, Ann Arbor, MI, USA). A BLAST search using the 806-bp ITS sequence showed 99% homology in GenBank to the ITS region of M. furfur AF246896 (bit score 1493; three mismatches and no gaps). A BLAST search using the 598-bp D1D2 large subunit DNA sequence showed 99% homology to M. furfur AY072790 (bit score 1094; two mismatches and three gaps). Neither sequence matched to either of the M. dermatis sequences in GenBank. The ITS region sequence of the isolate has been deposited in GenBank as AY 623429.

The patient returned for two more visits at 1 week intervals for further laboratory investigations. Clipplings and debris from the nail beds were collected for repeat microscopy and culture from different areas of lesions on the hand and toenails. At the second visit, one entire finger nail plate that had detached was prepared for histopathologic examination. Sections through this sample stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) revealed oval to spherical or elongated yeast cells with unipolar,
broad-based budding between deeper layers of keratin (Fig. 2). No hyphal filaments were seen, and there was no tissue reaction. Budding yeasts consistent with Malassezia were seen in KOH preparations from samples at both visits. Malassezia furfur was isolated from both subsequent samples, while Candida albicans was not recovered from either.

Following demonstration of the fungus in KOH-digested nail specimens and histopathologic sections, and isolation of M. furfur in culture on three occasions, oral ketoconazole at 200 mg/day was administered. After 12 weeks of treatment, complete clinical cure was achieved with normal nail bed and nail plate formation. Direct microscopy and culture of nail specimens at this stage yielded negative results.

Discussion

To our knowledge, none of the studies implicating M. furfur as an etiologic agent of onychomycosis [13–16] were based upon unequivocal evidence of repeated demonstration of the fungus in clinical specimens by direct microscopy, histopathologic examination, and repeated isolation of the fungus in culture with exclusion of other possible etiologic agents. In the absence of any evidence demonstrating keratinolytic character, M. furfur has, in fact, not been listed as one of the etiologic agents of onychomycosis in standard reference books of medical mycology, although it may exist as a colonizer of subungal debris of patients with nail infections [1–6]. We have carefully considered the following observations in order to evaluate whether M. furfur is a colonizer or an etiologic agent in the present case. (i) Presence of onycholysis, erosion, thickenings of nail plate and/or nail bed without evidence of another cause, and lack of clinical signs of nail psoriasis that can mimic onychomycosis. (ii) Repeated demonstration of budding yeast cells compatible with M. furfur in KOH wet mounts of damaged nails and histopathologic demonstration of the fungus showing penetration between deeper layers of keratin in PAS and H&E stained sections. (iii) Serial isolations on three consecutive occasions of numerous colonies of M. furfur from multiple specimens collected from different sites on the hands and toenails. (iv) No evidence of any dermatophytic or non-dermatophytic mold invasion in nails by direct microscopy and culture. (v) Negative mycological culture and direct microscopy following 12 weeks of ketoconazole treatment, resulting in growth of healthy nail plates with normal beds.

Notwithstanding the fact that we have not investigated the keratinolytic character of this isolate, it seems reasonable to infer from our observations that M. furfur was an etiologic agent rather than a colonizer in the present case of onychomycosis. As already stated, no predisposing factors were apparent in the case. It is not inconceivable that initially the etiologic agent in our case might have been a dermatophyte or another fungus that was eliminated following the 12 weeks of fluconazole therapy already administered before M. furfur was isolated. The Candida albicans recovered from the initial culture, but not the two subsequent cultures, could have been such an agent. If this assumption were correct, the nail damage caused by the putative fungus might have facilitated the subsequent invasion by M. furfur.

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References

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