

Candida as an Emerging Pathogen of Onychomycosis: A Study Report from North India

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Abstract: *Onychomycosis has a significant impact on the physical, psychosocial, and occupational problems of the patients worldwide including India. Immunosuppressive conditions have led to change in the epidemiological and clinic-mycological patterns of onychomycosis. The aim of the study was to assess the current trend of onychomycosis in North India. 103 consecutive clinically suspected cases of onychomycosis were studied. Detailed epidemiological and clinical data was collected in a designed performa. Nail shavings were subjected to 10%KOH for direct microscopy and culture. Results indicate that male and females were affected equally (1:1.2) with mean age of 33.3 years. Farmers and housewives were found to be most affected with DLSO as most common clinical presentation. 53.5% cases were positive by direct microscopy and 41.2% were culture positive and 36% cases by both the methods. Candida (47.5%) was the most common fungal pathogen followed by dermatophytes (40%) and NDM (12.5%). Candida albicans (32.5%) and Trichophyton rubrum (20%) were the most common species. Results indicate that Candida has emerged as important pathogen of onychomycosis necessitating the detailed diagnosis for appropriate therapy as it is entirely different from the dermatophytic infections and it can no longer be considered a mere cosmetic problem.*

Keywords: Onychomycosis, KOH, Dermatophytes, *Candida*, Diagnosis, Therapy

1. Introduction

Sudden increases in the incidences of onychomycosis have been observed worldwide, which has been related to increasing variety of etiologic factors, and environmental risk factors. Onychomycosis is a fungal infection of nail, its matrix, and other nail parts, and usually caused by dermatophytes. *Trichophyton rubrum* is most commonly responsible for it, but number of infection due to yeast and nondermatophyte moulds (NDM) is also increasing [1-2]. In India also, the incidence rate of onychomycosis in general population varies from 0.5 to 5%. However, scanty data is available in this regard from our country [3-6].

Though dermatophytes has been considered as a major pathogen but infections due to NDM and *Candida* has also been reported, worldwide [3, 7-8]. The etiology of onychomycosis may changes according to changing geographical area since distribution of fungi changes according to changing geographical area. The diagnosis mainly relies on direct KOH microscopy and culture with variable level of reliability. Hence, the diagnosis of onychomycosis becomes important to find out the agent involved for accurate therapy [3, 9]. The treatment of onychomycosis is also troublesome due to high resistance and low penetration of antifungal agents, including other factors affecting the outcome of treatment such as host age, fingernail or toenail, degree, concurrent therapy and the type of fungus [10-11].

The most common approach to treatment of onychomycosis includes mechanical, chemical, or surgical procedures along with suitable antifungals. In past, oral uses of griseofulvin and ketoconazole have been proved good [12]. However, oral griseofulvin is effective only against dermatophytes and has a poor compliance due to prolonged therapy. Ketoconazole is not preferred due to its side effects and drug

interactions. Now a days, terbinafine, fluconazole and itraconazole are the drugs of choice. These antifungals are expensive, and Candidal onychomycosis such as *Candida krusei* have shown the drug resistance.

The NDM's also does not respond well to systemic antifungal agents [13]. Since the spectrum of causative agents of onychomycosis is very wide and their management varies largely according to type of pathogen involved, therefore, clinico-mycological data is of utmost importance for the selection of an appropriate therapy. Therefore, present study was carried out to know about the clinical type and causal agent associated along with the age, sex, and occupation of onychomycosis patients in North India.

2. Materials and Methods

- **Study Cases:** The study comprised of 103 cases clinically suspected fungal nail infection as onychomycosis, attending Dermatology OPD of Smt. Sucheta Kripalini Hospital, New Delhi over a period of 18 months. A detailed history of occupation, duration of illness, predisposing factors etc. were obtained. Clinically the patients were classified into five categories viz. distal and lateral subungal onychomycosis (DLSO), proximal subungal onychomycosis (PSO), white superficial onychomycosis (WSO), candidal onychomycosis (CO), and total dystrophic onychomycosis (TDO). Written consent was obtained from each patient. Institutional Ethical Committee approved the study protocol.
- **Collection of Sample:** The affected nail area was washed with 70% alcohol and the nail material was collected on a sterilized paper with the help of a sterile surgical blade. The type and site of material to be collected was based on the clinical variety of onychomycosis. Repeat sampling and processing was done for each patient at least thrice.

