

Original Research Article

Clinico-mycological profile of onychomycosis in Assam, India

Nilakshi Borah^{1*}, Ajanta Sharma¹, Debeeka Hazarika²

¹Department of Microbiology, Gauhati Medical College, Guwahati-781032, Assam, India

²Department of Dermatology, Venereology and Leprology Tezpur Medical College, Tezpur, Assam, India

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*Correspondence:

Dr. Nilakshi Borah,

E-mail: drnilakshiborah@yahoo.com

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ABSTRACT

Background: Onychomycosis (OM) is a common nail disorder caused by dermatophytes, non-dermatophyte moulds and yeasts. The increased prevalence of OM along with the wide range of associated fungi necessitates the accurate laboratory isolation and identification of the causative fungus for optimal management strategies. The aim of the study was to evaluate clinical, epidemiological and mycological characteristics of OM in a tertiary care hospital in Assam.

Methods: It was a prospective hospital-based study. Hundred consecutive patients clinically diagnosed as OM were subjected to detailed history, clinical examination and investigations. Nail clipping and subungual debris of OM patients were subjected to KOH preparation and culture. Isolates were identified by colony characters, pigment production, microscopic morphology and biochemical tests.

Results: The maximum number of OM cases belonged to the age group 21-30 years with a male preponderance (M:F=1.7:1). The commonest clinical type of OM was distal lateral subungual onychomycosis (DLSO) (51%). Toe nails OM was the most common presentation (54%). Trauma (35%) was the most common predisposing factor. Farmers (agro industry labourers) (23%) were found to be the most commonly affected group. Non-dermatophyte moulds (NDM) accounted for 38(47.5%) of OM cases followed by yeast 27(33.8%) and dermatophytes 15(18.8%).

Conclusions: Early diagnosis and treatment is important to prevent distortion of the nails which can prevent loss of working efficiency of the commonly affected population of this region.

Keywords: Assam, Dermatophytes, Non-dermatophyte moulds, Onychomycosis

INTRODUCTION

Onychomycosis (OM) is the infection of nails caused by dermatophyte fungi, non-dermatophyte fungi or yeast which represents about 30% of cutaneous mycotic infections. It is the most common nail disease and accounts for approximately 50% of all onychopathies.¹ It affects approximately 5% of population worldwide.² It may reach up to 20% in high risk populations like miners and sportsmen etc.³ Various studies have reported the incidence to vary from 0.5%-5% in the general population in India.^{4,5} Various factors like increasing age, immunosuppression, diabetes mellitus, family history,

peripheral vascular disease, and disorders related to the skin such as hyperhidrosis, psoriasis, onychogryphosis, nail trauma, occupation and climate influence the etiology, progression and treatment outcome of OM.²

OM is caused by three groups of fungi, namely dermatophytes, nondermatophytic moulds and yeasts.⁶ Dermatophytes cause 90% of toenails and 50% of the fingernail onychomycosis.⁷ *Candida* species, particularly *Candida albicans*, prevail in finger infections.³ Non-dermatophyte moulds are rare, although few species are described as etiological agent of onychomycosis.⁸ Among the dermatophytes, the most common fungi reported is

Trichophyton rubrum followed by *T. mentagrophyte* var *interdigitale* and *Epidermophyton floccosum*. The non-dermatophytic fungi causing onychomycosis are *Scopulariopsis brevicaulis*, *Acremonium species*, *Aspergillus flavus* and *Aspergillus fumigatus*, *Fusarium oxysporum*, *Scytalidium dimidiatum*, *Onychocola canadensis* and *Geotrichum candidum*.⁶ The number of patients affected by OM and the number of fungi recognized as capable of parasitizing the nail plate directly is increasing all over the world.⁹ Several clinical types of OM have been recognized and recently a new classification has been proposed by Hay et al, comprising of primary and secondary OM. Primary includes clinical variants like distal lateral subungual onychomycosis (DLSO), white superficial onychomycosis (WSO), endonyx onychomycosis (EO), proximal subungual onychomycosis (PSO), mixed pattern onychomycosis (MPO) and total dystrophic onychomycosis (TDO).^{10,11} Toe nails are about 25 times more likely to be infected than fingernails.¹¹ Onychomycosis is persistent, intractable and poses serious concern to the clinicians as it often becomes a chronic source of recurrent superficial mycotic skin infections, besides causing considerable disfigurement.^{12,13} Relapse of OM depends on several factors, including genetic predisposition, reduced nail growth rate in elderly and underlying disease e.g., psoriasis, peripheral vascular diseases, diabetes, immunosuppression, HIV infection, and cigarette smoking.¹⁴⁻¹⁹

The etiologic spectrum of OM is largely dependent on the flora in the immediate environment of the individual. It is influenced by the geographic, climatic and occupational factors. Knowledge of the epidemiological and mycological characteristics is an important tool for control of this infection. The subtropical climate of Assam is known for its heavy rainfall and high humidity favouring the growth and dissemination of fungal pathogens. Moreover, a considerable proportion of the population of this region is agricultural and tea garden workers who are always exposed to these fungal pathogens in their working environment. This occupational hazard often leads to disfigurement of the nails decreasing the working efficiency and earning capabilities of the individuals which in turn creates a serious economic burden for the people of this region. Hence, the correct etiological diagnosis is important to select the best antifungal agent for treatment in a particular case.

