

## Original Research Article

# Clinico-mycological study of onychomycosis

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### ABSTRACT

**Background:** Onychomycosis is the most common infective nail disorder accounting for 30% of cutaneous mycoses. Though predominantly caused by dermatophytes, yeast and non-dermatophyte moulds have also been implicated. Aim of this study was to describe and analyse clinical and mycological pattern of onychomycosis.

**Methods:** One hundred patients with onychomycosis diagnosed by direct potassium hydroxide microscopy and culture were included. Nail specimens were collected for fungal culture as per standard procedure. An analysis of demographic data, clinical features and mycological results was made.

**Results:** Majority of the affected cases were between 19 to 85 years of age. Fingernail onychomycosis was seen in 41 patients, toenail was seen in 46 patients, concurrent involvement was seen in 13 patients. The predominant clinical pattern seen was distal lateral subungual type (70%). Culture was positive among 49% patients. Among these patients non dermatophyte growth was predominant (72%) showing *Aspergillus* species (31%) and *Fusarium* species (31%), *Cladosporium* species (4%) and *Scytalidium* species (2%). *Trichophyton* species (16%) and *Candida* species (16%) were also isolated. The clinico-etiological correlation revealed that a single pathogen could give rise to more than one clinical type of onychomycosis. Non-Dermatophyte moulds were the most common isolate followed by yeasts and dermatophytes. This is in contrast to earlier studies from a similar geographical region where dermatophytes were the commonest pathogen.

**Conclusions:** Non-dermatophyte moulds are emerging as the predominant isolate in onychomycosis. The clinical pattern of nail involvement showed no correlation with the fungal isolate.

**Keywords:** Non-dermatophyte moulds, Non dermatophyte onychomycosis, Onychomycosis, Dermatophytes

### INTRODUCTION

Onychomycosis is the most common nail infective disorder, caused by dermatophytes, non-dermatophytes and yeasts. It accounts for 30% of cutaneous fungal infections and 50% of nail disorders worldwide.<sup>1</sup> In most cases, this infection is caused by anthropophilic dermatophytes, in particular by *Trichophyton rubrum*, followed by *Trichophyton mentagrophytes* var *interdigitalis*. The non-dermatophyte moulds (NDMs) like *Fusarium spp* *Aspergillus spp*, can be involved in

onychomycosis as primary pathogens, as secondary pathogens or as contaminants.<sup>2</sup> Traditionally, dermatophytes have been predominantly attributed to cause onychomycosis. However, in the recent years, there has been a dramatic increase in non-dermatophyte onychomycosis (NDO), attributed to widespread use of broad-spectrum antibiotics, chemotherapy, and increase in elderly and immunocompromised population. The incidence of NDO is variable across the globe; it's isolation rates are low in Europe (5%) and United States (20%), however isolation of NDMs are higher in tropical

countries of Asia (40.5% to 68%) including India (13.6%-53.33%).<sup>3-9</sup>

Thus NDMs, previously considered as mere contaminants have emerged as potential pathogens in the tropical regions, in comparison to the traditional isolate of dermatophytes.<sup>10</sup> In the current study, the demographic data, clinical features and fungal spectrum of patients presenting with onychomycosis was reported. This study was designed to assess the most common pattern of onychomycosis and identify the causative agents.

## METHODS

The study was carried out in the outpatient department of St John's Medical College Hospital, Bangalore, India from September 2010 to September 2012. The climate of this place is tropical savanna type with distinct dry and wet seasons. The inclusion criteria were patients showing nail changes suggestive of Onychomycosis (OM) namely-onycholysis, onychodystrophy, subungual hyperkeratosis, thickening of the nail plate, Discolouration of the nail plate - melanonychia or leukonychia and paronychia which were positive for fungi on direct microscopy using potassium hydroxide. Patients whose nail clippings were negative for fungal elements on direct microscopy and those who had received antifungal treatment on the preceding one-month period were excluded.

