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Microbiology

A STUDY ON THE MYCOLOGICAL PROFILE OF ONYCHOMYCOSIS IN A TERTIARY CARE HOSPITAL IN NORTHEAST INDIA.

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ABSTRACT

BACKGROUND: Onychomycosis refers to fungal infection of nails with various etiological agents, involving dermatophytes, yeasts and moulds. It constitutes an important health problem because of its rising prevalence and under-diagnosis especially in developing countries.

AIMS: To study the isolation rate and mycological profile of onychomycosis.

SETTINGS AND DESIGN: Nail samples collected from patients attending the dermatology clinic were processed in the microbiology department of our institute

MATERIALS AND METHODS: Nail clippings and subungual scrapings of patients with onychomycosis were subjected to KOH preparation. Culture was done on Sabouraud's dextrose agar medium (SDA), SDA with 5% Chloramphenicol and Cycloheximide and Dermatophyte agar. Species identification was done by colony characteristics, pigment production, slide culture and LPCB stain.

RESULTS: Out of 187 cases, isolation rate of onychomycosis was found to be 60.42%. Dermatophyte (40.7%) was the most common isolate followed by yeast (34.51%) and NDM (Nondermatophytic mould) (24.78%). *Trichophyton* was the only dermatophyte that was isolated. Amongst the *Trichophyton* which could be speciated it was *Trichophyton rubrum* which accounted for majority of infection. *Candida parapsilosis* was the predominant yeast and *Aspergillus niger* was the commonest mould. The age group most commonly affected was 16-30yrs and males were commonly affected in our study.

CONCLUSION: The present study highlights the role of Non albicans candida and uncommon NDM apart from dermatophytes in onychomycosis and the need for microbiological confirmation in case of onychomycosis for appropriate management of onychomycosis cases and further epidemiological study.

KEYWORDS : Onychomycosis, Deramtophytes, *Trichophyton rubrum, Candida parapsilosis*

INTRODUCTION:

The term onychomycosis is derived from the Greek word "onyx", a nail and "mykes" a fungus[1]. "Onychomycosis" traditionally referred to as nondermatophytic infection of nail is now used as a general term to denote any fungal nail infection[2]. It is defined as the fungal infection of nails caused by dermatophytes, yeasts and nondermatophyte moulds. It is one of the commonest nail disorders and accounts for upto 30% of all superficial fungal infections [3].

Common clinical features include discoloration of the nail plate, hyperkeratosis and brittle nails. Although not life threatening, onychomycosis may have significant clinical consequences such as secondary bacterial infection, chronicity, therapeutic difficulties and disfigurement in addition to serving as reservoir of infection[2].

This infection can be caused by dermatophytes, yeasts and nondermatophyte moulds. Certain skin conditions such as psoriasis, lichen planus, onychogryphosis and nail trauma can mimic onychomycosis. Hence laboratory investigations are needed to differentiate accurately between fungal infections and the above mentioned skin diseases[4].

The present study was conducted to study the morphological patterns and to analyse the mycological and cultural characteristics of onychomycosis with respect to various etiological agents.

MATERIALS AND METHODS:

This was a cross sectional analytical study carried out on all clinically suspected cases of onychomycosis presenting to the dermatology OPD over a period of Jan 2015 – June 2017.

SPECIMEN COLLECTION:

Specimen collected were :- Nail and subungual scrapings from suspected cases of Onychomycosis.

