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Original Article

Non-dermatophyte moulds and yeasts as causative agents in onychomycosis

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Abstract

Background Although dermatophytes are the most common pathogens of onychomycosis, yeasts and non-dermatophyte moulds can also be found as causative agents.

Objective To find out relative frequency of non-dermatophyte moulds and yeasts as causative agents in onychomycosis.

Subjects and methods Forty patients of all age groups and either sex suffering from onychomycosis were subjected to fungal cultures. Demographic and clinical features of the patients were recorded. Nail scrapings were inoculated on fungal culture media and growth pattern studied.

Results Out of 40 patients, eight (16%) were positive for fungal culture. Amongst culture positive cases 4 (50%) were dermatophytes, all of them belonging to genus trichophyton, whereas 2 (25%) were positive for *Candida* spp. and 2 (25%) were positive for non-dermatophyte moulds belonging to *Scopulariopsis* spp. and *Aspergillus* spp. each.

Conclusion Dermatophytes remain the most common cause, but the role of yeasts and nondermatophye moulds should receive due consideration in a case of onychomycosis.

Key words
Onychomycosis, dermatophytes, yeasts, non-dermatophyte moulds

Introduction

Onychomycosis, a fungal infection of the nail plate occurs worldwide and accounts for upto 50% of all nail infections.[1-3] Its incidence has steadily increased in parallel with an expanding number of elderly persons and immunocompromised patients.[4] Although not life threatening, this may have significant clinical consequences such as secondary bacterial infection, chronicity, therapeutic difficulties and disfigurement in addition to serving as a reservoir of infection.[5] The symptomatic disease can be a source of embarrassment and potential cause of morbidity.[6] Common clinical features include discoloration of the nail plate, hyperkeratosis and brittle nails.[7] Onychomycosis may be classified into several types clinically: distal subungual onychomycosis, white superficial onychomycosis, proximal subungual onychomycosis, endonyx and total dystrophic onychomycosis.[8] Dermatophytes are the predominant pathogens[9] accounting for most of the cases. However, yeasts (especially *Candida albicans*)[10] and non-dermatophyte moulds may

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also be implicated especially in previously traumatized nails.[11,12] Tinea unguium i.e. onychomycosis due to dermatophytes is clinically indistinguishable from onychomycosis caused by non-dermatophytes. Moreover, certain other skin conditions, such as psoriasis, lichen planus, onychogryphosis and nail trauma can mimic onychomycosis. Hence, laboratory investigations are needed to differentiate between fungal infection and the above mentioned skin diseases. An accurate diagnosis, with relevant laboratory investigations is essential before starting treatment of onychomycosis[13] for best results. Fungal cultures are essential for accurate identification of the causative microorganism. This is of paramount importance because the clinical outcome of antifungal agents varies as to whether the aetiologic pathogen is a dermatophyte, yeast or a mould.[14] The antifungal agents with appropriate spectrum of activity can be used if the underlying fungal pathogen is identified correctly.[15]

Aim of this study was to find out the relative frequency with which non dermatophytic moulds and yeasts can be ascertained as a causative agent in cases of onychomycosis.

Material and methods:

Collection and transport of specimens Forty patients with clinical impression of onychomycosis were selected from outpatient clinic of Dermatology Department, Military Hospital, Rawalpindi, over a period of one year. These patients were selected with non-probability convenience sampling from all age groups, of both sexes with the nails affected either being finger or toenails. Patients with onychomycosis who were simultaneously suffering from other skin and/or systemic diseases like psoriasis, lichen planus, diabetes mellitus and peripheral vascular disease were excluded from the study. All the patients were interviewed and a thorough dermatological and systemic examination carried out. Demographic and clinical features of the patients were recorded on a specially designed proforma for each patient separately. Subungual keratinous debris were collected from all the patients by using steam autoclaved instruments after thorough scrubbing with 70% ethyl alcohol.[16] Then finely grained nail scrapings were placed on a clean glass slide for direct microscopy with a drop of 20% KOH. This was incubated at room temperature for 1 to 2 hours in a moist chamber. The presence of septae in the fungal hyphae and dichotomy with or without arthroconidia were kept in mind while examining the smear. Budding cells with pseudohyphae were also looked for which is typical for candida species.

A part of the affected nail scrapings was inoculated on to 3-4 different points of the fungal culture media with a sterilized platinum loop at 25-30º C. The most widely used fungal culture media Sabouraud's dexrose agar (SDA) + chloramphenicol and SDA + chloramphenicol + cyclohexamide were used for this purpose. Cultures were read initially at 24-48 hours (for non-dermatophytes) and later at 10-14 days (for dermatophytes). Repeat cultures were performed in cases where culture was negative for dermatophytes but positive for non-dermatophyte moulds or yeasts to rule out the possibility of contamination. Gross features of fungal colonies including colour, texture, topography of the surface of the culture, colour of reverse of the colony and the presence of a diffuse pigment were identified. A portion of fungal colony was then suspended in a solution of lactophenol cotton blue on a glass slide and viewed under microscope. Microscopic features

of the fungal colonies i.e. the presence of macroconidia and microconidia, their shape and appearance were then identified. The cultures were discarded after 21 days of inoculation.