Laboratory methods were as follows- the nail specimens were collected according to standard procedure, subjected to mycological study by direct microscopy using potassium hydroxide (KOH) 20% and lactophenol cotton blue preparation. Sabouraud dextrose agar along with chloramphenicol was used for culture. The culture medium was incubated at room temperature and was observed for fungal growth. Culture reading was done every day in the first week followed by every week in the following month. Based upon the colony morphology the fungus was broadly identified as yeast and mould. Yeasts were further identified by morphological tests which are germ tube test and chlamydospore fermentation and biochemical test i.e.; carbohydrate assimilation test and fermentation test. Moulds were further identified as dermatophytes and non-dermatophytes based on preliminary macroscopic and microscopic colony morphology using lacto phenol cotton blue.

Dermatophytes were grown on dermatophyte test medium and slide culture. Based on the size, number, shape and arrangement of conidia they are further identified as *Trichophyton*, *Epidermophyton* and *Microsporium*. Speciation of *Trichophyton* was done using trichophyton agar and invitro hair perforation tests. The moulds were identified to species level based on colony morphology and slide culture technique. The clinical and mycological findings were recorded and correlated. Data were analysed using SPSS statistics for Windows, version 17.0 (SPSS Inc, Chicago, IL, USA)

Chi-square test was the statistical method used for analysis of data.

## RESULTS

A total of 100 patients were enrolled in the study, whose age ranged from 19 to 85 years and the mean age was 43.86 years. Among the study patients 62(62%) were males and 38(38%) were females. Among the 62 males, 45% had toenail and 38% fingernail involvement. Among the 38 females, 47% and 44% had finger and toenail involvement respectively (Table 1).

The thumb and the great toe were most commonly involved in the upper and lower limb (Table 2).

**Table 1: Pattern of nail involvement in the study population.**

Nail involved	Male (%)	Female (%)	Both genders (%)
<b>Fingernail</b>	38	47	85
<b>Toenail</b>	45	44	89
<b>Both</b>	17	9	

**Table 2: Pattern of digital involvement in the study population.**

Fingernail	Males and females (%)	Toenail (%)	Males and females (%)
<b>Thumb</b>	56	Great toe	72
<b>Index</b>	14	Second toe	3
<b>Middle</b>	8	Third toe	5
<b>Ring</b>	4	Fourth toe	4
<b>Little</b>	18	Little toe	16

**Table 3: Distribution of Non-dermatophytes in the toenails and fingernails among males and females.**

Toenails	Great toe	2 <sup>nd</sup> toe	3 <sup>rd</sup> toe	4 <sup>th</sup> toe	5 <sup>th</sup> toe
<b>Males</b>	33%	22%	11%	22%	11%
<b>Females</b>	40%	40%	20%	20%	0
<b>Fingernail</b>	Thumb	Index	Middle	Ring	Little
<b>Males</b>	28%	16%	20%	16%	20%
<b>Females</b>	29%	0	14%	67%	0

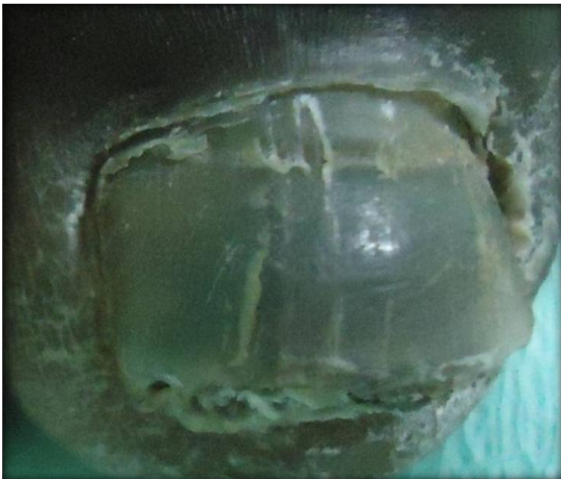
Distal and lateral subungual onychomycosis (DLSO) was the most common form of onychomycosis seen (70%) followed by, total dystrophic onychomycosis (TDO) 11%, mixed onychomycosis (MO) 11%, chronic paronychia (CP) 6%, subungual onychomycosis (SO)1% and proximal subungual onychomycosis (PSO)1% Among the 100 nail specimens cultured, 49% showed fungal growth. Among the culture positives, NDMs accounted for 72%, followed by 16% dermatophytes and 12% yeasts. Among the NDMs, the distribution was 31% *Aspergillus spp* (Figure 3), 31% *Fusarium spp*, 4%,

