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Non dermatophytic molds causing onychomycosis: a rising trend in North India

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ABSTRACT

Background: Onychomycosis is rarely life threatening but can affect patient's quality of life by its associated morbidity and cosmetic disfigurement that makes it an important public health problem. So the current study was undertaken to look for causes of onychomycosis including the non-dermatophytic molds that are normally considered as contaminants.

Methods: A total of 100 nail samples from clinically suspected cases of onychomycosis, were processed by direct microscopy of the KOH mount followed by two sets of culture on Sabourauds' Dextrose Agar and incubated at 25°C and 37°C and were examined once a week for a period of 4-6 weeks, to look for the fungal causative agent.

Results: Infection was more common among males and amongst age group 21-30 years (31%). The finger nails (57%) were more commonly involved than toe nails (43%). Onychomycosis was mostly caused by molds (55%), followed by dermatophytes (15.8%) and yeasts (9.3%). *Aspergillus niger* and *A. flavus* (13% each) were the most common molds, *T. verrucossum* (4.6%) was the most common dermatophyte, while *Candida albicans* (6.5%) was the most common yeast isolated.

Conclusions: There is a rising trend of non dermatophytic molds causing onychomycoses, Thus microbiologists should look for all the possible causes of superficial fungal infection.

Keywords: Onychomycoses, Non dermatophytic molds, Trichophyton, Curvularia

INTRODUCTION

Onychomycosis refers to the invasion of the nail plate by a fungus. It is mainly caused by dermatophytes, but yeast and non-dermatophyte molds (NDM) have also been associated, leading to variable clinical presentations. A number of factors contribute to the growing incidence of fungal nail infections, e.g. an increased aging population and an increasing number of immunocompromised state of patients. Onychomycosis and other nail diseases can adversely affect the appearance of the entire nail unit, but most often it is regarded as a simple cosmetic problem of relatively minor importance that hardly seek any

treatment in most of the cases. However, many patients with fungal nail infections experience serious physical, psychological, social, and occupational ill-effects. Onychomycosis affects approximately 5% of the population worldwide and represents 20-40% of the onychopathies and about 30% of cutaneous mycotic infections. Various workers have reported the incidence to vary from 0.5 to 5% in the general population in India. Although onychomycosis is rarely life threatening, its high incidence and prevalence and the associated morbidity makes it an important public health problem, thus it becomes important to look for the etiological agent and its treatment options. Although dermatophytes are still the major cause of superficial

infections, however, infections caused by nondermatophytes also may be common in India. Ignoring the NDM as a causative agent of onychomycoses might be due to the prevailing concept that all nondermatophytes are laboratory contaminants, and in general due to lack of mycological expertise in medical schools. In this paper, we have looked for the mycological spectrum of onychomycosis in a tertiary care hospital in North India.

METHODS

The study population comprised of 100 clinically suspected cases of onychomycosis (Figure 1 (A and B)), attending Skin and VD, Outpatient department of a tertiary care hospital in New Delhi, India over a period of one year (April 2013 to March 2014). Nail scrapings/clippings were obtained according to standard procedures. All specimens were subjected to direct microscopy in 20% KOH to determine the presence of fungal elements (Figure 2). The specimens were cultured in Sabouraud's dextrose agar with and without cycloheximide and incubated at 25°C and 37°C for 4-6 weeks with intermittent examination of the tube once a week for any growth (Figure 3 (A and B)). Fungal growth were identified according to standard procedures (Figure 4 (A and B)). Candida species were confirmed by germ tube test and growth on cornmeal agar and Hi Chrome agar (Himedia, Mumbai, India). In the process of diagnosis, C. albicans was considered as the primary pathogen on isolation as a single pure growth combined with a direct microscopy result showing yeast cells and pseudomycelia. Non- C. albicans spp. were considered as the primary pathogen on repeated (two or more) isolation if they were isolated singly (no other concomitant pathogen) and direct microscopy showing yeast pseudomycelia. Candida spp. were considered as secondary pathogens if they were isolated with dermatophytes or nondermatophytic pathogenic molds with microscopic findings of budding yeast cells.^{6,7} Nondermatophyte molds were considered significant if there is a positive KOH finding and they were isolated repeatedly (more than twice) in pure culture. Whereas all other NDM cases were considered to be contaminants. When there were growth of mixture of 3 or more fungi seen with a negative KOH mount than it was considered as contaminant grown.

