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Clinico mycological study of onychomycosis

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Abstract:
Background*: Onychomycosis is defined as fungal infection of nails caused by dermatophytes, yeasts and non dermatophytic filamentous fungi. It is generally considered as the disease of middle aged and elderly. Increase in the incidence of causative pathogens associated with onychomycosis in recent years is attributed to various factors like aging, expanding number of immune compromised patients and changing life style. The fungal pathogens are generally similar throughout the world however the pattern of dermatophyte infections may vary in different geographic locations due to multiple factors. Certain dermatophytes are endemic to specific geographical areas and with time these relations may change.*
Aim: *To study the clinico epidemiological profile of onychomycosis in 100 patients and to investigate the mycological profile of agents responsible for onychomycosis with direct microscopy using 20% KOH and culture on sabouraud's dextrose agar.*
Methods: *Material for the present study was obtained from patients who attended dermatology OPD in King George hospital, Vishakhapatnam during the period November 2018 to April 2019.*
Conclusion: *It is concluded that onychomycosis is due to dermatophytes, yeasts and non dermatophyte moulds. Dermatophytes are the major followed by yeasts. Trichophyton rubrum was the predominant species causing onychomycosis.*

I. Introduction:

Onychomycosis is defined as fungal infection of nails caused by dermatophytes, yeasts and non dermatophytic filamentous fungi.
It accounts for 30% of cutaneous fungal infections and 50% of nail disorders worldwide. [i]
Zias in 1972 proposed 4 clinical types of onychomycosis according to site of invasion
1. Distal subungual onychomycosis [DSO]
2. White superficial onychomycosis [WSO]
3. Proximal sub ungual onychomycosis [PSO]
4. Candidal onychomycosis [CO]

Clinically lesions range from asymptomatic streak of discolouration to massive destruction of nail plate. As compared to other superficial mycoses, it is more persistent and intractable and may pose a problem to affected individuals because it constitutes a chronic source for recurrent superficial mycotic skin infections at other places.

II. Material and Methods

The material for the present study was obtained from the patients who attended dermatology outpatient department King George hospital, Vishakhapatnam during the period from November 2018 to April 2019.

Selection of patients
Inclusion criteria
1. The patients included were clinically suggestive and mycologically confirmed cases of onychomycosis.
2. Patients willing to undergo necessary laboratory investigations for the diagnosis of onychomycosis.

Exclusion criteria
1. Patients already receiving antifungal therapy.
2. Patients having severely incapacitating systemic or skin diseases.

Methods

Clinical assessment

A detailed history of every patient along with complaints, duration of illness was taken and noted .The socio economic factors like occupation, income and education were recorded. The general physical examination and systemic examination were conducted in all cases. All the patients were also examined for other associated skin or systemic disease.

Collection of material

The affected nails were cleaned with 70 percent alcohol. Nail samples were collected using a sterile nail clipper. The nails were clipped as proximally as possible till the junction of healthy with the diseased nail was reached. The distal and outer most part of clippings are discarded.

In case of distal sub ungual onychomycosis the underlying nail bed and under surface of nail plate were scraped using a sterile scalpel blade. In case of white superficial onychomycosis, minute scrapings were taken from superficial areas of affected nail plate. In case of proximal sub ungual onychomycosis, nail scrapings were taken relatively from deeper layers of nail plate.

Transport of specimens

The specimens were transported in sterile paper packets which allow the specimens to dry out, thereby reducing bacterial contamination and also providing conditions under which the samples can be stored for long period without appreciable loss in the viability of fungus .This method enhanced review of specimen wherever there was a need and also for further processing.

Direct microscopic examination

A small quantity of nail sample was taken on a glass slide and treated with a drop of 20 percent KOH and slightly warmed. The sample was kept for 30 minutes. A small portion of the scraped material was placed in a few drops of 5 % KOH in a glass vial. The material was kept overnight. In both cases, a drop of softened nail material was placed on a clean glass slide, covered with cover slip and examined under low and high power of microscope for the presence of fungal elements.

Culture

- Isolation and confirmation of the etiological agents responsible for onychomycosis in the nail sample was carried out according to the standard microbiological procedures as described further. The nail samples were inoculated in to two sets of media i.e., Sabouraud's dextrose agar with chloramphenicol and Sabouraud's dextrose ager with chloramphenicol and cycloheximide.
- In case no growth seen in six weeks time, the sample was reported as negative. In most cases the culture was repeated more than once.
- In case where contamination suspected subculture done.

