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Onychomycosis: Fungal Isolates and Pattern of Infection in Kolkata, India

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The worldwide incidence of onychomycosis is increasing and it continues to spread and persist. Knowledge of the epidemiological and mycological characteristics is an important tool for control of this infection[1,2]. The study aimed to determine the prevalence of onychomycosis, to isolate the causative fungi and to determine the various clinical patterns. Over a period of two years (Jan. 2010 – Dec. 2012), 280 patients attending dermatology outpatient department with clinically suspected fungal nail infections, at Tertiary Care Hospital, Kolkata, were studied. Specimen from all the 280 suspected patients of onychomycos is were evaluated clinically; Potassium Hydroxide (KOH) examination and fungal culture were done. Onychomycosis commonly affects 31 to 40 years aged followed by 41 to 50 years age group. Males were predominantly affected, male to female ratio being 2 : 1. Finger nails were affected more frequently than toe nails with the ratio of 1.5 : 1. Distal and lateral subungualonychomycosis (48.21%) was the commonest clinical pattern. Direct microscopy of the nail clips in KOH examination was positive in 95% and culture was positive in 80.70% cases. Out of the samples cultured, dermatophytes were grown in 64.28% cases, non dermatophyte moulds in 15.71% and *Candida* spp. in 15% while 5% samples yielded no growth. Amongst dermatophytes, *Trichophyton rubrum* was the commonest clinical agent (30.36%); followed by *Trichophyton mentagrophyte* (20.36%). Amongst non dermatophyte moulds (NDM), *Aspergillus* spp. (12.14%) was the most prevalent species. Dermatophytes were main agents causing onychomycosis in the study area and direct examination along with culture is crucial in diagnosis of onychomycosis.

Key words: Onychomycosis, fungal isolates, pattern.

Onychomycosis accounts for up to 50% of all nail infections, which is caused by various species of dermatophytes, yeasts and moulds[1,2]. In onychomycosis, infected nails serve as a chronic reservoir of infection which can gives rise to repeated mycotic infections of the skin. It is of significance to suspectonychomycosis, perform mycological diagnosis and undertake treatment. This may help to prevent nail dystrophy and the spread of infection[3]. In addition to the physical effects of onychomycosis, psycho-social consequences may interfere with individual's personal and professional life. Clinical and mycological features of onychomycosis show variation with time and place[4].

Several factors have been attributed to the increased prevalence of the disease such as reduce peripheral circulation, diabetes, nail trauma and difficult to maintain proper nail hygiene[3]. Although it has worldwide occurrence, its frequency is variable depending on climate, occupation and socio-economic conditions[5].

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With this background the current study is being planned with the aim to determine the prevalence of onychomycosis, to isolate the causative fungi and to determine the various clinical patterns.

MATERIALS AND METHODS

Study population

Over a period of two years (Jan. 2010 – Dec. 2012) 280 patients with clinically suspected fungal nail infections, who attended dermatology outpatient department at Institute of Post Graduate Medical Education and Research, a tertiary care hospital, Kolkata, were incorporated under study.

Method

The patients were assessed by interview, clinical examination and specimens collected for microbiological studies. Information collected on demographic feature, patient history, and specific data related to risk factors for onychomycosis (age, gender, physical activities, occupation, history of trauma; predisposing diseases such as diabetes, cardiovascular disease, immunodeficiency disease and previous onychomycosis). The clinical appearance and location of onychomycosis (toenail / fingernail) were documented. Different clinical patterns were recorded separately.

