Study of Clinico-mycological profile of onychomycosis at a tertiary care hospital

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ABSTRACT

Introduction: 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 at a tertiary care hospital in Karimnagar.

Materials and Methods: The study was conducted at Department of Microbiology for a period of one year. 109 patients clinically diagnosed as OM were subjected to detailed history, clinical examination and investigations. Nail clippings and subungual debris of OM patients were subjected to KOH preparation and culture. Isolates were identified by colony characters, pigment production, microscopic morphology.

Results: The maximum number of OM cases belonged to the age group 21-30 years with a male preponderance. Higher incidence was noted in males (71.5%) than females (28.4%). Direct microscopy of the nail clippings in 40% KOH solution was positive in 56.8% and culture was positive in 75.2% cases. The common etiological agent was dermatophytes (45.12% cases) followed by yeasts (34.14% and non dermatophyte molds (20.7% cases). *T.rubrum* was the commonest among dermatophytes and *Candida albicans* among the yeasts.

Conclusions: Although onychomycosis is not life threatening, it can cause a significant negative impact on the quality of life of infected patients. Early diagnosis and treatment is important to prevent distortion of the nails which can prevent loss of working efficiency of the commonly affected population.

KEYWORDS: Onychomycosis, Dermatophytes, Nondermatophyte molds

INTRODUCTION:

Onychomycosis is fungal infection of the nails that causes discoloration, thickening, and separation from the nail bed, caused by dermatophyte fungi, non-dermatophyte fungi or yeast. It 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] There has been a worldwide increase in the incidence of onychomycosis with social, cultural and economical factors contributing to it. In developing countries, higher priorities in socioeconomic concerns and health issues for other diseases, have resulted in low awareness of onychomycosis. Though there is a clearly diseased appearance associated with this condition, it is often regarded as merely a cosmetic problem of relatively minor importance. ^[4]Several factors implicated to the increase in disease are reduced peripheral circulation, diabetes, nail trauma and improper nail hygiene.^[5] 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. Common clinical features include discoloration of the nail plate, hyperkeratosis and brittle nails. ^[6]Certain skin conditions such as psoriasis, lichen planus, onychogryphosis and nail trauma can mimic onychomycosis. ^[6] Hence laboratory investigations are needed to differentiate accurately between fungal infections and the above mentioned skin diseases. In India relatively less work has been done on onychomycosis as compared to western countries. The evolving role of nondermatophytic moulds has added a new dimension to the ountries. The evolving role of nondermatophytic moulds has added a new dimension to the clinical patterns of onychomycosis.

OM is caused by three groups of fungi, namely dermatophytes, nondermatophytic moluds and yeasts. Dermatophytes cause 90% of toenails and 50% of the fingernail onychomycosis. Previously regarded as contaminants, yeasts are now increasingly recognized as pathogens in fingernail infections, as are some moulds.^[7]

Common Fungal Agents Associated with Onychomycosis: $\ensuremath{^{[7]}}$

1.Dermatophytes: Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum

2. Nondermatophyte fungi: Acremonium species, Alternaria species, Aspergillus species, Fusarium species, Onycochola Canadensis, Geotrichum candidum, Cladosporium carrionii

3.Yeast: Candida albicans

Clinical Presentation and Patterns of Fungal Invasion in Onychomycosis:

Distal subungual onychomycosis : The most common variety of onychomycosis, distal lateral subungual onychomycosis (DLSO), is characterized by invasion of the nail bed and underside of the nail plate. It is best described as 'nail bed dermatophytosis'. The infecting organism migrates proximally through the underlying nail matrix. Mild inflammation develops, resulting in focal parakeratosis and subungual hyperkeratosis, with two consequences: onycholysis and subungual thickening. The nail bed becomes cornified and normal nail contour is lost. The thickened horny layer raises the free edge of the nail plate with disruption of the nail plate to nail bed attachment. DLSO may develop on the fingernails, toenails or both.

Proximal subungual onychomycosis : This is also known as proximal white subungual onychomycosis (PWSO) or proximal subungual onychomycosis (PSO). A relatively uncommon subtype, PSO occurs when organisms invade the nail unit via the proximal nail fold through the cuticle area, penetrate the newly formed nail plate and migrate distally resulting in subungual hyperkeratosis, proximal onycholysis, leukonychia and destruction of the proximal nail plate.

