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"Clinico- Mycological Profile of Onychomycosis –A Study in A Tertiary Care Hospital in Kolkata"

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Abstract: *Clinical and mycological features of onychomycosis show variation with time and place. Onychomycosis is a major public health problem with high incidence, associated morbidity and long lasting treatment with anti-fungal agents. A study was carried out on 118 patients having clinical diagnosis of onychomycosis, attending the dermatology out-patient department of R. G. Kar Medical College, Kolkata. Nail clippings were subjected to direct microscopy and cultured on Sabouraud's Dextrose Agar with antibiotics. The commonest age group affected was 21-40 years with a female preponderance and housewives were most commonly affected group. Finger nail involvement were more frequent and distolateral subungal onychomycosis (DLSO) was the most common clinical type observed . Out of 118 cases total KOH positivity was in 72 (61.01%) and culture positivity was in 62 (52.54%). Total culture positive cases were 62 and total number of fungi identified were 66 as dual pathogen had been identified in four cases . Among 66 culture positive isolates dermatophytes were 30 (45.55%), candida 21(31.82%) and non-dermatophyte moulds (NDM) 15 (22.73%) . T. rubrum was the commonest etiological agent. Among dermatophytes, Trichophyton rubrum, and among NDM, Fusarium moniliforme, were the most common isolates identified in our study.*

Keywords: *Onychomycosis, Dermatophytes, Non-dermatophyte moulds, Yeasts*

I. Introduction

Onychomycosis is traditionally referred to as non-dermatophytic infection of nail but now used as a general term to denote all fungal infection of nail.[1] Onychomycosis represents up to 30% of mycotic cutaneous infections and is the most common nail disease accounting for approximately 50% of all onychopathies.[2] Onychomycosis shows a worldwide prevalence rate of 2% to 50% and varies with age, time and geographic location [3] with male preponderance and more common in toe nails than finger nails.[4] Onychomycosis expresses itself in various clinical forms like-distal and lateral subungual onychomycosis (DLSO), white superficial onychomycosis (WSO), proximal subungual onychomycosis(PSO), endonyx onychomycosis and total dystrophic onychomycosis (TDO).[4]

Several factors implicated in the increase in disease are reduced peripheral circulation, trauma to nail, old age, immunosuppression, smoking, hyperhidrosis, nail destruction due to other primary dermatoses and improper nail hygiene.[5, 6]

In India relatively less work has been done on the onychomycosis as compared to western countries.[5, 7] The evolving role of non-dermatophytic moulds has added a new dimension to the clinical patterns of onychomycosis as they are relatively resistant to conventional antifungals.[7] There is a need for further studies on onychomycosis and other dermatophytosis in view of the introduction of several newer systemic antifungal drugs.[5]

The present study was undertaken to evaluate the demographic and clinico- mycological profile of onychomycosis cases attending dermatology out patients department of a tertiary care hospital in Kolkata.

II. Material And Methods

Uccessive out-patients diagnosed clinically as having onychomycosis were included in the study conducted uccessive out-patients diagnosed clinically as having onychomycosis were included in the study conducted 118 clinically suspected Onychomycosis cases attending the dermatology outpatient department of R G Kar Medical College during a period of one year (April 2014 –March 2015) were included in the study. Detailed history of the patients regarding age, sex, site of lesion, occupation, and associated illness was taken and patients were examined clinically for the type & site of the lesion and classified accordingly. The affected nail was cleaned with 70% alcohol and then collected aseptically according to clinical types. The sampled material was divided into 2 portions: one for direct microscopy with 40% KOH and the remainder for culture in

SDCA media both at 25°C and at 37°C. The culture tubes were examined at regular intervals for evidence of fungal growth and no culture was declared as negative before six weeks of incubation.

The culture isolates were further studied for colony morphology and microscopical examination of lacto-phenol blue mounts. Special tests like hair perforation test, urease production test, slide culture, culture in DTM media, gram staining ,morphology on corn meal agar, germ tube test were carried out using standard techniques[4] wherever necessary for identification of species.

III. Results

A total of 118 cases in the range of 3 –78 years were analyzed. The most common age group affected was 21-40 years with 51(43.22%) cases followed by 41-60 years with 45 (38.13%) cases, 0-20 years 17(14.4%)and the least common age group affected was above 60 years 05(04.23%) [Table 1].

Out of 118cases, the females 75 (63.55%) were slightly more affected than males 43(36.45%). 53 cases (44.91%) resided in rural locations while 65 cases (55.09%) were residents of urban areas.

Regarding occupation majority were housewives 26(22.03%)followed closely by farmer 18(18.64%), business 14(11.86%), students 13(11.01%), 11(9.32%)each were nurse and service,07(5.93%) teacher, 06(5.08%) tailor, 05(4.23%) driver and03(2.54%)were retired person.