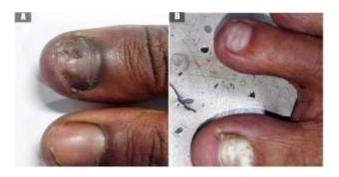


Figure 1: (A) Thumb nail, (B) Toe nail of a suspected case of onychomycoses.

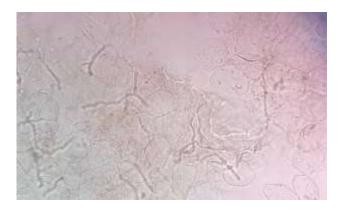


Figure 2: KOH mount of nail showing branching septate hyphae.



Figure 3: (A) Cottony greyish- white growth of *Rhizopus spp*, (B): White wrinkled colonies of *Scopulariopsis spp* on SDA slant.

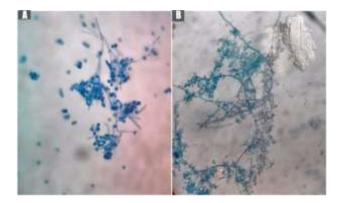


Figure 4: (A) LPCB mount of *Scopulariopsis spp* with round spiky conidia (B) LPCB mount of *T. mentagrophytes* under 40X.

Statistical analysis

The statistical analysis was carried out using SPSS software version 16.0. Data were presented as percentages and proportions. Chi-square test was applied when two or more set of variables were compared. The critical value of 'p' indicating the probability of significant difference was taken as <0.05.

RESULTS

In our study, male patients (75%) were more commonly affected than the female patients (25%) with a male female ratio of 3:1. The most commonly affected age group was 21-30 years (31%), followed by 51-60 years (20%). Patients with <10 years of age were least affected, while, infection in individuals within 51-60 years of age was high. (Table 1) On comparison of the affection of finger and toenails, it was found that finger nails were more affected (57%) than toe nails (43%). Only 3% of individuals had both the Finger and toe nails involvement. (Table 2). We found that, 83% of cases were positive by culture and/or microscopy, 79% were positive by culture, 63% cases were positive by microscopy and 59% were positive both by microscopy and culture, while, 16% were negative by both (Table 3).

Table 1: Patient demography.

Age Groups	Male (%)	Female (%)	Total (%)
0-10	1 (1.3)	1 (4)	2 (2)
11-20	7 (9.3)	1 (4)	8 (8)
21-30	21 (28)	10 (40)	31 (31)
31-40	11 (14.6)	7 (28)	18 (18)
41-50	12 (16)	3 (12)	15 (15)
51-60	17 (22.6)	3 (12)	20 (20)
>60	6 (8)	0 (0)	6 (6)
Total	75	25	100

Table 2: Clinical pattern of nails samples received

	Finger nails	Toe nails	Total n (%)
Male	42 (73.6)	33 (76.7)	75
Female	15 (23.4)	10 (23.3)	25
Total	57 (57)	43 (43)	100

Table 3: Microscopy and culture positivity of the clinical samples (n=100).

	No. of samples	Percentage (%)
Samples positive by KOH and/or Culture	83	83
Total KOH positive	63	63
Total Culture	79	79
Positive by both	59	59
KOH +, Cul -	4	4
KOH-, Cul+	20	20
Both Negative	16	16

In our study growth of non dermatophytic molds (NDM) predominated (55%), followed by dermatophytes (15.8%) and yeasts (9.3%) (Table 4). Amongst the NDM,

Aspergillus niger and A. flavus (13% each) were the most common isolates. Other NDM included Mucor spp. (7.4%), Alternaria alternata (4.6%), Rhizopus spp (4.6%), Penicillium spp. (3.7%), Fusarium spp. (2.8%), Bipolaris spp.(1.8%), A. fumigatus (1.8%) and Curvularia spp.(0.9%). Amongst the dermatophytes, T. verrucossum (4.6%) followed by T. rubrum (3.7%) were most commonly isolated followed by T. schoenleinii (2.8%), T. violaceum (1.8%), Microsporum spp (1.8%) and T. mentagrophytes (0.9%). Amongst the yeasts, Candida albicans (6.5%) was more common than NAC (2.8%). 18.6% of cases had no growth while, 0.9% had growth of contaminants seen.

