

Epidemiological, Clinical and Cultural Study of Onychomycosis

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Abstract Background and Objective: Onychomycosis is a common nail disorder caused by dermatophytes and non-dermatophyte molds and represents most of the mycotic cutaneous infections. The purpose of the present study was to know the incidence of onychomycosis on both age and sex, morphological pattern of the disease, predisposing factors, associated conditions and identification of fungus by direct microscopy and by culture methods to rule out in Indian population. Methods: A prospective study was conducted on 64 patients of onychomycosis. A detailed history and thorough examination was done in all patients. The samples were taken from involved nails and skin and subjected to potassium hydroxide (KOH) examination and fungal culture and identification of fungal growth on Sabouraud's dextrose agar (SDA) medium. Results: Distal sub-ungual onychomycosis (DSO) was commonest type 47(78.35%) patients, 10(16.6%) patients with candidal onychomycosis (CO), 2 (3.34%) patients with proximal subungual onychomycosis (PSO) and 1(1.71%) showing superficial white onychomycosis. The mycological observations showing positive fining with KOH were observed in 50(83%) and culture positive in 40(66.66%) cases. The most common predominant organism isolated is *Trichophyton rubrum* in 22(35%) cases followed by *trichophyton mentagrophytes* 6(10%) cases and the non-dermatophytes could be isolated in 3 cases (5%) only.

Keywords Dermatophytes, Onychomycosis, Nails, Culture, Kadapa, India

1. Introduction

Fungal infections of the nails also called onychomycosis are commonest nail disorder including dermatophytes, non-dermatophyte moulds or yeasts[1]. It is one of the commonest nail disorders occurs in 30% of the superficial skin infections[2]. *Tenia unguis* is clinically defined as the dermatophytic infection of the nail plate[3]. Onychomycosis affects approximately 5% of the population worldwide[4] but variable frequency depending on different climatic, professional and socioeconomic conditions. It represents 20-40% of onychopathies and about 30% of mycotic cutaneous infections[5]. Various Indian workers have reported incidence to be at 0.5-5% in the general population[6]. Yeasts earlier regarded as contaminants are now increasingly being recognized as pathogens in the fingernails[7]. Onychomycosis is rarely life threatening, its high incidence and prevalence and the associated morbidity makes it an important public health problem.

Dermatophytes cause 90% of toenails and 50% of the

fingernail onychomycosis[8]. *Candida* species, particularly *Candida albicans*, prevail in finger infections[9]. Non-dermatophyte molds are rare, although few species are described as etiological agent of onychomycosis[2].

Onychomycosis can be clinically confirmed by direct microscopy of potassium hydroxide (KOH) preparation. However a fungal culture is required to identify the specific genus and species of pathogens[10].

The present study was planned to know the incidence of both age and sex, morphological pattern of the disease, predisposing factors, associated conditions and identification of fungus by direct microscopy and by culture methods to rule out the individual fungal species involved.

2. Materials and Methods

This was a prospective study carried out from January 2011 to June 2012 in outpatients department and inpatients department of dermatology of RIMS hospital, Kadapa, A.P, India. The patients with clinical features of onychomycosis and microscopically proven to be positive for fungal elements were taken up for the study. A detailed history of every patient along with complaints and duration of disease was taken and noted in a specially designed case report proforma. History regards demographic details, family

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history, nature of onset, duration of treatment taken was collected. All the nails were examined in good light precise involvement of the nails and morphology of the disease. A detailed general physical, mucocutaneous examination was conducted in all patients. The physical examination features of the affected nails and nail folds were noted and recorded in a table form. All the patients were examined for any superficial fungal infections of the skin and any other associated skin or systemic disease especially psoriasis, lichen planus, HIV, and diabetes mellitus.

The suspected nails were cleaned with 70% alcohol and nails scrapings were taken with a sterile scalpel blade and collected in a black sterile paper using a sterile nail cutter or scrapper. The samples were also taken also taken from the associated fungal infections of skin and nail folds in cases of paronychia. The screening of samples was done by direct microscopy with 10% KOH were slightly warmed whenever required for rapid dissolution of the materials. 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.