*Cladosporium spp*, 2% *Scytalidium spp* and 2% showed dual growth of *Aspergillus spp* and *Scytalidium spp*. Among dermatophytes, *Trichophyton rubrum* (Figure 4)

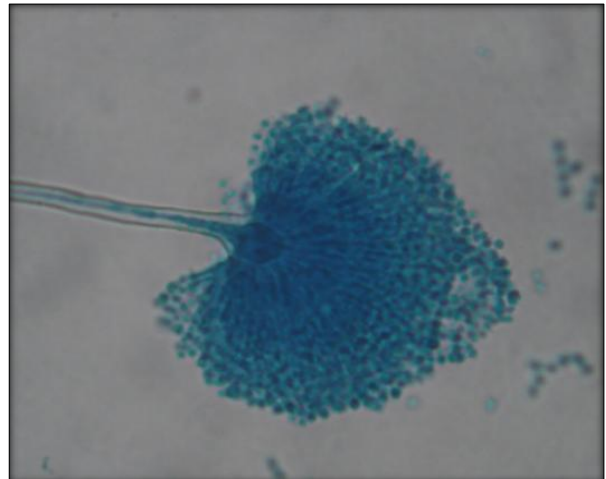
was the only isolate. Among yeasts, *Candida albicans* (Figure 5) was the only isolate.

**Table 4: Pattern of onychomycosis and organisms isolated.**

Organism isolated culture positive	Clinical pattern					
	DLSO	TDO	MO	CP	SO	PO
<i>Aspergillus spp</i>	10	2	2	0	1	0
<i>Aspg niger+Scytalidium</i>	0	0	1	0	0	0
<i>Candida species</i>	3	1	1	1		0
<i>Cladosporium species</i>	2	0	0	0		0
<i>Fusarium species</i>	10	2	2	1		0
<i>Scytalidium species</i>	2	0	0	0		0
<i>Trichophyton species</i>	5	1	2	0		0
Culture negative	38	5	2	4	0	0
<b>Total</b>	<b>70</b>	<b>11</b>	<b>11</b>	<b>6</b>	<b>1</b>	<b>1</b>



**Figure 1: Distal lateral subungual onychomycosis-scaling involving distal and lateral nail folds and subungual areas.**