First the affected area was cleaned with 70% ethanol. Nail and subungual scrapings were collected with a surgical blade in sterile petri dishes in microbiology department. The samples were subjected to microscopic examination and culture. The nail samples were subjected to 20% potassium hydroxide (KOH) examination and the softened nail materials were examined under both low and high power of the microscope for the presence of fungal elements. The details regarding the hyphae, spores, budding cells and pseudo-hyphae were noted. For culture all the samples were inoculated on:

1) SDA (Sabouraud's dextrose agar)
2) SDA with 5% Chloramphenicol and Cycloheximide
3) DA (Dermatophyte agar)

Cultures were incubated at 25degC and 37degC for 6 weeks and examined daily for the growth. The identification of isolate from the growth was done on the basis of colony morphology and wet mount microscopy with lactophenol cotton blue stain and slide culture technique. The morphological characteristics of the colony such as colour of the colony, type of the growth whether fluffy, cottony or creamy and the pigment produced on reverse were carefully observed and noted. For wet mount the material was taken from the growth with a wire loop and placed in a drop of lactophenol cotton blue stain on the glass slide. The material was evenly teased with a teasing needle known as 'spud needle' and observed under both low and high power of microscope. The details about the hyphae, the type of conidia and their arrangement were observed and recorded.[4,5]

The dermatophytes and nondermatophytic moulds were confirmed by slide culture technique. The following criteria were taken into consideration to consider nondermatophyte mould as pathogen [5]:

1) A Direct positive mycological examination presenting large and irregular septate hyphae
2) Growth of the same agent in pure culture in all three culture tubes
3) No development of dermatophytes.

The *Candida species* were identified by gram stain, germ tube test and chrom agar.

RESULTS : A total of 187 patients (83 Females and 104 males) were examined during the study period. Males were affected more than females. Majority were in the age group of 16 – 30yrs which accounted for 39.04% of the cases. Least affected age group was 5-15 accounting for about 2.67% of the cases. (Table 1, Fig 1 & Table 2, Fig 2).

Table 1 : Sex distribution in patients of onychomycosis

SEX	NO OF CASES	PERCENTAGE
Male	104	55.6%
Female	83	44.4%
Total	187	100%

Fig 1: Distribution of sex in terms of percentage

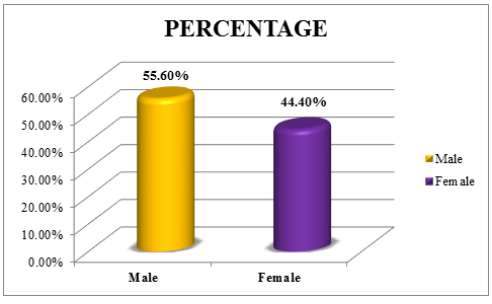


Table 2 : Age distribution in patients of onychomycosis

Age group (in years)	No of cases		Total	percentage
	Male	Female		
05 – 15	3	2	5	2.67%
16-30	40	33	73	39.04%
31-45	33	26	59	31.55%
46-60	16	15	31	16.58%
61-75	13	6	19	10.16%

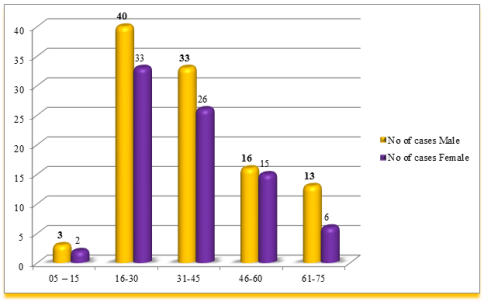


Fig 2: Bar diagram showing Age distribution

Out of 187 cases direct microscopy for fungal elements was positive only in 69 cases whereas fungal culture was positive in 113 cases (Table 3). Out of 113 isolated fungi, the most frequently isolated fungus was Dermatophytes (Table 4).

Table 3: Direct microscopy (KOH preparation) vs fungal culture in the diagnosis of onychomycosis (n=187)

Diagnostic tests	KOH positive	KOH negative	Total
Fungal culture positive	52	61	113
Fungal culture negative	17	57	74
Total	69	118	187

Fig 3 : Bar diagram showing comparision of Direct microscopy (KOH preparation) and Fungal culture.

