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A Clinico-mycological Study of Onychomycosis

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Abstract

Background and Objectives:

Onychomycosis is a chronic fungal infection of nails. The clinical diagnosis of onychomycosis needs to be confirmed by direct microscopy and culture for identification of specific pathogen and proper selection of antifungal treatment. Our aim is to study the morphological pattern of onychomycosis and to analyze the mycological and cultural positivity of onychomycosis with respect to various etiological agents.

Methods:

A prospective study was conducted on 200 patients of onychomycosis. A detailed history and thorough clinical 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 on Dermatophyte test medium (DTM) and Sabouraud's dextrose agar (SDA) medium. The identification of isolate was done from the growth on Sabouraud's dextrose agar (SDA) medium.

Results:

Distal and lateral sub-ungual onychomycosis (DLSO) was the commonest type (67%) and proximal Trichophyton mentagrophyte

subungual onychomycosis (PSO) was the least common (1%). Out of 200 KOH positive cases, 72 were culture positive on Sabouraud's dextrose agar (SDA) medium (36%) and 54 cases (27%) were positive on Dermatophyte test medium (DTM). *Trichophyton rubrum* was the most common (45%) isolate followed by (25%) and *Candida albicans* (14%). *Aspergillus niger* was only non-dermatophyte mould isolated from 7 cases (3.5%) of toe nail onychomycosis.

Conclusion:

Dermatophytes were the most common group followed by yeasts and non-dermatophyte moulds in the etiology of onychomycosis. *Trichophyton rubrum* was the most common isolate. Dermatophyte test medium (DTM) is a rapid and easy method to confirm dermatophyte infection.

Introduction

Onychomycosis is defined as the fungal infection of nails caused by dermatophytes, yeasts and non-dermatophytic fungi. It is one of the commonest nail disorders and accounts for up to 30% of all superficial infections of the skin [1]. Recently there has been a worldwide increase in the incidence of onychomycosis with social, cultural and economical factors contributing to it [2]. The disease per se is asymptomatic but it poses a serious concern to the clinician as it often becomes a chronic source of recurrent superficial skin infections. Besides this, the destruction and disfigurement of nail plate in onychomycosis can lead to self-consciousness and impairment in doing fine work [3].

The clinical diagnosis of onychomycosis can be confirmed by direct microscopy of potassium hydroxide (KOH) preparation. However a fungal culture is required to identify the specific genus and species of pathogens.

In the past most of the research work has been done on the superficial mycosis of the skin as compared to onychomycosis. In India relatively less work has been done on the onychomycosis as compared to western countries. The evolving role of non-dermatophytic moulds has added a new dimension to the clinical patterns of onychomycosis. There is a need for further studies on onychomycosis and other dermatophytosis in view of the introduction of several newer systemic antifungal drugs.

The present study was conducted to study the morphological pattern of onychomycosis and to analyze the mycological and cultural positivity in view of the paucity of literature on onychomycosis from this part of the country.

Methods

This was a prospective study carried out in the outpatient department

of dermatology on patients of clinically suspected cases of onychomycosis. The patients who were 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 proforma. History regarding occupation, hobbies and foot-wear were enquired into. History of contact with pets or cattle, history of fungal infection elsewhere on the body and history of fungal infection in other members of the family was checked in every patient. All the patients were asked about the history of any drug intake for prolonged periods with duration and type of drug with special reference to immunosuppressive drugs. A detailed general physical, systemic and mucocutaneous examination was conducted in all patients. The physical 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 infection of skin and any other associated skin or systemic disease especially psoriasis, lichen planus and diabetes mellitus.

The suspected nails were cleaned with 70% alcohol and nail scrapings were taken with a sterile scalpel blade and collected in a black coloured sterile paper. The scrapings were taken from the junction of healthy and diseased nails and also the friable sub-ungual debris was collected along with scrapings. In case of superficial white onychomycosis (SWO), minute scrapings were taken from the superficial layer of the affected area of nail plate and in case of proximal sub-ungual onychomycosis (PSO) nail scrapings were taken from the relatively deeper layer of nail plate. In case where both finger and toe nails were involved, the samples were taken from both sites and tested separately. The samples were also taken from the associated fungal infection of the skin and nail folds in cases of paronychia. The screening of the samples was done by direct microscopy with 10% potassium hydroxide (KOH). The samples with 10% KOH were slightly warmed whenever required for rapid dissolution of the material. The softened nail material was 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 inoculated into two sets of media for culture:

- Sabouraud's Dextrose Agar with Chloramphenicol (SDA).
- Dermatophyte Test medium (DTM).

Each set of media were incubated at 22- 30°C and 37°C and were examined for any growth of fungi for 4 weeks. The change of colour from yellow to red on dermatophyte test medium (DTM) indicated the growth of a dermatophyte. The identification of isolate from Sabouraud's Dextrose Agar was done on the basis of colony morphology and wet mount microscopy with Lactophenol cotton blue stain. The morphological characteristic of the colony including size, shape, margins, 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 then 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.