METHODS

The study was carried out for a period of one year from August, 2012 to July, 2013. The study group consisted of 100 consecutive patients attending outpatient department (OPD) of Dermatology, Venerology and Leprology. The formula used for calculating sample size was $n=4pq/d^2$ where 'd' is a relative allowable error of 0.1 and 'p' is 0.5. (As the prevalence rate of onychomycosis in general population of Assam is not known, 50% was considered

as the prevalence rate). The estimated sample size will be 100. An institutional ethical committee approval was taken, and a written informed consent was obtained from every patient enrolled in the study before sample collection. Clinically diagnosed cases of onychomycosis presenting with nail changes like onychodystrophy, onycholysis, subungual hyperkeratosis, melanonychia, leuconychia and thickening of nail plate were included in the study. Patients who had received treatment with antifungal agents within the last one month and pregnant women were excluded from the study. The suspected nails were cleaned with 70% alcohol. Full thickness clippings from the affected nails were collected following the protocol described by Reddy et al, 5-6 representative fragments of the nails were placed in a drop of 20% KOH for direct microscopy.^{20,21} Approximately 20 small pieces were inoculated in 20 different sites on the Sabouraud's dextrose agar (SDA) plates with chloramphenicol (0.5g/l), gentamicin (20mg/l) and cycloheximide (0.5g/l) and dermatophyte test medium. (Figure 1).



Figure 1: Inoculation of nail pieces in multiple sites on SDA with chloramphenicol (*T. mentagrophytes*).

Inoculated plates were incubated at 25°C and observed daily for fungal growth for as long as 4 weeks. In case of growth, the isolate was confirmed by characteristic morphology of the colonies, pigmentation and microscopic appearance of the fungus in lactophenol cotton blue mount (LCB). If a mould other than dermatophyte or yeast was isolated it was considered significant only if the appropriate fungal elements were seen on direct microscopy and a repeated culture showed growth of the same mould or at least 5/20 inocula have yielded the mould.²² Slide culture, hair perforation test and urease tests were performed whenever required.²³⁻²⁶

RESULTS

The age of the study population ranged from 9-72 years (mean age 34.24±14.53 years) with maximum patients (29%) belonging to the age group of 21-30 years. There was a slight male preponderance (M:F=1.7:1). Nail trauma (35%) was the commonest predisposing factor associated with OM. The demographic characteristics of the patients are shown in Table 1.

Table 1: Demographic characteristics of the 100 study subjects.

Demographic profile	Total =100 (%)
Sex	
Male	63
Female	37
Mean age (in years ± SD)	34.24±14.53
Predisposing factors	
H/O trauma	35
Occlusive foot ware	7
Steroid intake	5
Tinea pedis	5
Diabetes mellitus	4
Commercial swimming pool	4
Family history	3
Tinea manuum	3
Contact with fungal infection peron	2
Nothing suggestive	32

Fifty-four percent of the patients presented with toe nail OM, 36% with finger nail and 10% with both finger and toe nail OM. Toe nail OM was significantly more common in males (P value <0.0065) and finger nail involvement was more common in females (P value 0.0182) (Table 2).

Table 2: Relationship of nail involvement with gender.

Gender	Male	Female	P value*
Toe nail (n=54)	41	13	0.0065
Finger nail (n=36)	17	19	0.0182
Toe nail + finger nail (n=10)	5	5	0.4924

*Fisher’s exact test: level of significance P <0.05

Maximum number of OM cases was found during the period May to August. The prevalence was less in the winter months i.e. from September to April. Farmers (23%) were found to be the most commonly affected group. DLSO (51%) was the most common clinical type followed by PSO (25%), WSO (18%) and TDO (6%) (Figure 2). In DLSO and WSO types were more commonly seen in toe nails and PSO and TDO types in the finger nails. But, only DLSO type was significantly associated with toe nail OM (P value 0.0071) (Table 3). Of the hundred OM cases, 80 were culture positive. NDM (47.5%) were the commonest etiological agent followed by yeast (33.8%) and dermatophytes (18.8%). (Figure 3) Amongst the NDMs, Aspergillus niger (8.8%),

Cladosporium species (8.8%) and Fusarium solani (8.8%) were the most prevalent fungi. Among the yeasts, Candida species (25%) accounted for most cases. Trichophyton rubrum (8.8%) was the commonest dermatophyte isolated (Table 4). The association of clinical types of OM and various etiological agents are depicted in Table 5. All the three groups of fungi isolated, were more commonly associated with the DLSO type.