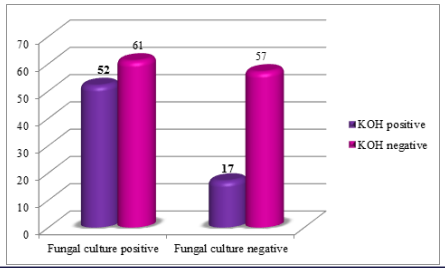


Table 4: The different categories of fungi isolated from onychomycosis cases with their comparative percentage of occurrence

Type of fungi	No of positive cases (n= 113)	Percentage
Dermatophytes (46)		
Trichophyton species	26	23%
T. rubrum	10	8.85%
T. tonsurans	4	3.54%
T. verrucosum	4	3.54%
T. schoenleinni	1	0.88%
T. soudanense	1	0.88%
Yeast (39)		
Candida parapsilosis	18	15.92%
Candida albicans	10	8.85%
Candida tropicalis	7	6.19
Candida glabrata	3	2.65%
Candida kefyr	1	0.88%
Moulds (28)		
Aspergillus niger	6	5.31%
Dematiceous fungi	4	3.54%
Cladosporium species	3	2.65%
Penicillium species	2	1.77%
Nigrospora	2	1.77%
Aspergillus fumigatus	1	0.88%
Acremonium species	1	0.88%
Chaetomium species	1	0.88%
Curvularia spp	1	0.88%
Fusarium species	1	0.88%
Monilia species	1	0.88%
Onychocola canadensis	1	0.88%
Paecilomyces spp	1	0.88%
Scedosporium spp (pseudoallescheria spp)	1	0.88%
Scopulariopsis spp	1	0.88%
Scytalidium species	1	0.88%

FIG 4: Distribution of Dermatophytes

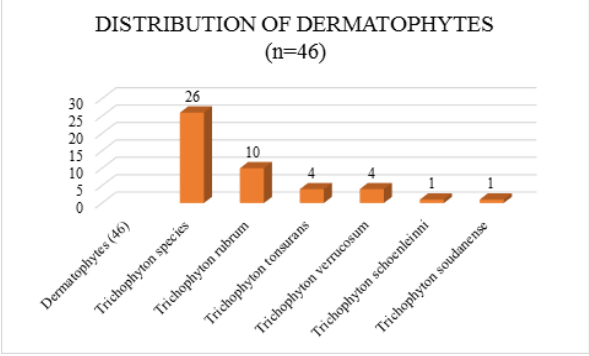


FIG 5: Distribution of yeast

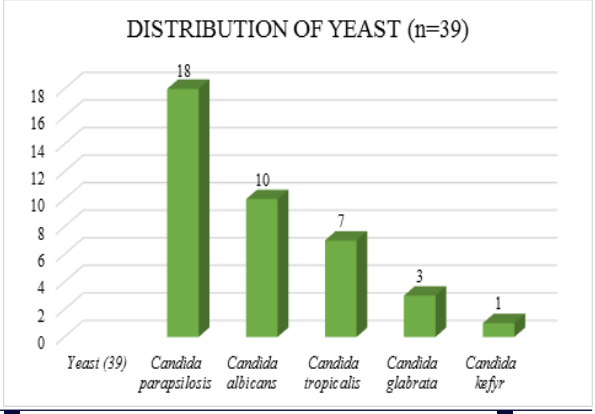
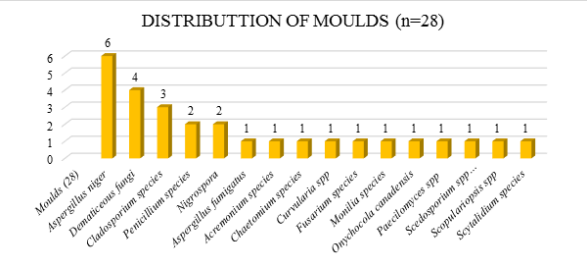


FIG 6: Distribution of moulds



DISCUSSION

The importance of onychomycosis is often underestimated. Far more than being a simple cosmetic problem, infected nails serve as a chronic reservoir of infection which can give rise to repeated mycotic infections of the skin[4]

