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Original Research Article

A study of etiology and epidemiology of onychomycosis from a tertiary care hospital in North India

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ABSTRACT

Background: Onychomycosis is the fungal infection of nail of which the incidence varies from 0.5-12% in India and around 5% worldwide. Onychomycosis is considered to be gender and age-related disease, being commoner in males and older adults in both genders. Apart from dermatophytes, other fungal agents like yeasts and non-dermatophyte moulds are increasingly being acknowledged as important etiological agents for the same. Our aim was to study the etiology of the nail infections in patients presenting to a dermatology department in a tertiary care hospital in central Delhi.
Methods: This study included nail samples from patients of various age groups with suspected onychomycosis attending dermatology clinics in our hospital over a period of 2 years. For all samples, KOH wet mount microscopic examination followed by culture on Sabouraud's dextrose agar was done. Growth of filamentous fungi and yeast obtained on SDA were identified using conventional microbiology techniques. Statistical analysis was performed using Epitools.
Results: Of the total 1061 nail samples received, maximum prevalence seen in patients of age group 21 to 50 years. The positivity of onychomycosis was 57.02%. Out of culture positive samples, 24.62% grew dermatophytes and 75.37% grew non-dermatophytes (31.40% yeasts and 43.97% non-dermatophyte moulds). *Trichophyton mentagrophytes* was the most common (59.73%) dermatophyte followed by *T. rubrum* (23.49%) while among non-dermatophyte moulds; *Aspergillus niger* (20.68%) was the most common followed by *A. flavus* (16.54%) and others. Common yeasts included *Candida* spp. (65.79%) and *Trichosporon* spp. (28.95%).
Conclusions: Non- dermatophytes are emerging as major etiological agents of onychomycosis which may be accounted various host factors. So, it becomes important to correctly identify the causative fungi to ensure appropriate treatment.

Keywords: Onychomycosis, Non-dermatophyte moulds, Dermatophytes

INTRODUCTION

Onychomycosis is the fungal infection that may involve any component of the nail apparatus as well as the adjoining mesenchymal tissue causing discoloration, thickening or separation of nail from the nail bed.[1,2] Constituting 30% of the cutaneous fungal infections, its incidence varies from 0.5-12% in India and around 5% worldwide.[3-8] Onychomycosis is considered to be gender and age-related disease, being commoner in males and older adults in both genders.[9]

Dermatophytes are known to cause onychomycosis, other fungal agents like yeasts and non-dermatophyte moulds are increasingly being acknowledged as important etiological agents for the same.[3-5]

Risk factors leading to increase in incidence of onychomycosis include reduced peripheral circulation, diabetes, nail trauma, poor nail hygiene, occlusive footwear, chronic smoking, intense sports participation, communal bathing, occupational exposure and working in the wet.[10,11] In spite of the general awareness among people to improve personal hygiene and living conditions, this problem continues to persist and spread.[3] As the persistence of nail infections can serve as a source of infections elsewhere in the body, therefore, early institution of treatment is necessary.[1] Frequent recurrences and relapses are seen in patients depending on genetic predisposition, occupation, lifestyle and immunosuppression. Treatment outcome depends on the treatment regime, type and degree of nail involvement and the type of infecting fungus especially the non-dermatophytes such as *Fusarium* spp. which are difficult to treat.[12,13] Therefore, it is important to correctly identify the causative fungi to ensure appropriate treatment.

The objective of this study was to know the etiology of the nail infections in patients presenting to a dermatology department in a tertiary care hospital in central Delhi.

METHODS

This study was carried out in the department of microbiology at Lady Hardinge medical college and associated hospitals, New Delhi over a period of 2 years starting from January 2017 to December 2018. It included nail samples from patients of various age groups with suspected onychomycosis attending dermatology clinics in our hospital. Nail clippings were transported in a sterile container or a paper envelope to microbiology laboratory for laboratory diagnosis. All the samples were first subjected to microscopic examination of their potassium hydroxide (20% KOH) wet mount preparation and inoculated on Sabouraud's dextrose agar (SDA) with antibiotics (chloramphenicol 0.05 mg/ml, gentamicin 0.02 mg/ml) with and without cycloheximide (0.5 mg/ml). Each sample was inoculated on 2 tubes of both the media and one tube from each set was incubated at 25˚C and other at 37˚C respectively and examined biweekly till 6 weeks. Slopes showing no growth after 6 weeks were discarded. Growth of filamentous fungi obtained on SDA were identified based on colony morphology, colour, texture of surface, topography, pigment on reverse and rate of growth. Microscopic examination of culture was done using lactophenol cotton blue (LPCB) preparation and slide culture was done in case morphology was not distinct in LPCB preparation. The growth of yeasts was confirmed by colony characteristics, LPCB mount and gram stain of colony. Various tests like germ tube test, colony color on chrom agar (HiMedia), morphology on corn meal agar with Tween 80, urease test was done to speciate isolated yeast.