Table 4: Fungal isolates obtained from the clinical samples.

	T. verrucossum T. rubrum	No. of isolates n (%) 5 (4.6) 4 (3.7)
Dermatophytes (15.8%)	T. schoenleinii T. violaceum Microsporum spp. T. mentagrophytes	3 (2.8) 2 (1.8) 2 (1.8) 1 (0.9)
Molds (55%)	A. niger A. flavus Mucor spp. Rhizopus spp. Alternaria spp. Penicillium spp. Fusarium spp. A. fumigatus Bipolaris spp. Curvularia spp.	14 (13) 14 (13) 8 (7.4) 5 (4.6) 5 (4.6) 4 (3.7) 3 (2.8) 2 (1.8) 2 (1.8) 1 (0.9)
Yeasts (9.3%)	Scopulariopsis spp. Candida albicans Non albicans candida	1 (0.9) 7 (6.5) 3 (2.8)
Negative (18.6%)		20 (18.6)
Contaminants (0.9%)		1 (0.9)
Total		107

DISCUSSION

In our study the infection was more common in males as compared to the females with a male: female ratio of 3:1, which was in accordance with studies by Veer et al, Lone et al and Sarma et al. 8-10 While few studies have shown higher prevalence of this infection in females. 11,12 The increased incidences in males, might be due to more of outdoor exposure, increased physical activity and occupational nail trauma in males as compared to females.

We found that a higher number of patients were in the age group of 21-30 years. High prevalence in this age group was also seen in many studies conducted from various parts of India. 9,10,13 The younger age group of prevalence might be due to more of occupation related trauma, and cosmetic consciousness amongst the young adults as compared to elderly individuals. However, in our study the next most common age group of infection was 51-60 years (20%) which showed an increasing incidence of this infection with an increasing age. A similar result was also seen in study conducted by Sarma et al. 10 This high incidence with an increasing age might be due to a decrease in immunity with age, due to repeated nail trauma, or decreased peripheral circulation and medical conditions like diabetes in elderly age groups.

Finger nails were involved in 57 (57%) cases as compared to toe nails (43%). While, both finger and toe nail involvement was seen in 3 (3%) cases. The toe nails have been reported to be more commonly involved in another study conducted by the authors in 2008 in our institute, reflecting a change in the trend of nail involvement.³ Similar findings with frequent involvement of finger nails were also seen in studies conducted by Lone et al in Kashmir, Sarma et al in new Delhi and Aghamirian et al in Iran.^{9,10,14} The lower incidence of toe nail onychomycosis could be because of lesser cosmetic awareness on their disfigurement, and less of occupation related trauma compared to finger nails.

Direct microscopy by KOH mounts is important for clinical diagnosis while culture is required to identify the pathogenic fungus. The results of both may vary as direct microscopy is relatively easy but subjective, while culture needs technical expertise. In our study, 83% cases were positive by KOH and/or culture. In studies which were conducted by Lone et al in Kashmir, Aghamirian et al in Iran, Kaur et al in New Delhi, Das et al in Kolkata and Jesudanam et al in Vishakhapatnam, 60%, 40.2%, 54.5%, 51.76% and 45.53% samples respectively were found to be positive by direct examination and/or culture. 9,14,16-18

In our study, culture positivity (79%) was more than microscopy (63%), similar to another study conducted by Das et al in 2008 in Kolkata, where 32.9% were positive by direct microscopy and 49.4% by culture. While, studies conducted by Lone et al in 2013 and Shenoy et al in 2008 found more positivity by direct microscopy than culture, with a positive result by microscopy in 56% and 53% and culture positivity in 40% and 35% respectively. 9,19 The variation in positivity by microscopy in different places might be due to varying technical expertise in microscopic examination in different places.

