Section: Microbiology

Original Research Article

ETIOLOGICAL AGENTS AND DEMOGRAPHIC CHARACTERISTICS OF ONYCHOMYCOSIS AT A TERTIARY CARE HOSPITAL OF MAHARASHTRA

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Abstract

Background: This is a study about fungal infections of nails, one of the important skin appendages. The study was conducted to formulate baseline data for the species occurrence of various fungal agents in patients with suspected onychomycosis attending the Department of Dermatology, Government Medical College, Aurangabad between January 2016 to June 2017.**Materials and Methods:** 113 clinically suspected cases of onychomycosis were subjected to mycological studies. **Result:** Total direct microscopy positivity was 70% and total culture positivity was 52%.Highest number of cases belonged to the younger age group of 21-30 years (28.3%) followed by the age group of 31-40 years (25.6%). Males (60.1%) were more affected than females (39.8%). Most common organism turned out to be T.mentagrophytes with 45.7% followed by T.rubrum with 40.6%. Thus, dermatophytes were the leading causative agent with 86.4% of the total agents isolated. Nondermatophytes contributed to 3.3% and yeasts accounted for 10.1% cases. **Conclusion:** Dermatophytes, mainly T.mentagrophytes were found to be the main culprit, causing considerable morbidity accompanied by cosmetic compromise in young population, mainly working outdoors.

INTRODUCTION

Onychomycosis, responsible for up to 50% of all nail diseases and 30% of all fungal infections is a chronic fungal infection of nails.[1,2] It derives its name from Greek word “onyx” meaning nail and “mykes” meaning fungus. It has varied worldwide distribution ranging from 2% to 50% with the incidence being particularly high in warm humid climates such as India.[1,3] But even in the more advanced Asian countries, onychomycosis has been highlighted only in the last decade or so.[1]

Dermatophytes is responsible for most (90%) cases of onychomycosis of toenails and at least 50% of fingernail infections,[4] main culprits being of genus Trichophyton, mainly Trichophyton rubrum and Trichophyton mentagrophytes. One other important organism includes Candida.[5] Chronic exposure to moisture and chemicals including detergents, breached local immunity due to trauma, contributes to Candidial onychomycosis.[6] Candidial onychomycosis (CO) lacks gross distortion and accumulated detritus mainly affects fingernails.[7]Nondermatophytes are less common causative pathogen in general population causing 1.5% to 6% of cases of onychomycosis, mostly seen in toenails of elderly individuals with a history of trauma.[3]

Compared to other superficial and cutaneous mycosis, onychomycosis is intractable and persistent, thus posing serious concern to the clinicians as it represents a chronic course of recurrent superficial fungal skin infections, besides causing considerable disfigurement.[8]

The present study was carried out to find out the various etiological agents responsible for onychomycosis along with its demographic pattern in patients from urban and rural regions of marathwada.

MATERIALSANDMETHODS

Source of Data: The present study was an observational, prospective study conducted at GMC, Aurangabad, post approval from ethical committee. The study population consisted of all the clinically suspected cases of onychomycosis who visited the Dermatology Outpatient Department during the period of January 2016 to June 2017.

Inclusion Criteria

Patients with clinical features of Onychomycosis presenting in Dermatology Outpatient Department.

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Exclusion Criteria

Patients who underwent treatment with systemic or topical antifungal agents within 4 weeks preceding the study period were excluded.

Specimen collection: A written, informed consent of the suspected cases were taken and complete history was recorded. General examination of all finger and toe nails was carried out. Patients showing sign of onychomycosis were subjected to sample collection. Diagnosis was made through 40% Potassium Hydroxide (KOH) preparations and culture of nail samples on various medias.[9]

Method: The procedure of sample collection was explained to the patient. After cleaning with 70% alcohol, nail scrapping/ clipping were collected in a sterile plain bulb/ sterile petri dish.

For nail clipping pre-sterilized nail clipper was used, while for nail bed scraping sterile scalpel blade no.15 was used. Whenever possible subungual debris were also collected.

Processing of Samples-

Microscopy: After 24 hours, KOH wet mount was prepared and microscopy done using low and high power of microscope.[7]. Fungal hyphae, arthrospores, yeasts and pseudohyphal forms were looked for, the next day.

Culture: All the nail samples were inoculated with strict adherence to all aseptic precautions as follows:-

1. Two slopes of Sabouraud Dextrose Agar (SDA) with antibiotic (Chloramphenicol) were inoculated. One was kept at room temperature (25^0C) for dermatophytes and molds. The other slope was incubated at 37^0C for yeasts.
2. One plate of Dermatophyte Test Medium (DTM) was inoculated for primary isolation of dermatophytes from the specimen and incubated at 25^0C
3. Sterile un-inoculated tubes of SDA slopes containing antibiotic and sterile DTM plates of the same lot were also incubated as controls with each batch, so as to rule out aerial contamination and to ensure media sterility.