Statistical Analysis was done using Epitools. The percentage and frequency distribution of fungal etiological agents causing onychomycosis was calculated. The performance of two different diagnostic modalities i.e., culture and microscopy that were used on the same sample was compared. Kappa and proportions of positive and negative agreement as well as McNemar's chi-squared value were calculated. Corresponding p values were also calculated for both kappa and chi-squared values. A p value<0.05 was considered to be significant.

RESULTS

A total of 1061 nail samples from patients clinically suspected to have onychomycosis (Figure 1 and 2) were tested over a period of 2 years. Among the 1061 patients, 571 (53.8%) were males and 490 (46.2%) were females. Majority of the suspected onychomycosis cases (52.3%) belonged to the age group 21 to 50 years (Table 1). Month wise distribution of the samples received is depicted in Figure 3 with maximum prevalence seen in the post monsoon months in this region.

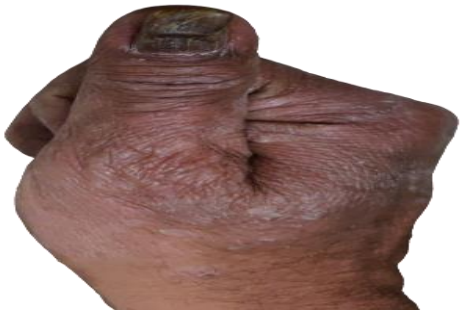

Figure 1: Dysmorphic and discoloured thumb nail of a patient.

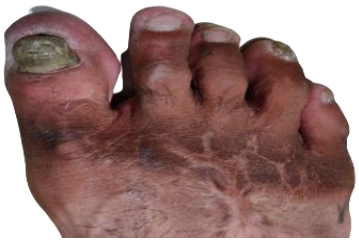

Figure 2: Dysmorphic and discoloured great toe nail of a patient.

Of the 1061 suspected cases of onychomycosis, 38.92% (413/1061) were confirmed by KOH wet mount microscopic examination (Figure 4), out of which 84.74% (350/413) samples were both microscopy and culture positive while 15.25% (63/413) of microscopy positive samples were culture negative (Table 2). Two fifty-five samples (24.03%) were negative on microscopic examination but were culture positive whereas 37.04% (393/1061) samples were negative on both microscopy and culture. For the samples that were culture positive and negative on microscopic examination

and when non-dermatophyte moulds were isolated, repeat samples from these cases were taken and only if they grew the same fungus, were considered positive. Thus, the positivity of onychomycosis was 57.02% (605/1061).

Table 1: Distribution of samples from different age groups.

Age (year)	No. of samples	Percentage (%)
0 to 10	6	0.56
11 to 20	112	10.55
21 to 30	272	25.63
31 to 40	283	26.67
41 to 50	173	16.30
51 to 60	130	12.25
61 to 70	65	6.12
>70	20	1.88
Grand total	1061	

Table 2: Agreement statistics between the two modalities for detecting onychomycosis.

Detection	Microscopy positive	Microscopy negative	Total
	No. (%)	**No. (%)**	**No. (%)**
Culture positive	350 (57.8)	255 (42.2)	605 (57.02)
Culture negative	63 (13.81)	393 (86.18)	456 (42.98)
Total	413 (38.92)	648 (61.08)	1061

The percentage of positive agreement and negative agreement between the two different diagnostic modalities i.e., Microscopy and culture was found to be 68.76 and 71.20 respectively. The overall agreement between the two modalities was found to be 70%. McNemar's chi square test was applied to check whether there is significant difference between agreements of these two. We found that there was a significant difference between the agreement of the two modalities (McNemar's χ^2=114.72 p=0.000). The Kappa statistics along with 95% C.I. was also calculated to check the proportion of agreement of the tests and was found to be 0.419 (0.368-0.469) with Z=14.56 and p=0.000, suggesting moderate level of agreement between these diagnostic modalities. Considering culture as gold standard for diagnosis of fungal infections, sensitivity and specificity of microscopy was found to be 57.85 (53.80-61.82%) and 86.18 (82.67-89.22%) respectively.