Results

A total of 200 patients with onychomycosis, who were microscopically positive for fungal elements, were studied. Onychomycosis was seen to affect all age groups ranging from 6 to 63 years. Majority of the patients (63%) were between 20 to 40 years of age. Only 3 cases were below 10 years and only 2 patients (1%) were more than 60 years. There were 143 (71.5%) male and 57 (28.5%) female patients. One hundred and twenty (60%) cases were from urban areas and 80 (40%) were from rural areas. Forty eight (24%) were students, 26 housewives, 22 farmers, 19 army and paramilitary persons, 12 labourers, 5 industrial workers and 36 others. The duration of the disease at the time of presentation varied from one month to 15 years. Forty eight (24%) patients had disease duration of less than six months, 58 (29%) had between 6 months to one year and others more than one year. Only five cases (2.5%) were having disease duration more than 10 years. One hundred and fifty six patients (78%) were having shoes with socks as predominant footwear and 44 (22%) had only shoes or chappals. There were 5 patients on systemic immunosuppressive drugs, 4 patients were taking oral steroids and 1 patient was on cyclosporine and azathioprine. There were 4 cases of psoriasis and 3 cases of lichen planus with nail involvement and 6 patients of diabetes mellitus. Seven patients (3.5%) had the history of preceding trauma to the affected nails. Disfigurement and discolouration were the main presenting complaints in all 200 patients. Pain and paronychia were the presenting complaints in 16 (8%) and 12 (6%) patients respectively. The associated fungal infection was present in 83 patients (41.5%). The commonest fungal infections were *Tinea mannum* (19%), and *Tinea pedis* (10%), whereas mixed pattern was seen in others. In majority of cases more than one nail were involved. Only finger nails were involved in 113 patients (56.5%), only toe nails in 34 (17%) patients and both finger and toe nails were involved in fifty three (26.5%) patients. The most common findings on examination of nail plate were discoloration and sub-ungual hyperkeratosis. Distal and lateral superficial onychomycosis (DLSO) was the most common pattern (67%) of nail dystrophy and proximal sub-ungual onychomycosis (PSO) was the least common (0.5%). Sub-ungual hyperkeratosis and onycholysis were present in 75% in 71% cases respectively.

Out of 200 patients, 72 (36%) were culture positive on Sabouraud's Dextrose Agar and 54 cases (27%) were positive on Dermatophyte test medium. The most frequent growth isolated on Sabouraud's Dextrose Agar was *Trichophyton rubrum* (**Table. 1**). *Aspergillus niger* was the only

non-dermatophyte isolated in seven cases. The observation showed that *T. rubrum* and *Candida albicans* involved mainly finger nails, and *A. niger* involved only toe nails. In mixed growth of *A. niger* and *T. rubrum* there were 4 cases of toe nail onychomycosis and one case of finger nail onychomycosis.

The commonest organism isolated from distal and lateral sub-ungual onychomycosis (DLSO) was *Trichophyton rubrum* (40%) followed by *T. mentagrophyte* (25%), *Candida albicans* (16%) and *Aspergillus niger* (11%). *Trichophyton tonsurans* and *Trichophyton schoenleinii* was isolated from two cases each of DLSO. In total dystrophic onychomycosis (TDO), *T. rubrum* was isolated from 60% cases, *T. mentagrophyte* from 30% cases, *C. albicans* (5%) and *A. niger* from 5% cases. *Trichophyton rubrum* was isolated from one case each of PSO and SWO.

Isolate	No. of Cases (n=72)	Percentage
<i>T. rubrum</i>	32	44.44%
<i>T. mentagrophyte</i>	18	25.00%
<i>T. tonsurans</i>	3	4.16%
<i>T. schoenleinii</i>	2	1.00%
<i>C. albicans</i>	10	13.88%
<i>A. niger</i>	3	4.16%
<i>A. niger</i> + <i>T. rubrum</i>	4	5.55%
Total	72	100.00%

Table 1: Results of fungal culture on SDA

Discussion

Onychomycosis affects all age groups. In our study 60% of patients were between 20- 40 years, 1.5% below 10 years and 1.5% above 60 years of age. The overall prevalence of onychomycosis in children has been reported 0.44% [4]. 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 [5]. There are mixed reports about the prevalence of onychomycosis in adults and elderly. Some authors have reported a high prevalence in the age group of 20-40 years [6,7,8,9] while others have reported a high prevalence above 55 years of age [10,11]. The high prevalence in adults can be attributed to more consciousness about discoloration and disfigurement of nails in adults as compared to elderly. The actual incidence of onychomycosis may be higher in elderly in our country but the disease being mostly asymptomatic and elderly being dependent upon others for medical help may not be presenting to the

