

Non-dermatophytes emerging as predominant cause of onychomycosis in a tertiary care centre in rural part of Punjab, India

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ABSTRACT

Background: Onychomycosis is most commonly caused by dermatophytes belonging to the genus *Trichophyton*. However, *Candida* spp. and non-dermatophyte moulds may also be responsible. Up to 10% of cases of onychomycosis are caused by non-dermatophyte moulds and these are becoming more common worldwide. **Materials and Methods:** A retrospective analysis for a period of one year was undertaken to know the prevalence of different fungi causing onychomycosis in a tertiary care hospital. Nail samples of fingers and toes collected from patients presenting with clinically suspected onychomycosis were processed by standard methods. **Results:** Of the 87 clinically suspected cases of onychomycosis, non-dermatophyte moulds were the most common pathogens isolated in 24 (53.33%) patients, followed by dermatophytes in 12 (26.66%) patients and *Candida* spp. in 9 (20%). Among the non-dermatophyte moulds, *Aspergillus flavus* was the most common isolate found in 15 (62.5%) cases. **Conclusion:** The predominant isolate obtained in this study was non-dermatophyte moulds. Among moulds, *Aspergillus* spp. was the most common isolate.

Key words: Non-dermatophyte moulds, onychomycosis, prevalence

INTRODUCTION

The term onychomycosis is derived from *onyx* and *mykes*, the Greek words for nail and fungus, respectively. It is a chronic fungal infection of the finger or toenails which leads to discoloration, thickening of nails and separation from the nail bed. Toenails are more commonly affected than fingernails as the former grow slowly, have less blood supply and are more often confined in dark and moist environments. Patients who have a history of nail trauma, hyperhidrosis, genetic predisposition, psoriasis, any concurrent fungal infection or distorted nails are more prone to onychomycosis. It is more commonly seen in people who use occlusive footwear, shared bathing facilities and in smokers.^[1,2]

Onychomycosis is found more commonly in older adults, its prevalence being 10% in general population, 20% in those older than 60 years and 50% in those older than 70 years.^[1] Elderly people are more prone to onychomycosis

due to its association with peripheral vascular disease, immunologic disorders and diabetes mellitus. Persons with diabetes have 1.9–2.8 times higher risk of onychomycosis as compared with the general population.^[3] Prevalence of onychomycosis in patients with human immunodeficiency virus (HIV) infection ranges from 15% to 40%.^[4]

According to the clinical presentation and mode of invasion of the nail, onychomycosis is divided into several classes which include distal and lateral subungual onychomycosis, proximal subungual onychomycosis (PSO), white superficial onychomycosis (WSO), total dystrophic onychomycosis (TDO) and endonyx subungual onychomycosis.^[5] Endonyx subungual onychomycosis

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How to cite this article: Sharma P, Sharma S. Non-dermatophytes emerging as predominant cause of onychomycosis in a tertiary care centre in rural part of Punjab, India. *J Acad Clin Microbiol* 2016;18:36-9.

Access this article online

Quick Response Code:



Website:
www.jacmjournal.org

DOI:
10.4103/0972-1282.184754

is rare and some nails have features from a combination of classes. For successful treatment, accurate diagnosis is essential which requires identification of physical changes and proper laboratory investigations.

Onychomycosis is most commonly caused by dermatophytes belonging to the genus *Trichophyton*; however, *Candida* and non-dermatophyte moulds may also be responsible. *Candida* onychomycosis (CO) is more common in fingernails and in patients with chronic mucocutaneous candidiasis.^[1] Up to 10% of cases of onychomycosis are caused by non-dermatophyte moulds and these are becoming more common worldwide.^[6] According to the recent studies, they have become the main causative organism in patient with onychomycosis and HIV infection.^[4]

MATERIALS AND METHODS

The present study was conducted over a period of one year from October 2014 to October 2015 in Microbiology Department of a Sri Guru Ram Das Charitable Hospital, which is a tertiary care teaching hospital. A total of 87 consecutive finger and toenail specimens were collected from patients presenting with different kinds of nail infections.