Out of 605 culture positive samples, 149 (24.62%) grew dermatophytes and 456 (75.37%) grew non-dermatophytes (31.40% yeasts and 43.97% non-dermatophyte moulds). Among the isolated dermatophytes, *Trichophyton mentagrophytes* was the commonest (59.73%), followed by *T. rubrum* (23.49%), *T. violaceum* (9.39%), *T. tonsurans* (4.69%), *Microsporum gypseum* (1.34%) and *Epidermophyton floccosum* (1.34%) (Figure 5).

Among the isolated non-dermatophyte moulds, *Aspergillus niger* (20.68%) was the most common followed by *A. flavus* (16.54%), *A. nidulans* (12.78%), *A. fumigatus* (9.02%), *A. terreus* (7.52%), *A. glaucus* (7.52%), *Penicillium* spp. (7.52%), *Alternaria* spp. (7.14%), *Acremonium* spp. (3.38%), *Fusarium* spp. (3%), *Cladosporium* spp. (1.5%), *Chaetomium* spp. (1.13%), *Aureobasidium* spp. (0.76%), *Scopulariopsis* spp. (0.03%), *Malbrachea* spp. (0.03%), *Phialophora* spp. (0.03%), *Curvularia* spp. (0.03%) (Figure 6).

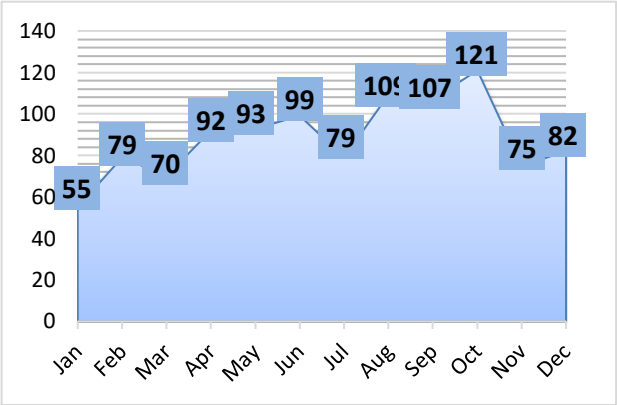

Figure 3: Month wise distribution of onychomycosis samples.

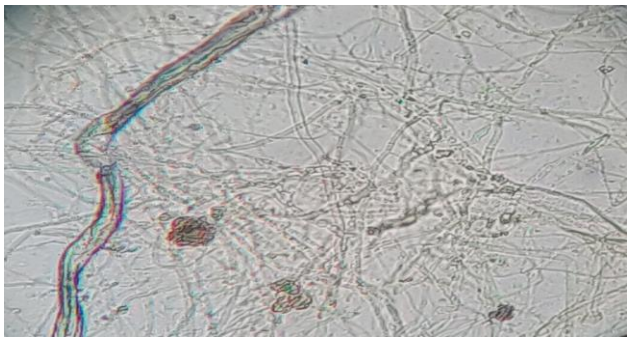

Figure 4: Microscopy (total magnification 400x)-KOH mount of a nail sample of thin hyaline septate branching hyphae.

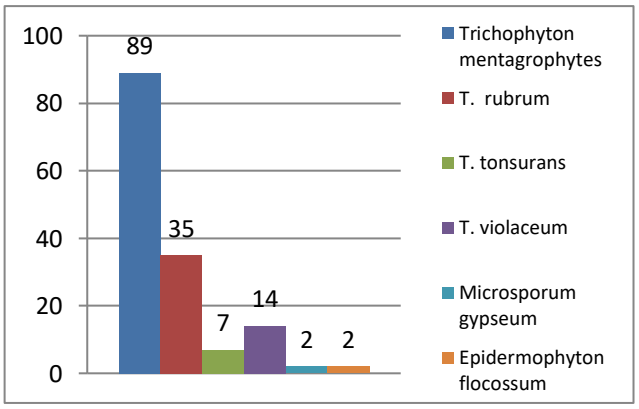

Figure 5: Dermatophytes isolated in culture from nail samples.