The study was approved by the ethics and scientific committee of the hospital.

Computer programme SPSS-10 was used to manage and analyze the data. Frequencies and percentages were obtained for the variables where applicable. Mean and standard deviations were calculated for continuous variables.

Results

Age of the patients ranged from 09-67 years with a mean of 31.44±11.83. Out of these 26 (65%) were males and 14 (35%) were females. Of these, 30 (75%) patients had distal and/or lateral onchomycosis and 10 (25%) had suffered from total dystrophic onchomycosis, whereas none of them had proximal or superficial white onychomycosis.

Of 40 patients, 6 (15%) had either acute or chronic paronychia, majority of them being female patients who had finger nail onychomycosis. Nine (22.5%) patients had clinical evidence of concomitant superficial fungal infection elsewhere i.e. tinea corporis, tinea cruris, tinea manuum and tinea pedis.

Positive fungal cultures were present in 8 (20%) specimens, 4 (50%) of these being dermatophytes all of them belonging to the genus *Trichophyton*, whereas 2 (25%) were positive for *Candida* spp and one each (12.5%) of *Scopulariopsis* and *Apergillus* spp. (non-dermatophyte moulds).

Discussion

Dermatophytes, yeasts and moulds are all potential pathogens in a case of onychomycosis.[17] Dermatophytes like *Trichophyton rubrum* and *T. mentagrophytes* are the most common causative organisms of onychomycosis but less commonly yeasts like *C. albicans* and non-dermatophytic moulds like *Fusarium* spp., *Scopulariopsis brevicaulis* and *Aspergillus* spp. can also cause onychomycosis.[18] However, some studies, implicate *Candida* species to be the most common microorganism especially in cases of fingernail onychomycosis in females.[19] Nail invasion by non-dermatophyte moulds is considered uncommon with a prevalence of 1.45% to 17.6%.[20]

Fungal culture of the subungual keratinous material provides final confirmation of the active cases of onychomycosis and enables to accurately pinpoint the causative organism.

In our study, fungal cultures were positive in 20% patients. These findings are in agreement with most other studies which showed dermatophyte as the predominant causative agent of onychomycosis as compared to yeasts and non-dermatophyte moulds.[21] Veer *et al*.[22] in a study found drematophytes to be the most common (29.5%) pathogen followed by non-dermatophyte moulds (13.6%) and *Candida* spp. (5.6%), whereas (51.1%) nail samples yielded no growth. In yet another study conducted in Tehran, Khosravi *et al* [23] also found dermatophytyes as the most common pathogen (48.4%) followed by *Candida* spp. (43.3%) and non-dermatophyte moulds (8.2%). However, Bokhari *et al*.[19] found that *Candida* was the most common (46%) cause of finger nail onychomycosis in young ladies followed by dermatophytes (43%) and non-dermatophyte

moulds (11%). This might be due to heavy wet workload to which those ladies were exposed which helped this yeast to flourish. Alvarez[24] also found candida to be the most common agent (40.7%) followed by dermatophytes (38%) and non-dermatophyte moulds (14%) with mixed etiology in 7.3% for onychomycosis.

Our findings show that the clinical impression of onychomycosis was proven in only 8 (20%) patients by positive fungal culture and we were unable to confirm the causative fungi in rest of the 80% cases. This may be because of lack of growth on fungal culture media due to old or non-viable fungal elements submitted for fungal culture. In addition, fault in the sample collection and fungal culture inoculation techniques might have contributed to this low percentage of positive yield on fungal culture. Other limitations of our study include limited number of patients with specific selection of age group and gender.

It would be ideal to confirm the diagnosis before starting treatment for onychomycosis. However, at places where the facility to grow fungi on culture media is not available, treatment is started on the basis of clinical experience. Under such situations, if clinical improvement does not occur, it is almost impossible to decide whether this represents treatment failure or an incorrect diagnosis to start with.[25] In our study half (50%) of the patients were positive for yeasts and non-dermatophyte moulds. Hence, yeasts and non-dermatophyte moulds should always be kept in mind while investigating and treating a case of onychomycosis and the common practice of discarding them as contaminant should be avoided.

Conclusion

Dermatophytes remain the predominant cause of onychomycosis but the role of yeasts and non-dermatophyte moulds as a causative microorganism should get due consideration.

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Authors Declaration

Authors are requested to send a letter of undertaking signed by all authors along with the submitted manuscript that:

The material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Journal of Pakistan Association of Dermatologists.*