Finger nails were more commonly affected 58(49.15%) compared to toe nails 45(38.13%), however in 15(12.71%) cases had both finger and toe nail involvement [Fig.1]. DLSO and WSO type of onychomycosis were found to be more in 41 -60 years age group 25 (55.56%) and 08 (17.78%) respectively whereas for the other three types of onychomycosis more common age group was 21-40 years [Fig. 2]

Out of 118 clinically suspected cases of onychomycosis, fungi were demonstrated by direct microscopy with KOH mount in 72(60.16%) . 40 (33.89%) were positive by both microscopy and culture. KOH positive and culture negative were 32 (27.11%), and KOH negative but cultures positive were 22 (22.64%). The KOH mount examination was found to be very useful with sensitivity 64.51%. [Table 2]

Out of 62 cultures positive cases total number of fungi isolated were 66 as dual pathogenic fungi were identified in four cases. Most frequently isolated fungi were dermatophyte 30(48.38%) followed by yeast 21 (33.87%) and non-dermatophytic molds isolates 15 (24.19%). Considering the clinical types of onychomycosis, most frequently isolated fungi were dermatophyte in DLSO, PSO and Endonyx type with 15(51.7%), 07(41.18%) and 03(60%) respectively. In WSO type, identified dermatophytes and yeasts were equal in number 05(41.67%) whereas in TDO type only 2 yeasts (66.67%) and 1(33.33%) non-dermatophyte mold were isolated [Table 3].

Among all dermatophytes identified, *Trichophyton rubrum* was the most common species 09 (13.63%), followed by T. tonsurans 06(09.09%), T. soudanense 05(07.57%), T. schoenleinii 03(04.5%),and 02(03.03%) each in all three species of T. mentagrophytes, T. verrucusum, and Epidermophyton spp. Candida albicans 08 (12.12%) was the 2nd most common causative agent in this study. Among the other Candida spp. Candida tropicalis 07(10.06%), Candida guilliermondii 03(04.54%), Candida parapsilosis 02(03.03%) were identified. Among non-dermatophytic molds most common isolated fungi Fusarium 15(22.72) and only 1 Aspergillous spp was identified during this entire study. [Table 4]

Table 1: Distribution of cases according to age group (n= 118)

	Total no	%
0-20 years	17	14.4
21-40 years	51	43.22
41-60 years	45	38.13
Above 60	5	4.23
Total	118	100

Figure 1: distribution of cases according to site of involvement (n =118)

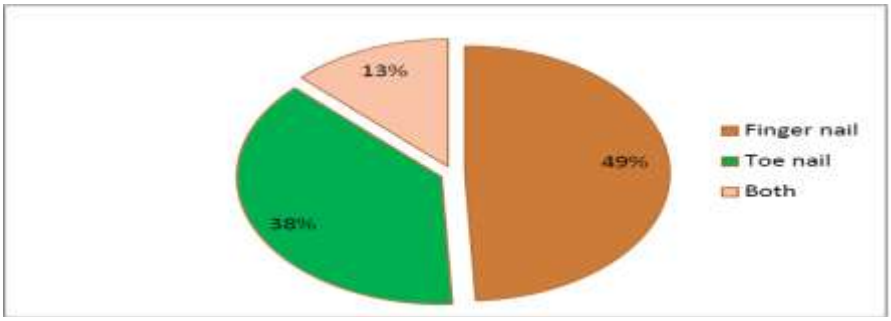


Figure 2: Column diagram showing distribution of cases according to clinical types and different age group (n =118)

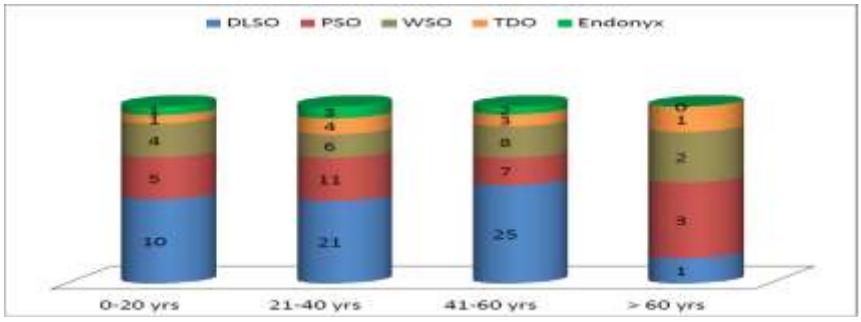


Table 2 Microscopy and culture positivity of 118 clinical samples

	Sample no (%)
Total KOH positive	72 (61.01%)
KOH positive & culture negative	32 (27.11%)
KOH negative & culture positive	22 (18.64%)
Total culture positive	62 (52.54%)
Both positive	33 (33.89%)
Both negative	24 (20.33%)