Fungal invasion of the proximal nail fold is often visible through the cuticle as a whitish yellow discolouration while the distal nail unit remains normal. Periungual inflammation may be quite marked and painful and in some cases associated with purulent discharge.

White superficial onychomycosis: White superficial onychomycosis (WSO); a less common variety, is a distinctive pattern in which the nail plate is the primary site of invasion. This is the surface infection of the nail primarily when the fungi invade the superficial layers of the nail plate directly. It can be recognized by the presence of well-delineated opaque, white islands on the external nail plate, which coalesce and spread as the disease progresses resulting in a rough, soft and crumbly appearance of the nails.

Total dystrophic onychomycosis (TDO) : In this type there is total destruction of the nail plate, which usually may

be the end result of the any of the four main patterns of onychomycosis. The entire nail unit becomes thick and dystrophic.

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 one year from January 2019 to December 2019.

SPECIMEN: 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 and sent in sterile petri dishes to Microbiology department.

The samples were subjected to microscopic examination and culture. The nail samples were subjected to 40% 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) Sabouraud's dextrose agar

(2) Sabouraud's dextrose agar with 5% chloramphenicol and cycloheximide.

(3) Dermatophyte test medium

Cultures were incubated at 25^0 C and 37^0 C for 6 weeks and examined daily for the growth.

Identification of isolate from the growth was done on the basis of colony morphology and wet mount microscopy with lactophenol cotton blue (LPCB) stain and slide culture technique. The morphological characteristics of the colony such as colour of the colony, type of the growth 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 teasing needles 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. Dermatophytes and nondermatophytic moulds were confirmed by slide culture technique.

The following criteria were taken into consideration to consider nondermatophyte mould as pathogen ^[8]:

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.

RESULTS:

Out of 158 cases of nail infection, 109(68.98%) samples were diagnosed to have Onychomycosis. Among 109 cases 78(71.5%) were males and 31(28.4%) were females, with a male to female ratio 2.5:1. Table 1 Out of 109 samples with OM, 62(56.8%) were positive for fungal elements through direct microscopy Figure 2, and 82(75.2%) were positive for fungal culture Table 3. Majority of patients 40 (36.6%) were between age groups 21-30 years followed by 31-40 years accounting 31 (28.4%), remaining patients fall in other age groups, 4(3.6%) are below 20 years and 34(31.2%) are from 40-70 years Table 2. Majority of patients with OM were agricultural workers accounting 38(34.8%), followed by housewives 22(20.1%) and remaining others accounted 49(44.9%).

In 61(55.96%) patients fingernails were infected and toe nails were infected in 37(33.9%) patients and both fingers and toenails were involved in 12(11%) patients. Distal lateral subungual onychomycosis (DLSO) is the commonest presentation in 56(51%) patients, followed by Proximal subungual onychomycosis (PSO) 29(26.6%) (Pic:1), Total dystrophic onychomycosis (TDO) was seen in 9(8.2%) and White superficial onychomycosis (WSO) in 13 (11.9%) patients.

Most commonly isolated fungi are dermatophytes 37(45.12%) followed by yeasts 28(34.14%) and by non dermatophyte molds 17(20.73%) (Table:4). Among the dermatophytes isolated, *Trichophyton rubrum* is the commonest isolate accounting 19(51.35%), 6(16.21%) were *Trichophyton mentagrophytes*, 5(13.51%) were *Epidermophyton floccosum* (Pic:3), and 2(5.4%) each were *Trichophyton tonsurans* and *Trichophyton verrucosum*. Among the isolated yeasts *Candida albicans* 17(60.71%) was the predominant species followed by *Candida parapsilosis* 5(17.85%), 3(10.71%) each of *Candida glabrata* and *Candida tropicalis*. *Aspergillus niger* 7(41.17%) was the commonest non-dermatophyte mold, followed by 4(23.52) *Fusarium spp* and 2(11.76%) *Cladosporium spp*. Table 5

Gender	No.of Cases	Percentage(%)
Males	78	71.5%
Females	31	28.4%
Total	109	100%



Age group	No of cases	Percentage(%)	
Below 20 yrs	4	3.6%	
21-30 yrs	40	36.6%	
31-40yrs	31	28.4%	
40-70yrs	34	31.2%s	