Microscopic examination of colonies

When growth became evident on the slope, macroscopic features of the colony such as colony morphology, texture of the colony, surface pigmentation were noted.

Microscopic examination of culture

Needle mount

As soon as satisfactory growth was obtained, a small amount of material was removed from the slope with a sterile bent dissecting needle and placed on a clean glass slide containing few drops of lactophenol cotton blue. The material was properly teased and a cover glass was then placed to delineate the exact morphological characteristics of fungus like septate hyphae, pseudo hyphae, macroconidia, microconidia.

Slide culture

This is the most successful method for examining details of conidial structure and form. In this method fungus was inoculated on to the four sides of agar, sandwiched between a glass slides and cover slip and was kept in a sterile petri dish with a moist atmosphere. The petri dish was incubated at room temperature and examined periodically for growth. When the growth was noted, the preparation examined under microscope. The overlying cover glass with growth of fungus was placed on a slide on which two drops of lactophenol cotton blue was applied to the preparation.

Germ tube test

The isolated candida species was treated with normal human serum and incubated at 37 degrees Celsius for 2 to 4 hrs. A drop of suspension was examined on a slide under the microscope. The germ tubes were seen along tubes as long tube like projections extending from yeast cells. In the cases of candida albicans germ tubes were formed within 2 hrs of incubation.

III. Result

Table 1: Distribution of onychomycosis patients according to the locality

Locality	Male		Female		Total	
	No of Pts	%	No. Of Pts	%	No .of Pts	%
Urban	40	76.92	44	91.67	84	84
Rural	12	23.08	4	8.33	16	16

Out of a total 100 patients studied, 84% were from urban area i.e., and 16% were from rural areas (Table I). A male preponderance was noticed in this study consisting of 52 males (52%) and 48 females (48%)

Table 2: Age and Sex distribution of onychomycosis patients

Age group (in years)	**Male**		**Female**		**Total**	
	No. of Pts	%	**No. of Pts**	**%**	**No. of Pts**	**%**
0-10	1	1.92	1	2.08	2	2
11-20	5	9.62	4	8.33	9	9
21-30	19	36.54	17	35.42	36	36
31-40	12	23.08	12	25.00	24	24
41-50	7	13.46	8	16.67	15	15
51-60	6	11.54	5	10.42	11	11
>60	2	3.85	1	2.08	3	3
Total	52	100.0	48	100.0	100	100

The age of patients ranged from 5 to 80 years, while majority i.e., 60 out of 100 patients were between 21 – 40 years of age. These observations were mentioned in Table 2

Table 3: Distribution of onychomycosis patients according to occupation

Category	No of patients	%
1)Associated with increased physical activity:	45	45
a)Labourers	32	71.11
b)Farmers	7	15.5
c)Mechanics	2	4.44
d)Vehicle operator	2	4.44
e)Tailor	2	4.44
2)Associated with wet work	25	25
a)House wives	8	32
b)Domestic helper	5	20
c)Washer men	4	16
d)Cooks	3	12
e)Hotel worker	5	20
3)OTHERS	30	305
a)Office personnel	10	33.3
b)Businessman	5	16.67
c)Students	5	16.67
d)Non specific	10	33.33

Table 4: Distribution of onychomycosis patients according to duration

Duration(years)	No of pts	%
0- 1	38	38
1-5	28	28
5-10	24	24
>10	10	10

The duration of disease varied from 3 months to 15years. 38 % of the patients i.e., more than one third of them had disease for less than one year.

Table 5: Clinical features of onychomycosis in patients

Clinical features	No of patients	%
Discoloration	100	100
Paronychia	32	32
Sub ungual hyperkeratosis	35	35
Onycholysis	26	26
Destruction of nail plate	3	3

Discolouration of nail plate was seen in all the 100 cases. Paronychia and subungual hyperkeratosis were seen in 32% and 35%.

Table 6: Previous treatment received

Treatment	No of patients	%
No treatment	60	60
Antifungals	20	20
Antibiotics	5	5
Steroids	6	6
Combinations	5	5
Home remedies	4	4

60% of the patients did not take any treatment, 20% patients had taken antifungal treatment.