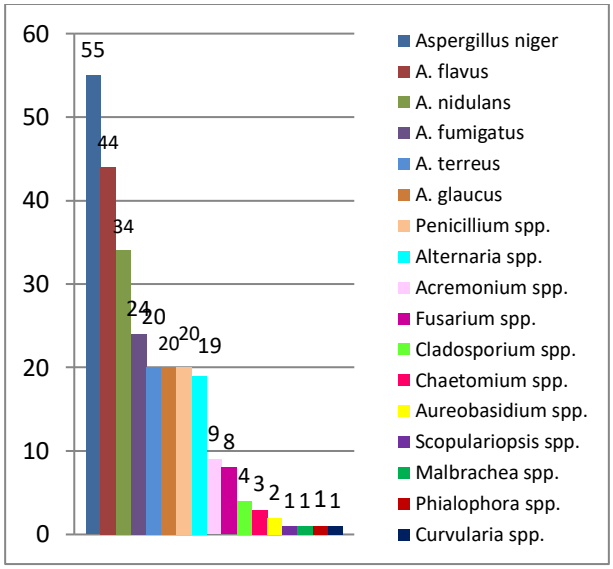

Figure 6: Non-dermatophyte moulds isolated in culture from nail samples.

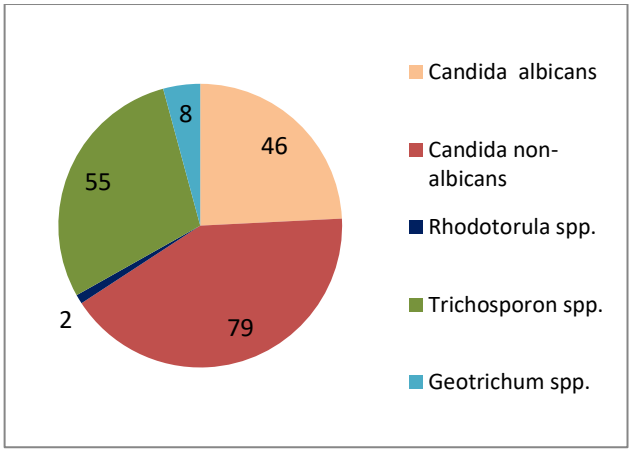

Figure 7: Yeasts isolated in culture from nail samples.

Candida spp. was the commonest yeast (65.79%) isolated, majority being *C. non-albicans* (63.2%). Other yeasts include *Trichosporon* spp. (28.95%), *Geotrichum* spp. (4.21%) and *Rhodotorula* spp. (1.05%) (Figure 7).

Table 3: Percentage of different non-dermatophyte moulds isolated from nails in different studies.

Non-dermatophytes isolated	Present study (%)	Narain et al[23] (%)	Motamedi et al[24] (%)	Nouripour et al[25] (%)
Aspergillus niger	20.68	7.44	69.3	2.6
A. flavus	16.54	8.10		77.3
A. nidulans	12.78	-		5.8
A. fumigatus	9.02	-		
A. terreus	7.52	14.06		1.95
A. glaucus	7.52	-		-
***Penicillium* spp.**	7.52	8.22	-	-
***Alternaria* spp.**	7.14	-	-	-
***Acremonium* spp.**	3.38	11.99	1.3	-
***Fusarium* spp.**	3	7.60	9.33	-
***Cladosporium* spp.**	1.5	-	1.3	-
***Chaetomium* spp.**	1.13	-	-	-
***Aureobasidium* spp.**	0.76	-	-	-
***Scopulariopsis* spp.**	0.03	20.51	1.3	-
***Malbrachea* spp.**	0.03	-	-	-
***Phialophora* spp.**	0.03	-	-	
***Curvularia* spp.**	0.03	-	-	-
***Absidia* spp.**	-	7.22	-	-
***Rhizopus* spp.**	-	7.60	-	-
***Scytalidium* spp.**	-	1.48	-	
***Mucor* spp.**	-	5.78	-	-
***Paecilomyces* spp.**	-	-	1.3	-
***Chrysosporium* spp.**	-	-	1.3	-

Table 4: Percentage isolation of dermatophytes from nail samples in various studies.

Dermatophytes	Present study (%)	Asifa et al 2017, Kashmir[11] (%)	Niranjan et al, 2012, Devangere[16] (%)	Banik et al, 2017, Shillong[1] (%)	Bitew et al 2019, Ethiopia[20] (%)
T. mentagrophytes	59.73	47.05	14.81	11.5	11.9
T. rubrum	23.49	38.23	33.33	16.9	13.4
T. violaceum	9.39	-	-	0.8	-

Continued.