hospital. Onychomycosis in our study was found to be more common in males than females. This has been observed by most of the workers from India and abroad [3,6,7]. However candidal 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 [12]. There were many patients in our study from the occupations which involve the work that predisposes to minor trauma and injuries. The importance of trauma as predisposing factor in onychomycosis is well established [2,13]. In India and other tropical countries where the majority of the people are generally barefoot, the incidence of toe nail onychomycosis is reported to be extremely low [9,14] but the majority of patients in our study were having shoes and socks as predominant footwear and there was no patient walking barefoot. All the eighty seven patients of toe nail onychomycosis were having shoes and socks as footwear whereas none of those with only shoes and chapel as footwear had toe nail onychomycosis. This highlights the fact that warm and moist environment provided by occlusive footwear promotes the growth of fungus and predisposes to onychomycosis. Similar observations have been reported from time to time [15,16]. The students formed the largest group (24%) in our study and this could be because of more concern about the cosmetic problem of nails in these patients.

In present study only finger nails were involved in 56.5% patients, only toe nails in 17% and both finger and toe nails in 26.5%. Most of the studies from India show that fingernail onychomycosis is more common than toe nail onychomycosis [7,8,17]. The low incidence of toe nail onychomycosis in our country may be attributed to open footwear and lesser concern for appearance of feet and toe nails in our people. The number of cases of total dystrophic onychomycosis (TDO) is more in our study as compared to other Indian reports [7,8,18]. Proximal sub-ungual onychomycosis (PSO) has been reported to be associated with HIV; however the only two patients in our study were healthy and non-reactive for HIV by ELISA.

Two types of culture media were used for isolation of fungi i.e. Dermatophyte test medium (DTM) and Sabouraud's Dextrose Agar (SDA) medium. Dermatophyte test medium is a cycloheximide containing culture medium and it also has phenol red as pH indicator. This medium is yellowish before inoculation but most of the dermatophyte release alkaline metabolites that turn the medium red in 7-14 days [19]. There is no growth of yeasts and moulds and therefore this medium can easily differentiate between the dermatophyte group from others. The growth rate on Sabouraud's Dextrose Agar (SDA) was 36%. The low growth rate on culture from nail material is well known. The most important explanation given for this by various workers is non-viability of fungal hyphae in the distal portion of the nail plate from where the samples are usually taken [10,20]. Out of total 72 culture- positive cases on Sabouraud's Dextrose agar (SDA) 54 cases (75%) were positive for dermatophyte growth on Dermatophyte test medium (DTM) and most of these were positive within 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 [18,21]. 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 [10,15]. It was the commonest organism isolated from finger nail onychomycosis and also it was the commonest cause of distal and lateral sub-ungual onychomycosis (DLSO) and total dystrophic onychomycosis (TDO) in our patients.

Trichophyton mentagrophyte is the second most common isolate (25%) in our study. It was isolated from both finger and toe nail onychomycosis. Although *T. mentagrophyte* has been reported to be usually associated with toe nail onychomycosis [17] but *T. rubrum* is still more common than *T. mentagrophyte* in toe nail onychomycosis. Out of the four cases of superficial white onychomycosis (SWO), the isolate obtained from only one culture positive case was *T. mentagrophyte*. This is the commonest dermatophyte implicated in the etiology of superficial white onychomycosis (SWO) [1]. *T. tonsurans* and *T. schoenleinii* were isolated in 4% and 2.7% cases respectively. These have been reported as less common isolates but *T. schoenleinii* may be an important causative agent in the parts of world where it is endemic [20,21]. There were ten cases (14%) of *Candida albicans* in our study. All these patients had chronic paronychia with nail involvement. Among all the yeasts, *Candida* species are reported most frequently [2,8].

The non-dermatophytes could be isolated in 3 cases (4.16%) only. The only non-dermatophyte isolate was *Aspergillus niger* from three cases of toe nails. *Aspergillus* sp. has earlier been reported from India as the cause of onychomycosis [22,23]. The non-dermatophyte moulds are opportunists and none of them is keratolytic. They virtually damage the toe nails especially which are damaged by disease or trauma [1,15]. The preference of non-dermatophytes for toe nails is explained by their presence in soil and slower linear growth of toe nails [11]. In our study there were three cases of mixed infections of *T. rubrum* and *Aspergillus niger*. Mixed infections are described in the literature but are uncommon [2, 12]. The most probable explanation for mixed etiology is that the diseased and dystrophic nails already damaged by dermatophytes are easily invaded by non-dermatophytic moulds.

Conclusions

Dermatophytes were the most common group followed by yeasts and non-dermatophyte moulds in the etiology of onychomycosis. *Trichophyton rubrum* was the most common isolate. Dermatophyte test medium (DTM) is a rapid and easy method to confirm dermatophyte infection.

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