Specimen collection, processing and identification

The specimens were obtained from clinically abnormal nails, by a vigorous scrapping of the nail bed, the underside of the nail plate and the hyponychyum, after cleaning the affected area with 70% ethanol. The samples of each patient were placed in separate sterile petridish and transported to the mycology laboratory. All specimens were examined by direct microscopy and culture. Scales scraped from the nails were examined for fungal elements, such as hyphae or blastoconidia, by direct microscopyin 40% KOH. Nail scrapings and clippings were inoculated on antibiotic containing Sabouraud dextrose agar (SDA) with and without cycloheximide at 27^0 C and at 37^0 C and examined daily for six weeks. At least three samples from each patient were processed. Growth in the culture medium was viewed as confirmation of dermatophytes as etiologic agents. In addition, the identification was confirmed by micromorphological aspects on slide culture. Confirmation of Candida species required observation of pseudomycelium under light microscopy with KOH and positive culture. Those who grew a particular mould other than dermatophyte consistently on two or more successive occasions with consistent filaments by direct microscopy and continued to grow the same mould consistently thereafter from the same nail and without growing a dermatophyte on any occasion were classified as opportunistic onychomycosis. Because of difficulty in discerning pathogens from contaminants, the guidelines followed were, if a dermatophyte was isolated on culture, it was a pathogen and if a nondermatophyte mould or yeast was cultured, it was significant only if direct microscopy was positive and repeatedly isolated[6-8].

RESULTS

There were total 280 suspected cases of onychomycosis. Of these 280 cases, 266(95%) were positive by direct microscopy and 226(80.70%) were culture positive (Table 1). Of these 280 cases, 188(67.14%) were male and 92(32.85%) were female, male to female ratio being 2:1. The commonest age group was 31 to 40 years followed by 41 to 50 years. Infections were less common in the age group below 30 years. The finger nails were more frequently involved 144(51.42%) cases followed by toe nails 94 (33.57%) and both in 42(15%) cases. Ratio of finger nail to toe nail infection was 1.5:1 (Table 2). Distal and lateral subungualonychomycosis (DLSO) was the commonest clinical pattern (48.21%) followed by proximal subungualonychomycosis (PSO) (23.21%) and then white superficial onychomycosis (WSO) (10.36%), total dystrophic onychomycosis (TDO) (10.00%) and paronychia (8.21%) (Table 3). The most common fungal isolates were dermatophytes

Table 1. Microscopy and culture positivity of the clinical samples (n=280)

Test result	Sample No.(%)
Total KOH positive	266 (95.00)
KOH positive, Culture negative	45 (16.00)
Culture positive, KOH negative	05 (1.70)
Total culture positive	226 (80.70)
Both KOH and Culture positive	221 (78.90)
Both KOH and Culture negative	14 (5.00)

Table 2. Age and gender-wise distribution of patients in relation to site of involvement

Age (years)	Male No.(%)	Female No.(%)	Finger nails No.(%)	Toe nails No.(%)	Both Finger & Toe nailsNo.(%)
<10	02(1.06)	-	02(1.39)	-	-
11-20	05(2.66)	-	05(3.47)	-	-
21-30	11(5.85)	06(6.52)	12(8.33)	05(5.32)	-
31-40	87(46.28)	38(41.30)	62(43.06)	42(44.68)	21(50.00)
41-50	64(34.04)	31(33.70)	47(32.64)	34(36.17)	14(33.33)
>50	19(10.11)	17(18.48)	16(11.11)	13(13.83)	07(16.67)
Total	188 (100.00)	92 (100.00)	144 (100.00)	94 (100.00)	42 (100.00)

(n=280)

Table 3. Clinical pattern of onychomycosis. (n=280)

Clinical patterns	Finger nails No.(%)	Toe nails No.(%)	Both Finger & Toe nailsNo.(%)	Total No.(%)
DLSO*	65(45.14)	49(52.13)	21(50.00)	135 (48.21)
PSO**	32(22.22)	19(20.21)	14(33.33)	65(23.21)
SWO***	18(12.50)	11(11.70)	-	29(10.36)
TDO****	13(9.03)	08(8.51)	07(16.67)	28(10.00)
Paronychia	16(11.11)	07(7.45)	-	23(8.21)
Total	144(100.00)	94(100.00)	42(100.00)	280(100.00)

*Distal and lateral subungualonychomycosis, **Proximal subungualonychomycosis,*** White superficial onychomycosis, ****Total dystrophic onychomycosis