Recently, there had been a noticeable worldwide increase in the incidence of onychomycosis. This has been related to a variety of etiological factors, including the rise in immunocompromised patients, an aging worldwide population and a rise in environmental risk factors secondary to life style changes[6,7]There is an increase in the spectrum of causative pathogens causing infections[6]

Though onychomycosis occurs worldwide, isolation rate varies. In our study isolation rate of onychomycosis was found to be 60.42%. This was in concordance with some studies conducted in India which showed isolation rate of 59.58%[8], 59.18%[4] and 64.34%[9] respectively. There were studies which recorded high isolation rate of 94.12%[6], 71.6% [10], 85.44% [11], 81.1%[12], 70.83%[13] and some low isolation rate 39.5%[2], 40% [7]. This variation, may be because many people do not seek medical advice for nail infections and also due to difference in the climatic condition of different regions[8].

In our study the commonest age group affected was 16-30yrs. High prevelance of onychomycosis in this age group has also been found in other studies by Venkatesh et al [13], Golia S et al [4], Lungran P et al[11], Ahuja S et al [2]and Adhikari L et al [6].This increased incidence in the younger population could be because they are more often exposed to occupation-related trauma, predisposing them to onychomycosis and in addition they may also be cosmetic conscious than the older age group thereby seeking medical care [6].

Furthermore in our study, Males (55.6%)were affected more than females(44.4%).A higher isolation rate in males worldwide may be due to common use of occlusive footwear, more exposure to outdoor conditions, and increased physical activity, leading to an increased likelihood of trauma. The importance of trauma to the nails as a predisposing factor for onychomycosis is well established [7]

In our study dermatophytes was the most common group followed by Yeast and then non dermatophytic moulds in the etiology of onychomycosis. Similar findings was observed in various other studies as well [2,4,10,13].

Trichophyton species was the only Dermatophyte that was isolated in our studies. There were 46 *Trichophyton species* out of which 26 *Trichophyton* could not be speciated and among the one which we could speciate, it was *Trichophyton rubrum* which accounted for the majority of the cases. In Some studies too it has been reported as most prevelant pathogen in onychomycosis[4,13].

Non-dermatophytic moulds (NDM) and yeasts are gradually emerging as an important etiological agent in onychomycosis. In our study Yeast was the second most common isolate. Golia S et al [4], Venkatesh et al[13], Adekhandi S et al[10] too reported yeast as the second most common isolate. Among the yeast *Candida parapsilosis* was most common and was isolated in 18 cases (46.15%) followed by *Candida albicans* (17.94%). Other non albicans candida species like *Candida tropicalis* (17.94%), *Candida glabrata*(7.69%) and *Candida kefyr* (2.56%) were also isolated as causative agents of onychomycosis. These findings correlates with the study of kaur et al[1] and Shanimole P et al[8].

Nondermatophytic moulds was isolated in 28 cases of onychomycosis. *Aspergillus niger* was the most common non dermatophytic mould in our study. Golia S et al [4], Adhikari L et al[6] , Venkatesh VN et al[13], Adekhandi S et al[10] and Lungran P et al [11] too reported *Aspergillus niger* as the most common nondermatophytic mould responsible for onychmycosis. Various other uncommon NDM were also isolated. Though few cases but still this highlights that even uncommon NDM are also emerging as an important etiological agents. This finding was in concordance with the study in Manipur by Lungran P et al[11].