The samples were incubated by using sterile spud 10-20 pieces of the materials are inoculated on the surface of Sabouraud's Dextrose Agar (SDA) and inoculated at 37°C for 2-4 days for isolation of yeasts, 26°C for 14-30 days for moulds/dermatophytes. SDA with chloramphenicol (5gms) + Cycloheximide (50gms) is used for isolation of dermatophytes. The morphological characteristic of the rate of the growth, colony size, shape, margins, colour of the colony, type of growth and the pigment produced on reverse were carefully observed and noted. Nail samples from 8 cases of onychomycosis have been selected for histopathological examination by hematoxylin and Eosin staining (H&E) and periodic acid-Schiff staining (PAS).

3. Results



Figure 1. a) Distal subungual onychomycosis showing discoloration, subungual hyperkeratosis and onycholysis b) Toenail onychomycosis with Tinea pedis c) Distal subungual onychomycosis with disseminated superficial fungal infection



Figure 2. Proximal subungual onychomycosis of index finger and distal subungual onychomycosis of middle and little fingers with Tinea manuum and also claw-hand deformity. The patient has Hansen's disease with type-2 reaction and is on chronic steroid therapy

A total of 60 patients with onychomycosis, who were microscopically positive for fungal elements, were studied. Onychomycosis was seen to be affect ranging from 15-70 years. Majority of the (36.70%) were between 21-30 years of age. Only 1 patient (1%) under 20 years of age was observed and only 2 patients (3.33%) were more than 60 years. There were 39 (65%) males and 21 (35%) female patients. 36 (60%) cases were rural areas and 24 (40%) were urban areas. 16 (26.6%) were housewives, 10 (16.6%) were laborers, 6 (10%) farmers and students and 16 (26.6%) others. The duration of the disease at the time of presentation carried from 7 months to 10 years. 39 (65%) patients had disease between 7 months to 1 year and 18 (30%) had between more than 1 year to 5 years. Only 3 (5%) cases were having disease duration 5 to 10 years. There were 3 (5.6%) cases showing positive family history and 57 (95%) cases negative family history. 33 (55%) patients showing finger involvement, 24 (40%) patients showing toe involvement and only 3 (5%) showing both involvement. The study shows 47 (78.35%) patients with distal subungual onychomycosis (DSO) [Figure 1(a,b,c)], followed by 10 (16.6%) patients with candidal onychomycosis (CO), 2 (3.34%) patients with proximal subungual onychomycosis (PSO) [Figure 2] and 1 (1.71%) showing superficial white onychomycosis.

Patients showing common clinical presentation of discoloration of nails were seen in 60 (100%) patients and 56 (93%) patients showing subungual hyperkeratosis. The predisposing factors includes 60 (100%) patients use of common bathing facilities, 7 (11.6%) patients had the history of trauma to the nails, 6 (10%) patients with past history of tinea pedis, history of contact with person suffering from mycotic infections were 4 (6.66%) patients, past history of tinea manum 3 (5%) patients and 1 (1.66%) patients with use of occlusive foot wear and deformities [Table 1]

The associated fungal infections were present in 38 (63.33%) patients. The commonest fungal infections were 9 (23.68%) Mixed infections, 6 (15.78%) with Tinea pedis and Tinea manum where as mixed pattern was seen in others. In our study, the common diseases observed were diabetes mellitus in 7 (11.66%), anemia in 6 (10%) cases and 2 (3.33%) case with HIV with pulmonary tuberculosis and leprosy [Table 2].