Sample collection

All samples were collected after thorough cleansing of the nail area with 70% alcohol to remove contaminants. For patients with distal subungual onychomycosis, nail material was obtained from the advancing infected edge closest to the cuticle, where the likelihood of viable hyphae is the greatest. For PSO, the normal surface of the nail plate was pared down with a no. 15 surgical blade at the lunula and the white debris was collected with a sharp curette from the deeper portion of the plate and the proximal nail bed. For WSO, the white spots on the nail were scraped and the white debris directly underneath was then collected. For *Candida* infection, the material closest to the proximal and lateral nail edges was obtained. If *Candida* onycholysis was suspected, the lifted nail bed and if necessary, the under surface of the nail plate was scraped. For TDO, any abnormal area of the nail plate or bed was used as a specimen.

Sample processing

Microscopic examination of the samples was performed following digestion with 20% potassium hydroxide (KOH). All samples were inoculated on Sabouraud's dextrose agar (SDA, HiMedia Laboratories) and SDA with 5% Chloramphenicol and Cycloheximide. Cultures were incubated at 25°C and 37°C and examined daily for 1st week and twice a week for six weeks.

Different fungi were identified based on microscopic examination with lactophenol cotton blue and culture characteristics which were confirmed by slide culture in case of dermatophytes and other moulds and by germ tube, sugar assimilation test in case of *Candida* species. Only those samples which showed microscopic evidence of fungi and grew on culture were considered for further evaluation. The following criteria were taken into consideration for confirming non-dermatophyte moulds as pathogens, (i) large and irregular septate hyphae in KOH mount on microscopy, (ii) the growth of the same agent in pure culture in at least three tubes of SDA and (iii) no growth of dermatophytes (iv) isolation from another sample repeated after an interval of two weeks.^[7-9]

RESULTS

Of the 87 clinically suspected cases of onychomycosis, during this period, 73 (83.9%) were from toenails and 14 (16.09%) from fingernails. The study included 59 (67.8%) males and 28 (32.1%) females with a mean age of 43 years. The prevalence of onychomycosis was higher in 78 patients (59.8%) older than 40 years and lower in nine patients (10.3%) younger than 20 years. In the present study, 39 (44.8%) patients were farmers, 18 (20.6%) were homemakers, 9 (10.3%) were students and 21 (24.1%) had other occupations. These 21 patients were above 65 years of age, and out of these 21 old patients, 12 (13.7%) were diabetic and 9 (10.4%) had some chronic ailment.

The most prevalent clinical pattern found in 26 (57.78%) patients was distal lateral subungual onychomycosis (DLSO). After DLSO, the next most prevalent clinical pattern was PSO, which was found in 10 (22.22%) participants, and clinical pattern of CO was seen in 9 (20%) patients, showing fungal growth as shown in Table 1.

Table 1: Correlation between the clinical patterns and type of fungus isolated

Fungal species	DLSO	PSO	CO	Total
Dermatophytes				
<i>T. mentagrophytes</i>	2	6	-	8
<i>T. rubrum</i>	4	-	-	4
Non-dermatophytes				
<i>A. flavus</i>	13	2	-	15
<i>A. fumigatus</i>	6	-	-	6
<i>Penicillium spp</i>	1	-	-	1
<i>Fusarium spp</i>	-	1	-	1
<i>Alternaria alternata</i>	-	1	-	1
<i>Candida spp.</i>	-	-	9	9
	26	10	9	45

DLSO: Distal lateral subungual onychomycosis; PSO: Proximal subungual onychomycosis; CO: *Candida* onychomycosis

Out of 87 clinically suspected onychomycosis, 45 (51.7%) samples grew fungi, among which non-dermatophyte moulds were the most common pathogens isolated in 24 (53.33%) patients, followed by dermatophytes in 12 (26.66%) patients and *Candida* species in 9 (20%). Among the non-dermatophyte moulds, *Aspergillus flavus* was the most common isolate found in 15 (62.5%) cases, followed by *Aspergillus fumigatus* found in 6 (25%) cases. *Fusarium solani*, *Alternaria alternata* and *Penicillium* sp. were found in one case each. Among the 12 dermatophytes, eight were *Trichophyton mentagrophytes* and four were *Trichophyton rubrum*. In 9 (20%) cases involving *Candida* species, *Candida albicans* was the most common 7 (77.7%) followed by *Candida parapsilosis* 1 (11.1%) and *Candida tropicalis* 1 (11.1%).