Figure 2: A. Distal and lateral subungual OM (DLSO), B. Proximal subungual OM (PSO), C. White superficial OM (WSO), D. Total dystrophic OM (TDO).

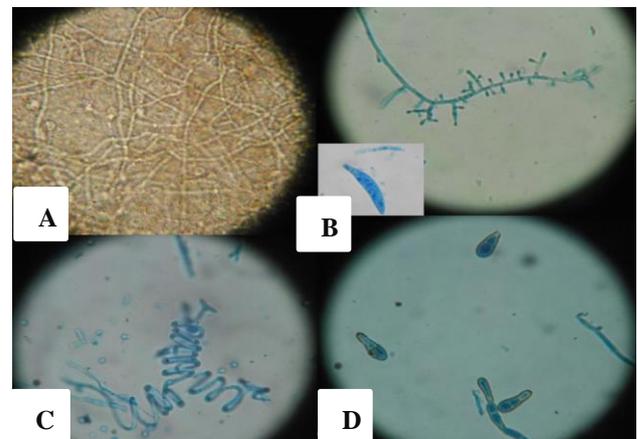


Figure :3 A. KOH mount showing dermatophyte hyphae (× 400), B. Teardrop shaped microconidia and macroconidia of *T. rubrum* (LCB×400), C. Spiral hyphae of *T. mentagrophytes* (LCB×400), D. Club shaped macroconidia of *E. floccosum* (LCB×400).

Table 3: Relationship of the clinical types with the type of the nail involved.

Clinical types	Total	Toe nail	Finger nail	Toe nail + finger nail	P value*
DLSO	51	29 (53.7%)	15 (41.7%)	7 (70%)	0.0071
PSO	25	10 (18.5%)	13 (36.1%)	2 (20%)	0.0889
WSO	18	13 (24.1%)	4 (11.1%)	1 (10%)	0.1717
TDO	6	2 (3.7%)	4 (11.1%)	0 (0%)	0.2127
Total	100	54 (100%)	36 (100%)	10 (100%)	-

* Fisher’s exact test: level of significance:P<0.05

Table 4: Etiological agents of onychomycosis.

Etiological agents	No (%), n=80
Non-dematophyte mould (NDM)	
<i>Aspergillus niger</i>	7 (8.8%)
<i>Cladosporium species</i>	7 (8.8%)
<i>Fusarium solani</i>	7 (8.8%)
<i>Penicillium species</i>	5 (6.3%)
<i>Chaetomium species</i>	3 (3.8%)
<i>Aspergillus flavus</i>	2 (2.5%)
<i>Scopulariopsis brevicaulis</i>	2 (2.5%)
<i>Aspergillus fumigatus</i>	2 (2.5%)
<i>Alternaria alternata</i>	1 (1.3%)
<i>Curvularia lunata</i>	1 (1.3%)
<i>Exophiala species</i>	1 (1.3%)
Yeast	
<i>Candida species</i>	20 (25%)
<i>Trichosporon beigelii</i>	7 (8.8%)
Dermatophytes	
<i>Trichophyton rubrum</i>	7 (8.8%)
<i>Trichophyton mentagrophytes</i>	4 (5%)
<i>Epidermophyton floccosum</i>	4 (5%)

Table 5: Relationship of clinical types with etiological agents.

Etiological agents	DLSO	PSO	WSO	TDO
NDM	17 (44.7%)	16 (42.1%)	2 (5.3%)	3 (7.9%)
Yeast	13 (48.1%)	7(25.9%)	6 (22.2%)	1(3.7%)
Dermatophyte	9 (7.9%)	0 (0%)	4 (26.7%)	2 (13.3%)

There was no significant correlation between the etiological agent and clinical type of OM, age and sex of the patient and involvement of the nail.