Table 1. Predisposing factors

Contributing factors	No. of Cases	Percentage
Past history of trauma	7	11.6%
Past history of tinea pedis	6	10.0%
Past history of tinea manum	3	5.0%
Use if occlusive foot wear	1	1.66%
Use of common bathrooms (Communal bathing facilities)	60	100%
History of contact with person suffering from fungal infections	4	6.66%
Deformalities	1	1.66%

Table 2. Associated conditions with Dermatitis & Diseases

Associated Dermatitis	No. of Cases (n=60)	Percentage
Tinea pedis	6	10%
Tinea manum	6	10%
Tinea corporis	2	3.33%
Mixed infections	9	15%
Paronychia	2	3.33%
Hyperhidrosis	3	5%
Acne vulgaris	2	3.33%
Melasma	4	6.66%
Scabies	2	3.33%
Lichen simplex chronicus (LSC)	1	1.66%
Polymorphic light eruption	2	3.33%
Fissure foot	2	3.33%
Verruca vulgaris	1	1.66%
Infective eczema	1	1.66%
Diabetes mellitus	7	11.66%
HIV with pulmonary tuberculosis	2	3.33%
Leprosy (ENL on high dose steroids)	2	3.33%
Anemia	6	10%

The mycological observations showing positive fining with KOH were observed in 50 (83%) and culture positive in 40 (66.66%) cases. The studies showing that most common isolated species were dermatophytes in 70% of the cases, among the most common predominant organism isolated is Trichophyton rubrum in 22 (35%) [Figure 4] cases followed by trichophyton mentagrophytes 6 (10%) cases [Table 3]

Table 3. Culture isolation in relation to the site of involvement

Fungal isolated	Finger nails	Toe nails	Both	Total	Percentage
Dermatophytes	18	7	3	28	70%
Trichophyton rubrum	14	6	2	-	-
Trichophyton mentagrophytes	4	1	1	-	-
Candida Species	4	4	-	8	20%
C.albicans	4	4	-	-	-
Non-dermatophytes	3	1	-	4	10%
Aspergillus species					
Aspergillus flavum	2	-	-	-	-
Aspergillus niger	1	1	-	-	-
Total	25	12	3	40	100%

To the total of 60 cases, histopathological examination was conducted in 8(13.33%) cases by standard and specific methods including H&E and PAS staining[Figure 3]. 6(10%) cases shown positive reaction with PAS staining showing

long slender hyphae and spores. 2 (3.33%) cases shown PAS negative and were subjected for special staining procedure by Grocott's stain[Figure 5], out of which one shown positive result of slender hyphae.

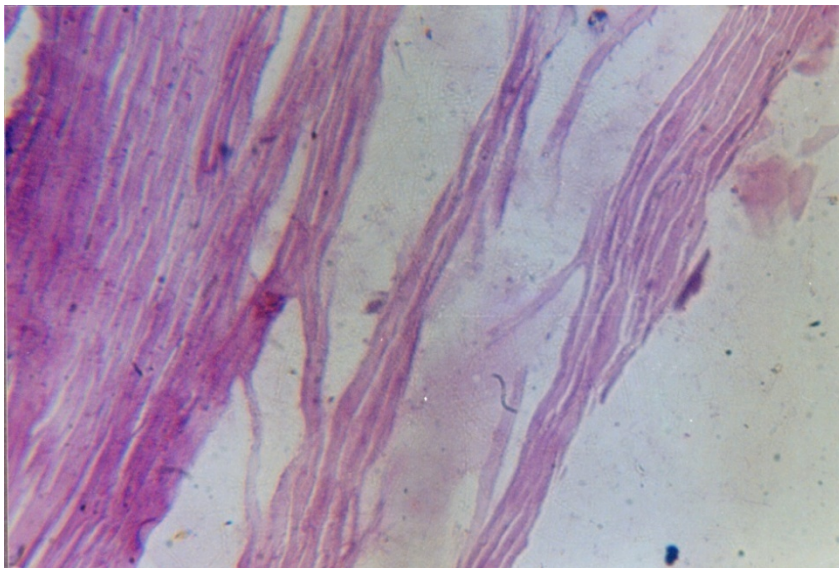


Figure 3. Trichophyton rubrum – Sabouraud dextrose agar (with double antibiotic) showing reverse view of colony of Trichophyton rubrum showing red wine pigmentation