Figure 3: Distribution of cases according to clinical type and mycological types (n=118)

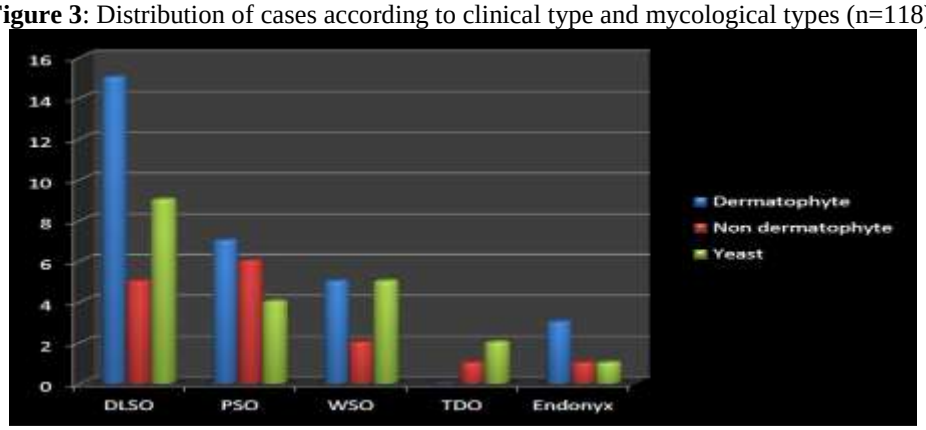
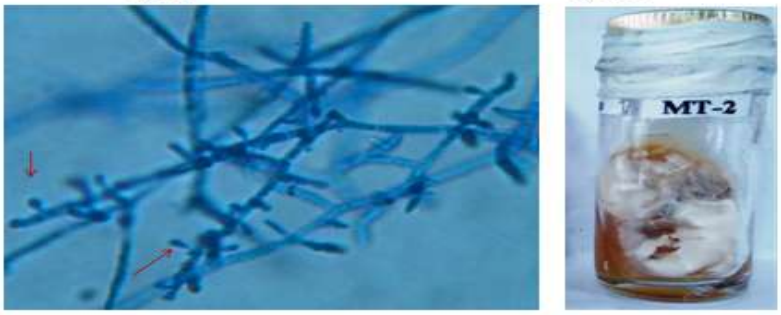


Table 3: Distribution of fungal species according to the different clinical types (n=118)

	Dermatophyte 30								Non dermatophyte 15					candida 21				
	T. kanagawense	T. rubrum	T. soudanense	T. schoenleinii	T. interdigitale	T. verrucosum	Epidermophyton spp.	Microsporum spp.	F. solani	F. oxysporum	F. moniliforme	F. dimerum	A.terreus	C. tropicalis	C. parapsilosis	C. albicans	C. guilliermondii	Total
	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %
DLSO	4(6.06%)	4(6.06%)	2(3.03%)	1(1.51%)	1(1.51%)	1(1.51%)	2(3.03%)	0(0%)	1(1.51%)	1(1.51%)	2 (3.02%)	1 (1.51%)	0 (0%)	2 (3.03%)	2 (3.03%)	3 (4.54%)	2 (3.03%)	29 (41.04%)
PSO	0(0%)	2(3.03%)	2(3.03%)	1(1.51%)	1(1.51%)	1(1.51%)	0(0%)	0(0%)	1(1.51%)	2(3.03%)	1 (1.51%)	2 (3.03%)	1 (1.51%)	3 (4.54%)	0 (0%)	0 (0%)	0 (0%)	17 (25.75%)
WSO	0(0%)	2(3.03%)	1(1.51%)	1(1.51%)	0(0%)	0(0%)	0(0%)	1(1.51%)	1(1.51%)	0(0%)	1 (1.51%)	0(0%)	0 (0%)	1 (1.51%)	0(0%)	3 (4.54%)	1 (1.51%)	12 (18.18%)
TDO	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(1.51%)	0(0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0(0%)	2 (3.03%)	0 (0%)	3 (4.54%)
Endonyx	2(3.03%)	1(1.51%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1 (1.51%)	0 (0%)	0 (0%)	1 (1.51%)	0	0 (0%)	0 (0%)	5 (75.75%)
Total	6(9.09%)	9(13.63%)	5(7.57%)	3(4.54%)	2(3.03%)	2(3.03%)	2(3.03%)	1(1.51%)	4(6.06%)	3(4.54%	5 (7.57%)	3 (4.54%)	1 (1.51%)	7 (10.60%)	2 (3.03%)	8 (12.12%)	3 (4.54%)	66(100)%

a) TDO b) DLSO



C) Microscopic morphology of Trichophyton rubrum. Pear shaped microconidia are attached lateral to hyphae (red arrow)

d) Colony morphology of T.rubrum

IV. 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 present study shows that onychomycosis is more common in age group of 21- 40 years (43.22%) which is comparable with other studies done by Ahuja S et al.[8] and Satpathi P et al.[3] . Least common age group in this study is above 60 years (4.23%) which is comparable to the study done by Lone R et al.[9]. The highest incidence in young adults aged 21-40 years may be due to increased physical activity and increased opportunity for exposure to the pathogens.