Table 5 : Various studies carried out to find out the prevelance & etiology of onychomycosis

Year	Study by	Duration of study	Total sample	Isolation rate	Most common age and sex	Most frequently isolated fungus group	Second most common group	Third most common group
2006	Adhikari L et al Sikkim [6]	2 year	34	32(94.12%)	21-30/M	Dermatophyte (18)*Trichophyton tonsurans*(44.44%)	Mould (10) *Aspergillus niger* (21.43%)	No yeast was isolated
2008	Ahuja S et al Delhi [2]	2 years	276	39.5%	21-30/M	Dermatophyte (39) *Trichophyton spp*(43.75%)	Yeast (18) *Candida spp*(11.94%)	Nondermatophytic moulds (23) *Aspergillus spp* (20%)
2009	SS Beena et al Kashmir [7]	3 year	95	38 (40%)	21-40/F	Dermatophyte(20)*Trichophyton mentagrophytes* (42.10%)	Mould (17) *Fusarium species* (13.15%)	Yeast (1) *Candida albicans* (2.63%)
2010	Adekhandi S et al [10] Uttarakhand	1 year	134	96 (71.6%)	20-40/M	Dermatophyte(56) *Trichophyton mentagrophytes*(20.90%)	Yeast (28) *Candida albicans*(11.94%)	Nondermatophytic moulds (12) *Aspergillus niger* (5.97%)
2010	Lungran P et al [11] Manipur	3 year	467	399(85.44%)	21-30/F	Dermatophyte (250)(54.58%)	Nondermatophytic moulds (176) *Aspergillus spp* (24.89%)	Yeast (33) *Candida spp*(6.77%)
2010	Shanimole P et al [8]	5 years	250	143(59.58%)	30-39/F	Nondermatophytic moulds (56) *Aspergillus niger* (67.8%)	Dermatophyte (52) *Trichophyton mentagrophytes* (53.8%)	Yeast (35) *Candida albicans*(74.3%)
2011	Samaddar D et al Kolkata [12]	1 year	106	81.1%	31-40/M	Dermatophyte (66) *Trichophyton mentagrophytes* (21.2%)	Nondermatophytic moulds (12) *Aspergillus Fumigatus* (3.7%)	Yeast (8) Candida spp(7.5%)
2012	Golia S et al Bangalore [4]	6 months	98	58(59.18%)	16-30/M	Dermatophyte (40) *Trichophyton rubrum* (43.84%)	Yeast (17) *Candida albicans* (22.30%)	Nondermatophytic moulds (6) *Aspergillus niger* (16.44%)

Year	Study by	Duration of study	Total sample	Isolation rate	Most common age and sex	Most frequently isolated fungus group	Second most common group	Third most common group
2014	Asifa N et al Kashmir [9]	1 year	129	83 (64.34%)	31-40/F	Yeast (46) *Candida albicans* (54.34%)	Dermatophyte (34) *Trichophyton mentagrophytes* (47.05%)	Nondermatophytic Mould (3) *Alternaria species* (3.61%)
2014	Venkatesh VN et al [13] Karnataka	1 year	168	119 (70.83%)	16-30/F	Dermatophyte (98) *Trichophyton rubrum* (30.25%)	Yeast (13) *Candida species* (10.02%)	Nondermatophytic moulds (8) *Aspergillus niger* (3.36%)
2015	Present study	2 year 7 months	187	113 (60.42%)	16-30/M	Dermatophyte (46) *Trichophyton species* (23%)	Yeast (39) *Candida parapsilosis* (15.92%)	Nondermatophytic moulds (28) *Aspergillus niger* (5.31%)

CONCLUSION

The present study shows that *Trichophyton species* is the commonest etiological agent of onychomycosis. However Yeast and nondermatophytic moulds are also emerging as important etiological agent of onychomycosis in our geographical area. The study also highlights that non albicans candida and uncommon NDM are also emerging as an important etiological agents. Various such studies has to be conducted to find out the prevalence of all these uncommon but important pathogens. Though onychomycosis is mainly a cosmetic problem, mycological study is necessary. Without proper diagnosis and treatment, onychomycosis becomes chronic and more difficult to treat. Triazole group of antifungals as fluconazole, are frequently prescribed by the clinicians are ineffective against dermatophytes. Hence proper diagnosis of the etiological agent of onychomycosis is necessary for targeted treatment and also to differentiate from other nail disorders.

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