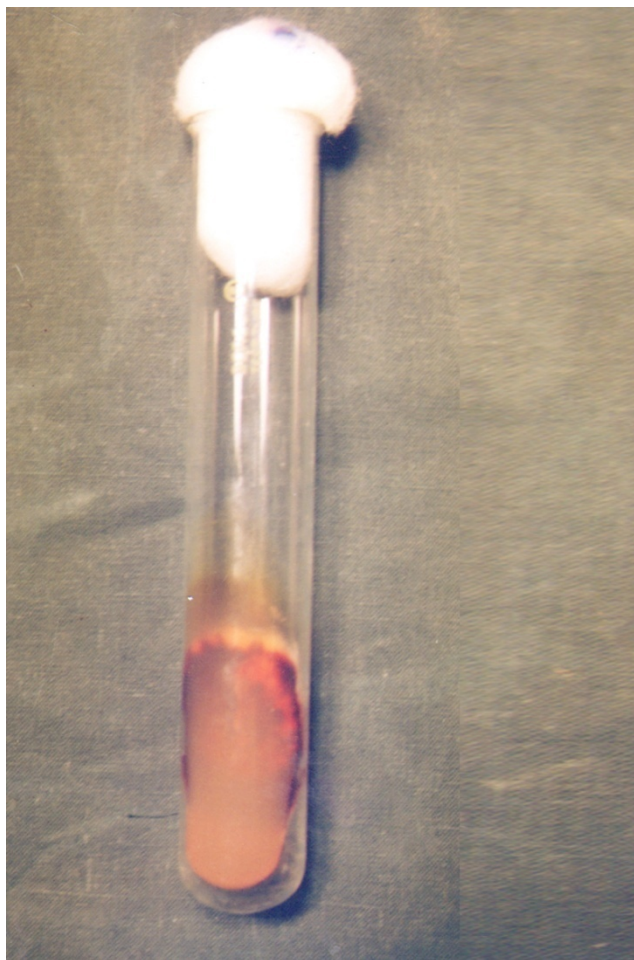


Figure 4. H&E – 280X: H & E stain of Fungal Nail

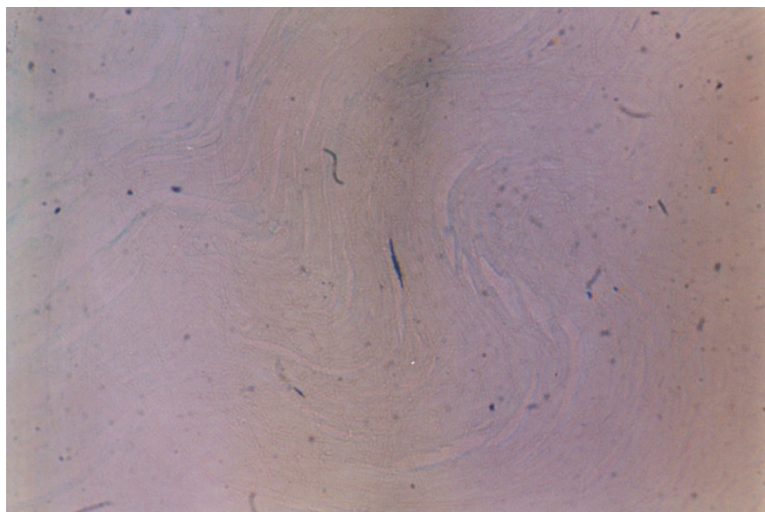


Figure 5. Grocott's stain – 280X: Black fungal hyphae are observed

4. Discussion

Onychomycosis affects all age groups. In our study 36.70% of patients were between 21-30 years, 1% below 20 years and 3.33% above 60 years of age. The overall prevalence of onychomycosis in children has been reported 0.44%[11]. The low prevalence of onychomycosis in children is attributed to difference in nail plate structure, lack of cumulative trauma and increased growth rate of nail plate with subsequent elimination of fungus[12]. There are mixed reports about the prevalence of onychomycosis in adults and elders. Some authors reported a high prevalence in the age group of 20-40 years[13,14,15] while others have reported a high prevalence above 55 years of age[16,17]. The incidence of onychomycosis may be higher in elderly in India but the disease being mostly asymptomatic and elderly being dependent upon for medical help may not be presenting to the hospital. Onychomycosis in our study was found to be more common in males 65% than females 35%. This has been found observed by most of the workers from India and abroad[13,18,19]. However distal subungual onychomycosis was more common in females. This has been attributed to greater burden of wet work with increased trauma facilitating easy entry to fungal pathogens[10,20]. In our study the common predisposing factor preceding to the development of onychomycosis observed were associated with wet work 43.3%. The predisposing factor of our study correlates with the study reported by Madhuri et al[21]. In this study common clinical presentation observed in all types of onychomycosis was nail discoloration (100%) and subungual hyperkeratosis in 93%. This concurs with the study done by Ramesh et al[22]. The housewives formed of the largest group (26.6%) in our study and this could be associated with wet work associated with constant trauma could explain the probable reason for the high prevalence of onychomycosis in the wet work occupation workers (43.3%). Increased physical activity (26.6%) with trauma facilitating easy entry of the fungal pathogens could probably explain the second contributing factors.