DISCUSSION

In the present study, maximum number of the cases (29%) belonged to the age group of 21-30 years. A similar higher prevalence in the same age group was reported by previous workers.²⁷⁻²⁹ Grover reported a higher prevalence among the age group 20-40 years.³⁰ But there are reports where 31-40 years was the most commonly affected age group.²⁷ Increased prevalence in younger age could be due to their frequent exposure to occupation related trauma predisposing to OM or may be due to their more cosmetic concern than the elderly. Low prevalence in children may be due to a difference in nail plate structure, lack of cumulative trauma, and increased growth rate of the nails with subsequent elimination of the fungus.³¹

A male preponderance found in the present study is similar to the findings reported by various authors.^{20,28-30,32} The males are more exposed to greater outdoor physical activity and hence, more prone to trauma.

Females were reported to be primarily affected in a study done by Jesudanam et al.²⁷ But there are reports depicting equal prevalence in male and female.^{11,33}

In the present study, trauma was the most common predisposing factor, followed by occlusive footwear. Various studies also found trauma to be the most common predisposing factor.^{20,27} Trauma causes distortion of the nails facilitating easy entry of fungus.³⁴ Toe nail was seen more commonly involved than the finger nails. Similar finding was also reported by Adhikari et al.²⁹ Increased prevalence in toe nail may be because of increased chances of occupation related trauma facilitating easy entry of fungus. Greater use of occlusive footwear may be another reason for toenail involvement. But, finger nails were seen to be more commonly involved as reported in studies done by Kaur et al, Jesudanam et al, Scher et al.^{11,27,35}

In the present study, toe nail was involved more frequently in males and finger nail in females which is comparable to other studies.³⁵ It may be the result of increased trauma and longer use of occlusive footwear in males compared to females and greater burden of household wet work using hands mostly in female results in more involvement of the finger nails.³⁶

Occupationally, farmers were most commonly involved which is conforming to other studies.²⁸ Reddy et al, reported highest prevalence amongst housewives.²⁰ Increased physical activity with trauma and maceration from wet work and contact with carbohydrates could probably explain the increased prevalence in farmers and housewives. The habit of working barefooted leading to exposure to soil saprophytes increases the burden of OM in farmers.

DLSO was the most common type of OM. The higher prevalence of DLSO pattern has been reported in various studies.³⁰⁻³² In the present study, DLSO was more commonly seen in toe nails, conforming to clinical patterns observed in other studies.²⁹

In the present study, NDM (47.5%) were the commonest etiological agents followed by yeast (33.8%) and dermatophytes (18.8%). Reports reflecting increasing trend in isolation of NDM from onychomycosis have come up recently.^{37,38} The hot and humid tropical and subtropical climate may be a factor responsible for the causative role of moulds. High rate of NDM could also be justified by their presence in the local environment of work and frequent exposure to soil saprophytes. However, the highest prevalence of individual etiologic agent was *Candida* species with a total of 20 isolates (25%). Similar findings were also reported in other studies done by Jesudanam et al and Efuntoye et al.^{27,39} Isolation rate of different etiologic agents were compared and observations were listed in Table 6.

Table 6: Prevalence of various etiologic agents causing onychomycosis.

Authors	Place	Year of study	Etiological agents		
			Dermatophyte	NDM	Yeast
Jesudanam et al ²⁶	Visakhapatnam	1998-99	38.2%	3.37%	56.74%
Agarwalla et al ²⁷	Nepal	2006	75%	3.8%	21.2%
Grover ²⁹	Bangalore, Jorhat	2003	23.7%	22.1%	16.9%
Barua et al ³⁰	Assam	2012	28.8%	56.6%	14.4%
Sujatha et al ³¹	Bangalore	1997-99	25.71%	28.57%	5.71%
Das et al ³²	Eastern India	2008	50%	22.72%	27.27%
Bokhari et al ³⁷	Pakistan	1999	43%	11%	46%
Efuntoye et al ³⁸	Nigeria	2002-06	26.4%	40.6%	22.2%

In this study, isolated organisms were found to be more commonly associated with the DLSO type conforming with other studies.^{28,38} As DLSO is much more commoner than the other types, etiologic agents are also more commonly seen with this type.

All the three groups of organisms i.e. NDM, yeasts and dermatophytes were most commonly isolated from toe nails. Barua et al, found NDM and dermatophytes to be common in finger nails and yeast in toe nails of green tea pluckers.³¹ Sujatha et al, isolated maximum no. of yeast from fingernails.³² Jahromi et al, found NDM to be more associated with toe nail.³⁷ Jesudanam et al, isolated *Candida* species predominantly from finger nails, while dermatophytes were isolated more frequently from the toe nails.²⁷

CONCLUSION

The present study found a high rate of NDMs as compared to other studies which can be justified by their presence in the local environment. This highlights the fact that the causative agents of OM differ according to geographic and ecological variations. Hence, focus should be given on understanding the mode of acquiring fungal infection, factors that enhance or facilitate growth and survival especially with respect to NDMs, which is becoming increasingly important as causative pathogen.

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