In the present study females to male ratio is 1.75:1, which is similar to other studies done by Pukhrambam PD et al.[10] and Puri N et al.[11] Female preponderance may be due to increase awareness among female population regarding medical advice and due to greater exposure of wet work as well as trauma while doing work.

Analyzing the occupational relationship with onychomycosis it is observed that the bulk of patients in our study were either house-wife (26) or farmer (22). Together they comprises of 40.67% of the total cases which is in accordance with other studies [3,11] Repeated trauma to nail, excessive perspiration and contact with the soil saprophytes might be the reason why these sections of population suffer more from onychomycosis.

Both the finger and toenails are affected in 12.71% patients. Although toe nails have been reported in the literature, to be more commonly involved[1], our finding is in accordance with many other studies [12, 13] in which finger nails are found to be more frequently affected. Finger nail involvement is commoner in females also confirmed by other authors.[14]Such pattern of involvement may be due to greater involvement of women in household works. Moreover fingernail infection can have a negative impact on their job and thereby compelling them to report to a doctor early. In India and other tropical countries where the majority of the people are barefooted, the incidence of toe nail onychomycosis is reported to be extremely low due to ignorance among those people.

Distal-lateral subungual onychomycosis is the most common pattern found in our study similar to the findings of other studies .[3,5] Although direct microscopy can provide clues about the identity of the microorganism, careful matching of microscopic and culture results are necessary for confirmation of the diagnosis. In the present study KOH positivity was found in 61.01% and culture positivity was 52.54% which is close to the findings of Rubeena Lone et al.[9] However, in the study which was conducted by Das et al.[15] showed direct microscopy positive only in 32.94%, while culture positivity was in 49.4% cases. We observe direct microscopic examination (60.16%) to be more sensitive than culture positivity (52.54%). But culture positivity

helps in the identification of genus and species of different fungi and always remains the gold standard. Many authors have reported a higher positivity than ours, [16] whereas some have a lower rate of positivity.[3]

In the culture isolates, mixed growth are obtained in 4 samples. This finding is similar to the findings of other studies.[5,10] 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.

In our study the most common fungi isolated in culture are dermatophytes followed by candida and NDM. In a study by Ahmed R *et al* [17] in Karachi, dermatophytes were found to be 50% which is almost comparable to our study (dermatophytes 45.5%). Though yeasts have been quoted in literature as being responsible for many cases of onychomycosis worldwide,[18, 19] but studies conducted in different parts of India and abroad have also reported dermatophytes as the commonest cause of onychomycosis. [1, 10] This difference could be attributed to changes in global climate and humidity favouring the of growth different pathogens over the years.

T. rubrum is the most common isolate identified in our study which corroborates well with the report of other Indian authors[4] and also by other countries e.g. Finland,[20] Spain.[21] UK,[22]. The high prevalence of onychomycosis caused by T. rubrum might be due to its ability to adapt to the hard keratin of nail. The present study shows candida to be the second most common cause of onychomycosis in which *Candida albicans* being the commonest species. This can be attributed to the fact that epidemiology of onychomycosis varies from one geographical region to other.

Candida albicans was reported as the commonest cause of paronychial onychomycosis.[23] This is reflected in our study where all paronychial cases grew *Candida albicans* on culture. *Candida albicans* previously regarded as contaminant yeast, now is recognized as important pathogen in fungal nail infection.

V. Conclusion

Onychomycosis can no longer be considered a simple cosmetic nuisance confined to the nails. It is a significant and important disease which can generate many physical, physiological and occupational problems, considerably impairing patient quality of life.

In this study the combined sensitivity of direct microscopy and culture was greater than direct microscopy and culture alone. This emphasizes the need of performing both tests. 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. Moreover clinico-epidemiological data can also be helpful in public awareness and development of diagnostic, preventive and treatment strategies.

Generally without proper treatment, onychomycosis becomes a chronic disease and more difficult to treat due to added secondary bacterial infection. Hence if the surveillance of this infections and the awareness level in the common population is increased, the burden of this type of disease can be reduced to much lower level.

VI. Limitation & scope

Diagnostic methodology and fungal susceptibility testing lag behind therapeutic advances. Our attention in future should be focused on antifungal susceptibility testing of dermatophyte as it has been observed that patients are not responding to commonly used antifungal which indicate emergence of drug resistance among wild strains of dermatophytes. This also emphasize the need of more advanced diagnostic mode like molecular characterization for better diagnostic purpose.

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