- **Culture Study:** The material was inoculated on Sabouraud's dextrose agar (SDA) with and without cycloheximide and was incubated at 30°C for a period of 4 weeks. Chloramphenicol and gentamicin were incorporated in SDA to prevent bacterial contamination. The fungal isolate was identified by rate of growth, colony morphology, and Lactophenol cotton blue (LCB) mounts. *Candida* isolates were identified by Gram's staining, germ tube test, sporulation on corn meal agar, Tween-80 Rice infusion agar media, and sugar fermentation and assimilation. NDM's were considered a pathogen if isolated on a repeat culture at least on one occasion with consistent direct microscopic findings [14].
- **KOH Microscopy:** The remaining material was placed on a clean sterile glass slide, and was incubated at 37°C with 10% KOH to emulsify the lipids. Later, slides were examined under microscope for the presence of fungal elements.
- **Treatment:** All patients were treated with terbinafine or itraconazole or fluconazole with nail lacquers (amorolfine or cyclopirox olamine) and were followed subsequently. Fingernail infection was treated for 6-12 weeks while 12-24 weeks therapy was given for toenail infection.
- **Statistical Analysis:** All the experiments were repeated three times to confirm the reproducibility of experiments. Results were analysed statistically by Student's *t* test using SPSS 11.05.

3. Results

Onychomycosis was seen to affect almost all age groups ranging from 6 to 72 years with a mean of 33.3 years. The male to female ratio was 1:1.2. The age group of 31 to 40 years was the most commonly affected followed by age group of 41 to 50 years (Figure 1).

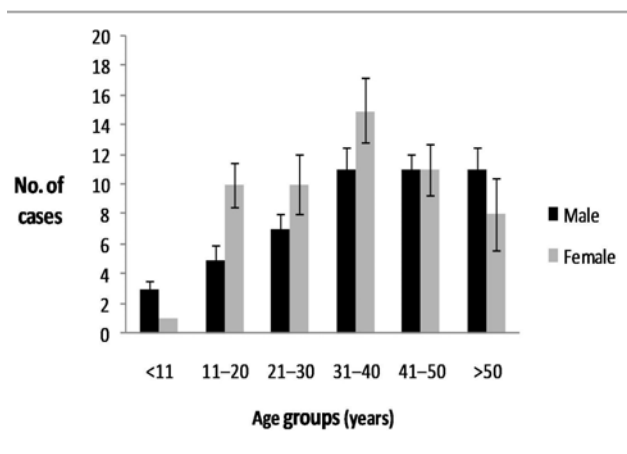


Figure 1: Figure showing the percentage distribution of age groups having onychomycosis infections.

Samples collected were subjected to microscopy (KOH) and culture. It was observed that out of 103 samples, 52 samples were positive by KOH while 40 samples were positive by culture. 35 samples were positive by both the methods (Table 1).

Table 1: Total number of samples showing the presence of fungi by direct KOH microscopy, culture, and both the methods for the diagnosis of onychomycosis

Total Samples	10% KOH	Culture on SDA	Both KOH & culture
n=103	52	40	35
% detection	53.56	41.2	36

In culture, *Candida* 19 (47.5%) was the most common isolate followed by dermatophytes 16 (40%) and NDM 5 (12.5%) (Table 2).

Table 2: Percentage isolation of different fungal pathogens from nail samples of onychomycosis patients after culture on SDA and identification by LCB (n=40).