Table 4. Distribution of fungal isolates in clinical samples. (n=280)

Fungus	IsolateNo.(%)
Dermatophytes -	
Trichophytonrubrum	85 (30.36)
Trichophytonmentagrophyte	57 (20.36)
Epidermophytonfloccosum	38 (13.57)
Non-dermatophytic moulds -	
Aspergillus spp.	
(*Aspergillus fumigatus*	21 (7.50)
Aspergillus flavus)	13 (4.64)
Fusarium spp.	06 (2.14)
Acremonium spp.	04 (1.43)
Yeast like fungi-	
Candida albicans	42 (15.00)
No growth	14 (5.00)
Total	280(100.00)

180(64.28%) of which *Trichophyton rubrum* were 85 (30.36%), *Trichophyton mentagrophyte* 57 (20.36%) and *Epidermophyton floccosum* were 38 (13.57%). Non-dermatophytic moulds constituted 44(15.71%) of the fungal isolates of which *Aspergillus* spp. were 34(12.14%), followed by *Fusarium* spp. 6(2.14%) and *Acremonium* spp. 4(1.43%). Out of the total fungal isolates 42(15%) were *Candida albicans* and 14(5%) samples showed no growth (Table 4).

DISCUSSION

Onychomycosis is a chronic infection of the nails; nowadays considered a serious problem for public health, in view of its high occurrence in the worldwide population[9]. Although this disorder is not serious in terms of mortality, it has significant clinical consequence given it's infectious nature, aesthetic consequences, chronicity and therapeutic difficulties[10]. The two conventional methods for fungi identification are direct microscopy under KOH and fungal culture. The microscopic method is more sensitive for showing the presence of fungi, but the isolation of a specific genus and species of the pathogen requires fungal culture, which is often not very fruitful. Direct microscopy examination positivity in the present

study was 266(95%). Culture was positive in 226(80.70%) cases, including 5(1.70%) cases with negative direct examination and 221(78.90%) cases with positive direct examination, corroborated with other studies[10-12]. In the present study, onychomycosis was found to be commonest in the age group 31-40 years followed by 41-50 years and infections were less common in the age group below 30 years in accordance with most of the studies[1,12-14]. Higher incidence was noted amongst males than females, the ratio being 2 : 1, which is similar to most of the studies[12,13]. Higher incidence in males may be because they are more exposed to outdoors with greater physical activity and are more prone to trauma. In the present study more cases of finger nail onychomycosis found than toe nails with a ratio of 1.5 : 1, which is similar to other studies[1,12,14]. Incidence of increased finger nail onychomycosis may be because of the increased chances of occupation related trauma and the more common causes of toe nail onychomycosis due to use of occlusive footwear. It was observed that, thumb finger and greater toe nail onychomycosis has been reported frequently, this is in agreement with other studies, because of its bigger size predisposing to increased trauma[1,11,12]. The present study showed, most common clinical pattern was distal and lateral subungualonychomycosis (DLSO), which was similar with other studies[12,15,16]. In the present study the most common isolated causative agent was dermatophytic mould *Trichophyton rubrum* 30.36%[3,11-13,15] and other dermatophytic mould *Trichophyton mentagrophyte* and *Epidermophyton floccosum* isolated 20.36% and 13.57% cases respectively. In non dermatophytic moulds predominant species were Aspergillus spp. (12.14%) and other isolated non dermatophytic moulds were *Fusarium* spp. 2.14% and *Acremonium* spp. 1.43%[2,3,16]. *Candida albicans* is reported as the commonest cause of paronychialonychomycosis, which is comparable with other studies[3,11,15]. Yeast is now increasingly recognized as pathogen in finger nail infections[12].

CONCLUSIONS

Dermatophytes were main agents causing onychomycosis and Distal and lateral subungualonychomycosis was the most common clinical pattern found in the study. In the perspective of increasing prevalence of onychomycosis during last decades as well as the role of various types of climate, socio-economic and occupational situations, regional investigations for determining causative fungal agents and its prevalence is necessary.

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