**Figure 3: Conidiophore, vesicle and conidia of *Aspergillus spp* (40x).**

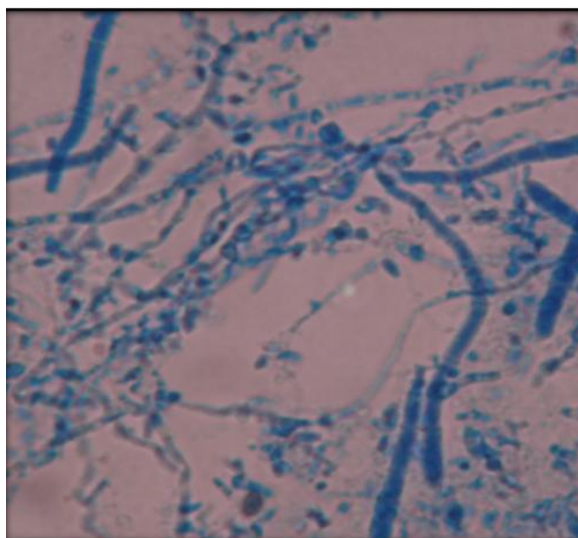


**Figure 2: Total dystrophic onychomycosis- total destruction of the nail plate.**

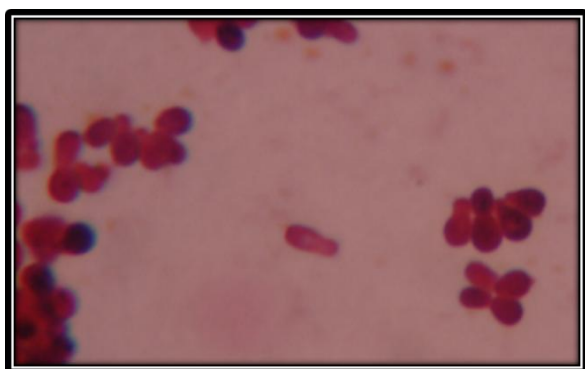


**Figure 4: Potassium hydroxide mount-showing septate hyphae.**





**Figure 5: Dermatophyte-macroconidia and microconidia of *Trichophyton rubrum* (40x).**



**Figure 6: Potassium hydroxide mount showing budding yeast cell of *Candida* spp.**

## DISCUSSION

Among the 100 cases of suspected onychomycosis the male to female ratio was 2:1. This result is comparable to Patwardhan and Perea et al.<sup>7,11</sup> This is probably attributable to outdoor occupation of males, subjecting their nails to higher chances of trauma. The majority of the studied patients were between the ages 18 and 40 yrs. (46%). The higher prevalence in this age group could be secondary to occupation-related trauma and health seeking behaviour. This is in accordance with other studies.<sup>7,12,13</sup> However in developed countries this entity is considered to be an age-related infection that is more prevalent among males in the older age groups.<sup>14,15</sup> A higher toe nail involvement compared to finger nail involvement (Table 1) was noted. Similar findings were noted among several other studies.<sup>4,8,14</sup> This is attributable to slow growth of the toenails, which facilitates the invasion of the fungus and is perhaps supported by factors such as trauma and poor circulation.

Female patients had predominant fingernail involvement (Table 2), attributable to frequent household work. This

result is agreeable with other studies.<sup>16</sup> Males had predominant toenail involvement (Table 2), these findings are consistent with other studies.<sup>4,7,11,15</sup> This is attributable to open footwear and exposure to soil saprophytes.

Among the fingernails, the thumb (Table 2) was frequently involved probably due to occupation related trauma and household work.<sup>7,16</sup> Among the toenails, the great toe (Table 2) was most commonly involved, possibly due to its greater size predisposing to more frequent trauma. Similar findings were observed by other authors.<sup>7,14</sup>

Fungal cultures were positive among 49% patients and negative among 51% patients. Similar isolation rates were noted by Patwardhan et al.<sup>7</sup> Among culture positive samples, 72% were NDMs, 16% Dermatophytes and 12% were Yeasts. Similar pattern of isolation was noted in recent studies.<sup>5,6,17,18</sup>

The presence of superficial fungal infection was seen among seven patients, majority of whom had tinea pedis. *T. rubrum* was most commonly isolated in this group, which is in accordance with previous studies.<sup>14,15</sup> The use of open footwear was seen among 41 patients among whom NDMs were predominantly isolated. This is attributed to the minimal protection that open footwear offers against the external environment which exposes the nail to soil saprophytes. Similar results were noted in several studies.<sup>17,18</sup>

The common clinical pattern of onychomycosis seen was DLSO, followed by TDO, MO, CP, SO and PSO. This finding is in accordance with reports by Garg et al, and Patwardhan et al.<sup>7,13</sup>

The isolation pattern of the organisms varied between clinical patterns of OM (Table 4). Thus clinico- etiologic correlation reveals that a single pathogen could give rise to more than one clinical type of OM. Conversely. a given clinical pattern of onychomycosis can be caused by different organisms. Similar findings were noted by other authors.<sup>19-22</sup> Interestingly, periungal inflammation in association with nail dystrophy was a finding noted among OM secondary to NDMs. This finding was noted in several other studies too.<sup>15-17</sup>