DISCUSSION

The fungi known to cause onychomycosis differ among geographic areas, primarily due to differences in climate.^[10] Risk factors for onychomycosis include positive family history, increasing age, poor health, prior trauma, warm climate, participation in fitness activities, immunosuppression (e.g. HIV, drug induced), communal bathing and occlusive footwear.

Clinical features of onychomycosis may resemble a large number of other nail disorders. Therefore, laboratory diagnosis of onychomycosis must be confirmed before beginning a treatment regimen. However, a negative mycological result does not rule out onychomycosis because direct microscopy may be negative in up to 10% of cases and culture in up to 30% of cases.

The isolation rate of fungi from patients with onychomycosis was 51.7% in our study, against 64% in Rajasthan,^[11] 39.5% in Delhi^[12] and 47% in Pakistan^[13] and 86.9% in Turkey.^[14] Variation in prevalence of onychomycosis worldwide may be because many people do not seek medical advice for nail disfigurement^[15] and also due to difference in the climatic conditions of different regions.

In the present study, participants older than 40 years showed the highest prevalence, 52 (59.8%), and patients younger than 20 years showed lower prevalence 9 (9.6%), which is in accordance with other studies from India^[11] and abroad.^[16] In this study, suspected onychomycosis was more common in males (67.8%) than in females (32.1%). These results are consistent with the studies done elsewhere.^[12,14,17] A higher incidence of onychomycosis in males worldwide may be due to common use of occlusive footwear, more exposure to outdoor conditions and increased physical activity, leading to an increased likelihood of trauma. The

importance of trauma to the nails as a predisposing factor for onychomycosis is well established.^[18]

In the present study, 39 (44.8%) patients were farmers and 18 (20.6%) were homemakers, whereas farmers have more exposure to outdoor conditions and increased physical activity leading to an increased likelihood of trauma, homemakers are also more prone to this condition due to frequent contact with soap and water.

In the present study, non-dermatophyte moulds were isolated in 24 (53.33%) patients, with *A. flavus* being the most common isolate followed by *A. fumigatus*, *F. solani*, *A. alternata* and *Penicillium* spp. Of the nine cases (20%) involving *Candida* species, *C. albicans* was the most common followed by *C. parapsilosis* and *C. tropicalis* which is in accordance with many studies.^[12,19] In nine cases of CO, females (8) were affected more than males (1). All of the females were homemakers and *C. albicans* was predominantly isolated from their fingernails. Household work (e.g, cutting and peeling vegetables, washing utensils, cleaning the house/laundry) may chronically expose homemakers to moist environments and make them more prone to injury, thus facilitating easy entry of fungal agents. Among the 12 dermatophytes, eight were *Trichophyton mentagrophytes* which is consistent with other reports^[12,19] and four were *T. rubrum*. However, in a study from eastern India, *T. rubrum* has been reported as most common cause of onychomycosis due to dermatophytes.^[20]

DLSO was the most common clinical type (26/45), which is comparable to other studies.^[19] PSO was the second most common type (10/45) in our study although a greater incidence has been reported in some studies,^[18] whereas in other studies, a lower incidence has been reported.^[21]

For proper management of onychomycosis, diagnosis and accurate treatment play a key role. In the present study, patients suffering from onychomycosis due to dermatophytes and *Aspergillus* spp. responded well to treatment with Terbinafine and topical application of Ciclopirox. Patients who had infection either with *Candida* spp. or *Alternaria* spp. or *Penicillium* spp. responded better to Itraconazole; however, the patient who was suffering from infection due to *Fusarium* spp. did not respond to either Terbinafine or Itraconazole.

Several newer diagnostic methods have been introduced such as polymerase chain reaction-based methods and non-invasive methods such as optical coherence tomography, confocal laser scan microscopy, matrix-assisted laser desorption time of flight mass spectrometry and phase contrast hard X-ray microscopy^[22] which may help in early diagnosis and better management of infection. However,

as the spectrum of disease is based on various aetiologic agents, culture will remain the gold standard in identifying the species causing the infection.

CONCLUSION

Onychomycosis due to non-dermatophyte moulds is more common in our area. Clinical examination alone is not sufficient for diagnosing onychomycosis. As there are several fungi, which cause the infection, it is necessary to perform culture for appropriate treatment. Males are at more risk than females to develop onychomycosis, especially farmers and sportspersons. Homemakers are more prone to develop CO due to frequent contact with soap and water.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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