In present study only finger nails were involved in 33(55%) patients, only toe nails in 24(40%) and both finger and toe nails in 3 (5%). Most of the house wives from India show that fingernail onychomycosis is more common than toe nail onychomycosis[14,23]. Sabouraud's Dextrose Agar (SDA) culture medium was used for the isolation of fungi and the growth rate on SDA were 83% for dermatophyte growth observed in first two weeks of inoculation. The most common isolate obtained in our study was *Trichophyton rubrum*. It has been reported as most prevalent pathogen in onychomycosis by many workers[24,25]. The high rate of isolation of *T.rubrum* can be explained on the basis that can be explained on the basis that it has greater capacity to infect the nails because it can easily colonise on the hard keratin[26,27].

Trichophyton mentagrophyte is the second most common isolated dermatophyte (6.66%) in our study. It was isolated from both finger and toe nails onychomycosis. Although *T. mentagrophyte* has been reported to be usually associated with toe nail onychomycosis[23] but *T.rubrum* is still more common than *T.mentagrophyte* in the toe nail onychomycosis. Out of four cases of distal subungual onychomycosis (DSO), the isolate obtained from 47 cases were shown positive towards *T. rubrum* and *T.mentagrophytes*. This is the commonest dermatophyte implemented in the etiology of distal subungual onychomycosis (DSO)[9]. There were 4 cases (6.66%) of *Candida albicans* in our study. All these patients had chronic paronychia with nail and toe involvement. Among all the yeast, *Candida* species are reported most frequently reported[14,28].

The non-dermatophytes could be isolated in 3 cases (5%) only. The only non-dermatophytes isolate was *Aspergillus flavum* from 2 cases of finger nail and 1 case of *Aspergillus niger* of finger nail. *Aspergillus* sp. has earlier been reported from India as the cause of onychomycosis[29]. In our study there were 9 (15%) cases of mixed infections of *T. rubrum* and *Aspergillus flavum*. Mixed infections are described in the literature but are uncommon[21]. The most possible

explanation for mixed etiology is that the disease and dystrophic nails already damaged by dermatophytes than non-dermatophytes.

5. Conclusions

Onychomycosis is caused by the dermatophytes of fungi and non-dermatophytes moulds. *Trichophyton rubrum* was most common isolate. Although the onychomycosis is not a life threatening, it can be source of pain and discomfort; it can also pose a risk for the patients, their families and others in contact with them. Onychomycosis can no longer consider a simple cosmetic nuisance confined to the nails. It is a significant and important disease, which can generate many physical, psychological and occupational problems considerably impairing patient's quality of life.

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