Out of the 100 cases in our study, growth of NDMs predominated (55%), followed by dermatophytes (15.8%) and yeasts (9.3%). The usual commonest causative agents of superficial fungal infection i.e. dermatophytes was found to be replaced by the NDMs, which were

previously thought to be common laboratory or environmental contaminants. Our result was similar to two other studies conducted by Singh et al and Bassiri-Jahromi et al, showing a rising trend of NDM in causing superficial mycoses. However many other workers have found dermatophytes as the commonest causative agent for onychomycosis in their studies. 8,10,15,20

We isolated NDMs in 55% of cases, while in studies conducted from various parts of India by Lone et al, Lakshmanan et al and Grover et al, NDMs were isolated in 31.6%, 24.4% and 34% respectively. 9,22,23 Amongst the NDM, in our study, A. niger and A. flavus (13% each) were the most common isolates. A. niger was also the commonest NDM isolated in other studies conducted by Adhikari et al in Sikkim in 2009, Kaur et al in 2007 in Delhi and Grover at al in 2003 in Bangalore. 13,16,24 While Bassiri-Jahromi et al in Tehran have isolated mostly A. fumigatus (27.6%).²¹ Many other NDMs isolated in our study were Bipolaris spp., Alternaria spp. Scopulariopsis spp., Penicillium spp., Curvularia spp., Mucor spp. and Rhizopus spp. (Table 4) Scopulariopsis spp. is known to cause onychomycoses, and rarely causes pneumonia and septicaemia. Bipolaris spp. and Curvularia spp. have been found to usually cause pulmonary, cutaneous, CNS infections. While, Alternaria spp. has been associated with sinusitis, asthma, cutaneous, eye and ear infections. Rhizopus spp and Mucor spp. usually causes cutaneous, subcutaneous, gastrointestinal and rhinocerebral mucormycosis.

Amongst the dermatophytes in our study, *T. verrucossum* (4.6%) followed by *T. rubrum* (3.7%) were most commonly isolated. Kaur et al in a previous study in 2007 from the same institute had reported *T. mentagrophytes* as the most common agent from the cases of onychomycoses showing a change in trend in prevalence of the dermatophyte infection in our hospital over the period of a decade. Many studies have shown *T. rubrum* as the most common dermatophytic cause of onychomycosis. 8-10,13

In the current study *C. albicans* was isolated in 6.5% and *NAC* was isolated in 2.8%. In other studies conducted by Sarma et al and Lone et al 40% and 6.6% of *Candida spp.* were isolated.^{9,10} While, in a study by Adhikari et al in Sikkim, not a single case of onychomycoses due to *Candida spp.* was isolated.¹³

Unlike any other Indian studies on onychomycoses, our study shows that onychomycoses is a very common health problem in north India, with a changing trend in etiological agents from the dermatophytes (earlier sole cause) to NDMs which being the major cause of onychomycoses currently. The diagnosis onychomycosis by a NDM is difficult as compared to onychomycosis caused by dermatophytes and yeasts, as consecutive cultures from repeated nail scrapings for reliable laboratory results becomes substantial.² According to some authors an untreated nondermatophytic onychomycosis could be an important and dangerous portal of entry for deep-seated and disseminated mycosis, which are difficult to treat in immunocompromised patients, making the correct diagnosis and rapid treatment of non-dermatophytic onychomycosis essential. ^{25,26}

CONCLUSION

The changing spectrum of infections as shown in our study with an increasing role of Non- Dermatophytic Moulds, (earlier thought to be laboratory or environmental contaminants), as a causative agent of onychomycoses, makes it imperative to look for all the possible causes of superficial fungal infection. A proper diagnosis, consisting of both clinical and mycological examinations, may aid the clinician in selecting the appropriate therapy. In addition, improving knowledge and awareness about the epidemiological and mycological features of onychomycosis and availability of their better diagnostic facilities would go a long way in decreasing the morbidity and improving the patient's quality of life.

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Ethical approval: The study was approved by the

Institutional Ethics Committee

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