Table 7: Associated fungal infections in case of onychomycosis

Clinical type	No of patients	%
Tinea corporis	11	11
Tinea pedis	7	7
Tinea manuum	2	2
Combinations	2	2
Total	22	22

Co existing fungal infections over other sites of body were seen in 22% of the cases. Tinea corporis was the commonest in 11% of the cases.

Table 8: Site of involvement in onychomycosis

Site	unilateral	bilateral	total	%
Finger nails	24	10	34	34
Toe nails	15	5	56	56
Both	8	2	10	10
Total	83	17	100	100

Toe nails were affected in 56% .Finger nails in 34% patients

Table9: Clinical types in onychomycosis

Type	male	Female	total	%
Distal sub ungual	42	28	70	70
Candidal	10	18	28	28
White superficial		1	1	1
Proximal subungual		1	1	1

Distal sub ungual onychomycosis was seen in 70% of patients. Candidal onychomycosis was seen in 285 patients, white superficial type seen in 1%.

Table 10: Culture isolates in relation to site of involvement

Fungus	Finger nails	%	Toe nail	%	both	%	Total	%
Dermatophyte	8	27	42	79.25	6	75	56	62.22
T.rubrum	6	20	24	45.28	4	50	34	37.7
T.mentagrophytes	1	3.45	16	30.19	1	12.50	18	20
T.violaceum			1	1.89	1	12.50	2	2.22
E.floccosum	1	3.45	1	1.89			2	2.22
yeasts	21	72.41	5	9.43	2	25	28	31.11
C.albicans	20	68.97	2	3.77			22	24.4
Other candida	1	3.45	3	5.66	2	25	6	6.67
moulds			**6**	11.32			**6**	**6.67**
Aspergillus			2	3.77			2	2.22
Curvularia			2	3.77			2	2.22
Geotrichium			1	1.89			1	1.11
Pencillium			1	1.89			1	1.11
Total	29	100	53	100	8	100	90	100

IV. Discussion

A prospective study was conducted among 100 clinically and mycologically confirmed cases of onychomycosis. The observations analysed and discussed below

People living in urban areas appear to be associated with higher prevalence of onychomycosis. Majority (84%) of the patients with onychomycosis in present series were from urban areas. People living in urban areas are more cosmetic and health conscious with easy accessibility to health services and generally wear closed foot wear, could be some of the reasons responsible for an increased prevalence of onychomycosis among people living in urban areas as observed in present series (84**%)**[i,ii]

Onychomycosis affects both males and females; however male preponderance is noticed in this study where in 52% being males and 48% females, similar observations were noted in other studies**.**[iii] The increased prevalence in males is due to the fact that males are more prone to trauma in the work atmosphere and due to more occlusive foot wear.[iv]

The commonest age group affected in present series was between 21 – 30 yrs followed by 31- 40 yrs and this feature was also observed by several Indian studies[v]**.** The higher prevalence of onychomycosis among 21- 40yrs can be attributed to factors such as increased physical activity, increased exposure to wet work and shoe wearing habit, however in the western countries the disease is more prevalent in elderly age group above 40years. The incidence of onychomycosis increases with age, reasons for which may include poor peripheral circulation, diabetes, repeated nail trauma, long exposure to pathogenic fungi, sub optimal immune function.

The duration of disease varied from 3months to 15 yrs. The prolonged duration could be explained by asymptomatic in majority and hence only a few people seek medical advice. The asymptomatic and indolent course is also observed by other workers.[vi]

In 85% of the patients in the present study, the infection started primarily in the nail itself and in the remaining, it was preceded by fungal infection on other parts of the body. Discolouration is the earliest feature of the fungal infection. In the present study this is seen in 100% cases, sub ungual hyperkeratosis and onycholysis is seen in 35% and 26%. According to Ramesh et al subungual [vii] hyperkeratosis and nail discolouration were most common clinical signs, the presence of which should arouse a clinical suspicion of onychomycosis. Paronychia was seen in 32 cases, where in pain, swelling and redness of the proximal nail fold were observed. The association of candidal infection with paronychia is well established and has been reported in many studies.[viii]