Fungal pathogens	No (%)
Dermatophytes	
<i>Trichophyton rubrum</i>	8 (20)
<i>T. mentagrophytes</i>	3 (7.5)
<i>T. verrucosum</i>	2 (5)
<i>T. violaceum</i>	1 (2.5)
<i>Microsporum gypseum</i>	2 (5)
Yeasts	
<i>Candida albicans</i>	13 (32.5)
<i>Candida parapsilosis</i>	3 (7.5)
<i>Candida tropicalis</i>	2 (5)
<i>Candida krusei</i>	1 (2.5)
NDM	
<i>Aspergillus spp.</i>	3 (7.5)
<i>Alternaria</i>	1 (2.5)
<i>Fusarium</i>	1 (2.5)

Candida was isolated from 26.7% of fingernail infections, while dermatophytes were isolated from 16.4% of toenails (Figure 2). Yeasts remained to be most common isolates as compared to dermatophytes and NDM.

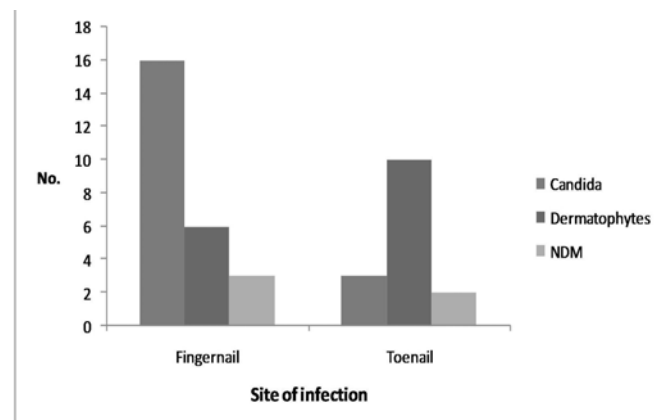


Figure 2: Figure showing the distribution of etiological agents of onychomycosis cases in fingernail and toenail infections.

The correlation of the different fungal pathogens with that of clinical categories was also observed. DLSO was the most common clinical presentation of onychomycosis (47.5%), out of which 63.15% were dermatophytic and 36.85% were Candidal infections. This was found to be statistically significant using the Chi-square test (Table 3).

Table 3: Correlation of the major fungal pathogens isolated from clinical samples with the type of clinical presentation of onychomycosis (n=40).

Pathogen	Clinical type				
	DLSO	CO	TDO	PSO	WSO
Dermatophyte	12	0	3	1	0
Candida	7	9	2	0	1
NDM	3	0	2	0	0
Total	19	9	8	1	1

$\chi^2=9.96$, df=1, $P<0.005$

Rate of occurrence of CO and TDO was found to be comparable. The distribution of the different clinical types of Onychomycosis with occupation type was also observed. Housewives showed the highest rate of onychomycosis and *Candida* was isolated from their nail samples. All the patients responded clinically to antifungal therapy. However, two patients were lost to follow up (Table 4).

Table 4: Table showing the distribution of *Candida* isolates among patients of onychomycosis associated with different professions.

Occupation	Total	Candida	Others
Laborers	26	2	6
Office workers	22	2	2
Housewives	19	10	1
Students	12	1	1
Farmers	10	0	9
Health care workers	7	2	1
Others	7	2	1
Total	103	19	21

4. Discussion

Onychomycosis is one of the commonest dermatological problems of the nail apparatus by fungi with toenails caused by *Trichophyton rubrum* being most affected [15]. Increasing awareness about this disease, and the availability of new and more effective antifungal drugs in recent time, has led to patients to seek treatment. However, most of time, treatment is prescribed without mycological confirmation of infection. This led to confusion over fungi, whether are primary or secondary pathogens. Moreover, the efficacy of antifungal agents also remains suspicious since drugs are given usually for undefined durations. Worldwide dermatophytes account for majority of onychomycosis cases and India is no exception. However, the data regarding the etiology of onychomycosis and its prevalence in particular geographical area is lacking. In the present study, an attempt was made to understand the etiology and distribution of onychomycosis and host factors in population of North India.