A high isolation rate of NDMs were noted in our study (72%). Similar high isolation rates are reported from Malaysia (45.4%), Pakistan (68%) Teheran (40.5%), Thailand (51.6%) and Sri Lanka (45.8%).<sup>6,18,23-25</sup> This is in contrast to studies done in temperate countries, where the isolation rates of NDMs varied from 1.49% and 33.5%.<sup>26,27</sup> This variation is a result of geographic differences in mould distribution and humidity. In addition, use of broad-spectrum antibiotics, immunosuppression, occupational trauma and aging population predispose the nails to the invasion of NDMs. Among NDMs the isolation rates of various species vary

considerably in different studies. The most common organisms isolated worldwide are *Scopulariopsis brevicaulis*, *Scytalidium dimidiatum*, *Fusarium spp*, *Aspergillus spp*, and *Acremonium spp*. The highest isolation rates were of *Aspergillus spp* (31%) and *Fusarium spp* (31%) is in accordance to recent case reports and epidemiological studies.<sup>5,26-30</sup>

Although study is not a comprehensive epidemiological survey this study demonstrated that 29.3% of onychomycosis cases are due to NDMs, which is 3 times more than the isolation found in the last study conducted in Bangalore, India.<sup>12</sup> The fact that non-dermatophyte moulds is the most common pathogen implicated for onychomycosis in this population, poses a challenge as its response to oral antifungal is unpredictable. The current available antifungal agents are more effective against dermatophytes and yeasts. Avulsion of the effected nail and treatment with oral antifungal may be the best available option in cases of infection with non-dermatophyte moulds.

## CONCLUSION

A single pathogen could give rise to more than one clinical type of OM and a given clinical pattern of onychomycosis can be caused by different organisms. The presence of paronychia in association with OM is suggestive of NDM etiology. Although, it is difficult to ascertain the role of NDMs as primary pathogen in deformed nail they should be considered as important pathogens, when evaluating patients with cultures that are negative for dermatophytes, or in those experiencing treatment failures. The results of this study would be very valuable for the dermatologists working in this area, and further epidemiological investigations should be performed in every country in order to determine the fungal species associated with OM.

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## REFERENCES

- Williams HC. The epidemiology of onychomycosis in Britain. *Brit J Dermatol.* 1993;129(2):101-9.
- Elewski BE. Onychomycosis: pathogenesis, diagnosis, and management. *Clini Microbiol Rev.* 1998;11(3):415-29.
- Tosti A, Piraccini BM, Lorenzi S. Onychomycosis caused by nondermatophytic molds: clinical features and response to treatment of 59 cases. *J Am Acad Dermatol.* 2000;42(2):217-24.
- Ghannoum MA, Hajjeh RA, Scher R, Konnikov N, Gupta AK, Summerbell R, et al. A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. *J Am Acad Dermatol.* 2000;43(4):641-8.
- Motamedi M, Ghasemi Z, Shidfar MR, Hosseinpour L, Khodadadi H, Zomorodian K, et al. Growing incidence of non-dermatophyte onychomycosis in Tehran, Iran. *Jundishapur J Microbiol.* 2016;9(8).
- Farwa U, Abbasi SA, Mirza IA, Amjad A, Ikram A, Malik N, et al. Non-dermatophyte moulds as pathogens of onychomycosis. *J Coll Physicians Surg Pak.* 2011 Oct 1;21(10):597-600.
- Veer P, Patwardhan NS, Damle AS. Study of onychomycosis: Prevailing fungi and pattern of infection. *Ind J Med Microbiol.* 2007;25:53-6.
- Lone R, Bashir D, Ahmad S, Syed A, Khurshid S. A Study on Clinico- Mycological Profile, Aetiological Agents and Diagnosis of Onychomycosis Government Medical College Hospital in Kashmir. *J Clini Diagnostic Res: JCDR.* 2013;7(9):1983-5.
- 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 Clini Microbiol.* 2016;18(1):36-9.
- Gianni C, Cerri A, Crosti C. Non-dermatophytic onychomycosis. An underestimated entity? A study of 51 cases. *Mycoses.* 2000;43(1-2):29-33.
- S, Ramos MJ, Garau M, Gonzalez A, Noriega AR, del Palacio A. Prevalence and risk factors of tinea unguium and tinea pedis in the general population in Spain. *J Clin Microbiol.* 2000;38(9):3226-30.
- Grover S. Clinico-mycological evaluation of onychomycosis at Bangalore and Jorhat. *Ind J Dermatol Venereol Leprol.* 2003;69:284-6.
- Garg A, Venkatesh V, Singh M, Pathak KP, Kaushal GP, Agrawal SK. Onychomycosis in central India: A clinico-etiological correlation. *Int J Dermatol.* 2004;43:498-502.
- Jain S, Sehgal VN. Onychomycosis: an epidemiological perspective. *Int J Dermatol.* 2000;39:100-3.
- Cheng S, Chong L. A prospective epidemiological study on tinea pedis and onychomycosis in Hong Kong. *Chin Med J.* 2002;115(6):860-5.
- Gelotar P, Vachhani S, Patel B, Makwana N. The prevalence of fungi in fingernail onychomycosis. *J Clini Diagnostic Res.* 2013;7(2):250.
- Bokhari MA, Hussain I, Jahangir M, Haroon TS, Aman S, Khurshi K. Onychomycosis in Lahore, Pakistan. *Int J Dermatol.* 1999;38:591-5.
- Leelavathi M, Tzar MN, Aawiah J. Common Microorganisms Causing Onychomycosis in Tropical Climate. *Sains Malaysiana.* 2012;41(6):697-700.
- Das NK, Ghosh P, Das S, Bhattacharya S, Dutta RN, Sengupta SR. A study on the etiological agent