Dermatophytes isolated	Present study (%)	Asifa et al 2017, Kashmir[11] (%)	Niranjan et al, 2012, Devangere[16] (%)	Banik et al, 2017, Shillong[1] (%)	Bitew et al 2019, Ethiopia[20] (%)
T. tonsurans	4.69	3.61	7.41	7.7	9.6
T. verrucosum	-	-	-	1.5	0.96
T. soudanense	-	-	-	-	4.8
T. schoenleni	-	-	-	0.8	0.96
M. gypseum	1.34	-	-	-	-
M. canis	-	-	-	-	-
E. flocossum	1.34	2.94	-	2.3	17.5

DISCUSSION

Onychomycosis is a common public health problem which comes with psychosocial effects, occupational discomfort, permanent disfigurement of the nail coupled with high cost of long-term treatment.[14] The prevalence of this infection varies according to geographical, racial, climatic, economic and cultural differences; therefore, it is important to assess its true burden in a particular region and identify its current etiological profile. In the present study, the prevalence of onychomycosis was confirmed in 57.02% samples analysed; which is almost similar to the findings reported by Kaur et al (54.5%) from the same region, Niranjan et al (55%) from Karnataka and Chetana et al (59.1%) from Puducherry, South India.[15-17] Other authors like Banik et al from North east India on the other hand have reported a higher prevalence (65%).[1] There is a wide variation in prevalence rates reported by authors worldwide like 28.3% from Brazil, 56.4% from Tehran, Iran and 60.4% from Ethiopia.[18-20] Among the patients included in our study, there was a male preponderance 53.8%, however, there are mixed reports about the prevalence of onychomycosis with respect to gender.[1,16,17] This comes from certain activities and occupations involving a particular gender like males involved in outdoor activities prone to trauma and women involved in wet work.[15] In the present study, the highest prevalence of onychomycosis was seen in the age group of 20-50 years, uncommon in elderly and least in children less than 10 years of age which is comparable to other studies.[15,17,20] The increased number of onychomycosis cases during post monsoon may be related to the favourable climatic conditions (increased temperature and humidity) seen during monsoon and the nail infections presenting late clinically.[21,22]

In the current study, the occurrence of onychomycosis due to non-dermatophyte moulds (43.97%) was much higher than the most commonly recognized etiological agents like dermatophytes (24.62%) and yeasts (31.40%). This is contrary to most previous studies from this region and worldwide where dermatophytes have been the most common isolates.[1,15-17,20] There are several authors who are reporting an increase in prevalence of non-dermatophyte moulds, most common isolate being *Aspergillus* species from onychomycosis samples like Narain et al from Uttar Pradesh (41.60%), Motamedi et al (29.3%) and Nouripour et al (33.2%) from Iran (Isolates mentioned in Table 3).[23-25]

Amongst dermatophytes, *T. mentagrophytes* (59.73%), was the commonest to be isolated, followed by *T. rubrum* (23.49%), *T. violaceum* (9.39%), *T. tonsurans* (4.69%), *Microsporum gypseum* (1.34%) and *Epidermophyton floccosum* (1.34%). The results are similar to those reported by Asifa et al from Kashmir and Kaur et al from Delhi who also reported *T. mentagrophytes* as the commonest isolate (47.05 and 79.2% respectively).[11,15] However, many authors from the India and abroad have reported *T. rubrum* as the most common isolate like 16.9% by Banik et al from Shillong, 33.33% by Niranjan et al from Devangere and 13.4% by Bitew et al from Ethiopia (as shown in Table 4). Such diversity in distribution of fungal species results from varying environmental conditions in different geographical terrains.[1,16,20,26]

However, to develop a complete understanding of the predisposing factors for onychomycosis and correlating demographic profile of patients with the infection pattern and prevalence, a more extensive community-based study spanned over a long duration of time is desirable. Considering these facts, we had certain limitations in our study, first of it being a short duration study of two years only. Secondly, ours being a tertiary care centre limited the inclusion of only those patients that visited our centre, missing out people with onychomycosis in the community and also that section of poor, illiterate or daily wage workers who are either ignorant or unaware of their infection or cannot afford to lose their daily wage on account of visiting the tertiary care centres.

CONCLUSION

Non-dermatophytes are emerging as major etiological agents of onychomycosis. This may be accounted to an increase in immunosuppressive conditions that makes the host vulnerable to the infection by the so-called commensal, contaminants or colonizer fungi. However, unlike dermatophytes which are diagnostically significant even with single isolation, non-dermatophyte moulds require further confirmation to be characterized as true pathogen. So, one should be aware of these potential pathogenic agents. With increasing prevalence of non-

dermatophyte moulds as causative agents of onychomycosis, it becomes more important to correctly identify the isolates so that the appropriate therapeutic decisions are taken.

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