In the present study, 103 clinical cases of onychomycosis were included. The samples were processed for microscopy and culture to find out the prevalence of etiological agent. Almost all age groups were affected by onychomycosis with the most common age group of 31-40 years. Similar results have been shown in various studies from middle and South India indicating the same age group most affected [8, 16-19]. Possibly, high prevalence rate in increasing age group could

be manifold like more exposure, slower nail growth, larger surface area, and high prevalence of associated risk factors such as diabetes mellitus, tinea pedis etc [3, 5]. The females were also observed to be slightly more affected than males, probably because a significant number (18.5%) in this study comprised of housewives, though most of the studies have reported high incidence rate in males [2, 8, 14]. Further, farmers were found to be more affected followed by housewives than any other occupations ($p \leq 0.01$) suggesting that possibly due to increased incidence of occupation related trauma.

In case of involvement of site, toenails (41.8%) and fingernails (40.8%) were affected, almost equally, and both the sites were affected in 17.5% of cases. However, conflicting reports exist regarding the site of infection involved. Gupta et al., have reported that toenail infection is more common than that of fingernail infection possibly because of the use of occlusive footwear, which are associated with increased perspiration and trauma [20-21]. However, contrary to these reports, fingernails have been shown to be more affected than toenails by other workers [3, 8, 16, 19].

To find out the etiological agent, microscopy and culture was done and was confirmed by both the methods in 57 patients (55.3%) by direct microscopy and/or culture. These results are in corroboration with previous studies where both the methods have been shown to be useful in diagnosis [3, 22]. Bhalla et al., have shown the 54.5% prevalence rate out of 400 clinically suspected cases of onychomycosis by both the methods [23]. In another study, 49.5% were found to be positive and the most common etiological agent was found to be dermatophytes only [24]. However, reports from other parts of India have shown variable rate of diagnosis. In a study from North East India, 23.7% cases and in central India, 26.6% cases were found to be positive for onychomycosis by these methods. A similar study from Maharashtra, India reported that 29.5% cases were positive by both the methods [16, 23-24]. Since the environmental conditions of North India are entirely different from rest of India, it may be the reason for this variability.

In recent time, a significant change in trend of etiological agent has been observed throughout world including India where number of onychomycosis cases caused by NDM and yeasts has been increased tremendously. Although *T. rubrum* was considered a primary pathogen, but lately NDM and the yeasts, mainly *Candida albicans* has also emerged as the important pathogen associated with onychomycosis [3, 6-7]. *Candida* and dermatophytes were the common fungi isolated from fingernails and toenails, respectively. In the present study, results have shown that 47.5% cases were associated with *C. albicans* only as compared to other agents. *Candidal* onychomycosis was the most common type of infection associated with fingernails of housewives. Possibly chronic exposure to water as seen in the housewives (52.6%) and chefs (10.5%) may be the most common occupational risk factor as reported in previous studies [21]. Further, as females harbour this organism in their intestine or vagina, and have more cosmetic concerns, that may be the reason for high number seeking medical attention [14, 16, 25]. Similar

results have been reported worldwide, where yeasts were implicated as major pathogen associated with onychomycosis [3-4, 6, 26-27]. In another report from India, yeast was demonstrated in 40.4% cases as compared to 49.5% cases caused by dermatophytes [24]. In clinical presentation, DLSO (54.2%) was the most common clinical presentation and *T. rubrum* was the most common pathogen associated with that. Similar results have also been shown by other studies [7, 16, 19].

5. Conclusions

In this study, no direct correlation was found in occurrence of onychomycosis with that of age, sex, occupation, and etiological agent. Although *Candidal* onychomycosis was found to be more common than that of dermatophytes but it can also present as any of the clinical type since as much as 17.5% cases of the *Candida* onychomycosis were presented as DLSO (Table 3). However, the typical features of *Candidal* onychomycosis like nail plate involvement, nail bed/ folds thickening with yellow-brown discolouration resulting in characteristic drumstick appearance of digits, transverse striations (Beau's line), nail plate dystrophy etc. may be completely absent. Since newer emerging pathogens shows more antimicrobial resistance such as *Candida krusei* intrinsic resistance to fluconazole. Therefore, a laboratory diagnosis to ascertain the exact etiological agent becomes extremely important for selection of appropriate therapy. For example, *Candidal* onychomycosis is a common condition and clinical diagnosis is often confused with a dermatophytic infection because of the varied clinical presentation. Therefore, exact diagnosis becomes imperative to choose an appropriate therapy.

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