- and clinico-mycological correlation of fingernail onychomycosis eastern India. Ind J Dermatol. 2008;53(2):75.
20. Rafiq AN, Zakaria AS, Khondker L, Khan MS, Doulah S, Hazra SC. Clinico-mycological correlation in onychomycosis in a tertiary level hospital. J Pak Assoc Dermatol. 2016;15(23):277-83
  21. Yuil R, Manuel J, Castro MR. Clinico-etiological correlation and factors associated with onychomycosis. Dermatología Cosmética, Médica y Quirúrgica. 2011;9(3):221-7.
  22. Kaur R, Kashyap B, Bhalla P. Onychomycosis - epidemiology, diagnosis and management. Ind J Med Microbiol. 2008;26:108-16.
  23. Ungpakorn R, Lahaprathan S, Reangchainam S. Prevalence of foot diseases in outpatients attending the Institute of dermatology, Bangkok, Thailand. Clin Exp Dermatol. 2004;29:87-90.
  24. Bassiri-Jahromi S, Khaksar AA. Nondermatophytic moulds as a causative agent of onychomycosis in Tehran. Indian J Dermatol. 2010;55:140-3.
  25. Ranawaka RR, de Silva N, Ragunathan RW. Non-dermatophyte mold onychomycosis in Sri Lanka. Dermatol Online J. 2012;18(1):7.
  26. Bonifaz A, Cruz-Aguilar P, Ponce RM. Onychomycosis by molds. Report of 78 cases. Eur J Dermatol. 2007;17(1):70-2
  27. Moreno G, Arenas R. Other fungi causing onychomycosis. Clin Dermatol. 2010;28(2):160-3.
  28. Ahmadi B, Hashemi SJ, Zaini F, Shidfar MR, Moazeni M, Mousavi B, et al. A case of onychomycosis caused by *Aspergillus candidus*. Med Mycol Case Rep. 2012;1(1):45-8.
  29. Zarei F, Mirhendi H, Fakhim H, Geramishoar M. The first case of onychomycosis due to *Aspergillus uvarum*. Mycoses. 2015;58(4):239-42.
  30. Nouripour-Sisakht S, Mirhendi H, Shidfar MR, Ahmadi B, Rezaei-Matehkolaei A, Geramishoar M, et al. *Aspergillus* species as emerging causative agents of onychomycosis. J Mycol Med. 2015;25(2):101-7.

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