Table 2: Age distribution (total cases=109)

Diagnostic parameter	No of cases	Percentage(%)
KOH positive	62	56.8%
Culture positive	82	75.2%

Table 3: KOH mount Vs Culture distribution (n=109)

ТҮРЕ	No of isolates	Percentage
Dermatophytes	37	45.12
Yeasts	28	34.14
Non-dermatophytic molds	17	20.73

Table 4: Morphological distribution of Isolates (n=109)

Name of the isolate	No of isolates	Percent- age		
DERMATOPHYTES (n=37)				
Trichophyton rubrum	19	51.35		
Trichophyton mentagrophytes	6	16.21		
Epidermophyton floccosum	5	13.5		
Trichophyton tonsurans	2	5.4		
Trichophyton verrucosum	2	5.4		
YEASTS (n=28)				
Candida albicans	17	60.71		
Candida parapsilosis	5	17.85		
Candida glabrata	3	10.71		
Candida tropicalis	3	10.71		
NON-DERMATOPHYTIC MOLDS (n=17)				
Aspergillus niger	7	41.17		
Fusarium spp	4	23.52		
Cladosporium spp.	2	11.76		

Table 5: Distribution of Isolates



Figure 1: Proxymal subungual onychomycosis:

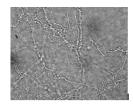


Figure 2: KOH mount Dermatophytes



Figure 3: Epidermophyton floccosum LPCB mount

DISCUSSION:

Onychomycosis occurs worldwide and appears to be a variable entity presenting in different forms in different parts of the world with every country and every region of country having its own characteristics of presentation. 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.

In our study, the rate of onychomycosis was 68.98% which is near concordance with study by Saroj Golia et al. (74.5%)^[9] but in contrast to study by Suryawanshi RS et al. (58.41%)^[10]. There are studies which showed a higher incidence of 82% by Martinez et al ^[11] and 86.9% by Kaur et al ^[12] and even 94.12% by Adhikari et al. ^[13] Our study showed a male preponderance of 71.5% to females 28.4%, which correlates with studies by Smita Kulkarni et al ^[14], Borah N Sharma et al ^[15], Saroj Golia et al ^[9], Suryawanshi RS et al ^[10].

The Commonest age group affected in our study were 21-30years, which is in concordance with the other studies by Borah N Sharma et al ^[15], Moumita Sarkar [16]. Agricultural workers are the major occupational group affected in our study which is similar to other studies by Smita Kulkarni et al. ^[14], Borah N Sharma et al. ^[15], Saroj Golia et al ^[9] etc.

The commonest organism isolated in our study is Trichophyton rubrum accounting to 51.35% which has been the same in studies by Saroj Golia et al.^[9], Smita Kulkarni et al.^[14], Moumita Sarkar^[16]. The high rate of isolation of *T.rubrum* can be explained on the basis that it has greater capacity to infect the nails because it can easily colonise on hard keratin. Among the yeasts isolated *Candida albicans* was the predominant organism accounting for 60.71% which also in concordance with studies by Saroj Golia et al ^[9], Smita Kulkarni et al. ^{[14].} Among the non dermatophytic fungi *Aspergillus* was the predominant organism followed by *Fusarium spp.* which is in contrast to studies by Suryawanshi RS et al ^[10], Leelavathi et al ^{[17].}

CONCLUSION

Onychomycosis can no longer be considered a simple cosmetic problem confined to the nails. Even with apparently optimal diagnosis patients are not cured by current therapies owing to misidentification of the pathogen, presence of a second disorder, characteristics of the nails, presence of a high fungal inoculum and/or drug resistant microorganisms, compromised immune system of the host, diabetes mellitus or peripheral vascular disease.

In this study the combined sensitivity of direct microscopy and culture was greater than direct microscopy and culture alone. Fungal cultures are of paramount importance in all suspected cases of onychomycosis because the antifungal agent with appropriate spectrum of activity can only be used if the underlying fungal pathogen is identified correctly. Diagnostic methodology and fungal susceptibility testing lag behind therapeutic advances. We should turn our attention to these problems.

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