Toe nails 56% were more frequently involved in comparison to finger nails 34% in present series which is in agreement with other studies, while Velez et al reported an increased involvement of finger nails compared to toe nails in their studies. Toe nails are about seven times more frequently affected than fingernails due to three times slower growth rate.[ix] The predominant involvement of the toe nails could be because of greater susceptibility of toes to trauma with subsequent invasion of the nails by fungus. Farmers while working in the fields wear closed rubber shoes and this combination of occlusion, perspiration, occupational trauma and exposure to soil saprophytes favours the growth of the fungus. Also, peripheral vascular disease appears to be the predisposing factor. A large proportion of our patients were engaged in manual labour that entails a higher risk of trauma to the nails and making prone to dystrophic subungual onychomycosis. Walking barefoot, wearing ill-fitting shoes, nail biting (onychophagia), and working with chemicals further predispose Indian patients to onychomycosis. [x]

Distal sub ungual onychomycosis was the most common clinical type noted in many studies including the present study where in DSO was seen in 70% followed by candidal onychomycosis in 28% of the patients and white superficial onychomycosis and proximal sub ungual onychomycosis each in 1% of the patients. Trauma predisposing to onychomycosis may be the cause of this highest presentation of DSO.

Candidal onychomycosis (28%) was the second commonest type and this is in conformity with the study of Dogre et al[xi]. While some authors reported candida species as the common pathogen of onychomycosis. In the present series candidal onychomycosis was more frequently seen among those whose occupation mainly involved with wet work (25%).

Prose et al[xii] reported that proximal subungual onychomycosis is nearly pathognomic of HIV infection and is more frequently seen when the cd4 count falls below 450/mm3. In the present study, proximal subungual onychomycosis and white subungual onychomycosis were observed in one each respectively and both the patients were tested positive for HIV antibodies.

Daniel et al[xiii] reported that host factor may be important in favouring white subungual onychomycosis which include decreased immune function; response against the fungus secondary to HIV infection is probably involved. Several studies report that occupations play a major role in causation of onychomycosis**.**[2] In this study 45% of the patients had occupations associated with increased physical activity. Trauma inflicted to the nails as a result of hard work facilitates easy entry of fungal pathogens. Further, 25% of the patients had occupations associated with wet work is suggestive of the fact that people involved in chronic wet work are prone to develop candidal paronychia and candidal onychomycosis. Among patients with occupations involving increased physical activity the major clinical type seen was DSO and among patients with wet work candidal onychomycosis.

In the present series, all the hundred cases were subjected to both direct microscopy and culture. In 97% of the cases direct microscopy was positive while 90% of the cases were culture positive. According to Kaur et al[xiv] the two conventional methods for identification were direct microscopy and presence of fungal culture, while 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.

Dermatophytes are the chief etiological agents causing nail infections has been established by previous studies**.**[3]In the present study dermatophytes were responsible for 62.2% of the total in cases. Among the dermatophytes T.rubrum was the most common dermatophyte isolated in about 37.7% of the cases, followed by T.menatagrophytes in 20% of the cases among the dermatophytes. Also among DSO clinical type of onychomycosis, T.rubrum was the most common isolate obtained from 53% of the cases**.** Other dermatophytes isolated were T.violaceum and E.floccosum. In the present study 2 patients were tested to be positive for HIV antibodies. In one patient mentagrophytes var interdigitale was isolated and clinically presented as white superficial onychomycosis. While in another patient T.rubrum was isolated and clinically it presented as proximal subungual onychomycosis.

In the present in dermatophyte were isolated predominantly from toe nails in 79.25% and finger nails in 27.59%. Yeasts were isolated from finger nails in 72 .41% of cases and from toe nails in 9% of the cases. This is similar to studies of Clayton et al observations showing candida species responsible for 1-32% of toe nail infections and 50 -72% of finger nail infections

In the present study yeasts were the second most common group of organisms isolated in 31.1% of the causative organisms. Among candidal onychomycosis, candida albicans were isolated in 75% cases.

In the present study nondermatophyte moulds were isolated in 6.66% of the cases. The rates of isolation of moulds in onychomycosis are reported between 1.5-6% by various authors and in present study similar observations were made. Aspergillus and curvularium being isolated in 2 cases each, while pencillium and geotrichium being isolated 1 case each. Moulds were predominantly isolated from toe nails in the present study and this is in accordance to other studies.

V. Conclusion

Although onychomycosis is not life threatening, it can cause a significant negative impact on the quality of life of infected patients. This study highlights that microbiological confirmation in case of onychomycosis is essential before the initiation of antifungal treatment.

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