Culture tubes and plates were examined daily for the first week and on alternate days thereafter till 4 weeks of incubation. The culture was labeled as sterile if no growth was observed at the end of 4 weeks, and was discarded. On DTM, change of color of media from yellow to red was looked for. On SDA, rate of growth, appearance, size, texture, color on obverse and pigmentation on the reverse was observed.

In this study, presence of fungus, either in KOH and/or fungal culture was considered as a positive case of Onychomycosis.

Because of difficulty in differentiating pathogens from contaminants, following guidelines were used 1) If a dermatophyte was isolated on culture, it was a pathogen regardless of KOH result 2) if a nondermatophyte mould (NDM) or yeast showed growth on culture, it was significant only if direct microscopy was positive along with culture OR Direct microscopy was negative but isolated repeatedly on culture.[10,11]

All culture positive growth was subjected to microscopic examination using Lacto Phenol Cotton Blue (LPCB) for further identification. Slide culture was performed whenever necessary to confirm genus and species level identification.

On LPCB: Shape, morphological features of fungal isolates like hyphae, conidiophores, macroconidia and microconidia, and their relation to hyphae were noted. Presence of special hyphae like spiral hyphae, favic chandelier, racquet hyphae etc were noted.

Slide Culture: So as to gain a better morphological view, growths were subjected to slide culture using Cornmeal agar block when direct LPCB was inconclusive.[12]The growth pattern, morphology of hyphae, conidia and spores for suspected dermatophytes and nondermatophytes was observed using LPCB mount of slide culture growth.

For Candida spp (Yeast like):

a) Colonies were provisionally identified by development of creamy and pasty colonies on SDA
b) This was followed by microscopic examination with Gram stain. Slide culture was performed to look for presence or absence of chlamydospores, blastospores, pseudohyphae.
c) Germ tube testing to grossly classify into C.albicans/C.dublinensis & Non albicans candida.
d) Incubation at 42^0C to differentiate between C.albicans & C.dubliniensis as both are germ tube positive.
e) CHROMagar inoculation to identify the species depending on different colored growth.

RESULTS

A total of 113 clinically suspected cases of Onychomycosis were studied.

The total direct microscopy positivity was 70% and total culture positivity was 52%.

There were six KOH negative but culture positive samples, all of which turned out to be dermatophytes. Thus, overall fungal isolation rate for onychomycosis in this study was 75% (85 cases) [Table 1]

The Chi-square statistic is 23.2875. The p-value is <0.00001. The result is thus statistically highly significant.

Sensitivity and specificity of KOH mount against Culture was 89.8% and 51.8% respectively.

Age: The age of study population ranged from 9 years to 65 years (mean age of 29 years), with maximum cases clustered between 21-40 years of age group. [Figure 1]

Sex: Male preponderance was seen, with male to female ratio of 1.5:1 [Figure 2].

Occupation: [Figure 3]

Site: [Figure 4]

Pathogen: [Table 2]

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Table 1: Statistical data of KOH positivity and Culture positivity

	Culture Positive (%)	Culture Negative (%)	Total (%)
KOH Positive (%)	53 (47)	26 (23)	79 (70)
KOH Negative (%)	6 (5)	28 (25)	34 (30)
Total (%)	59 (52)	54 (48)	113 (100)

Table 2: Shows the predominant pathogen isolated

Species		Number of isolates	Percentage
A)Dermatophytes:-		51	86.4
	T. mentagrophytes	27	45.7
	T. rubrum	24	40.6
B)Nondermatophytes:-		2	3.3
	A.niger	1	1.6
	Scytalidium spp	1	1.6
C)Yeasts:-		6	10.1
	C.albicans	6	10.6
TOTAL		59	100

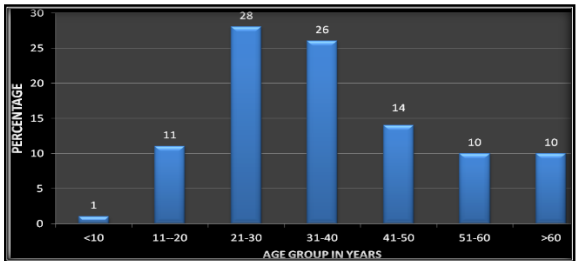


Figure 1: Shows age distribution of onychomycosis cases

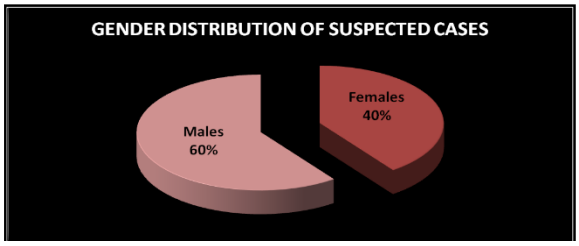
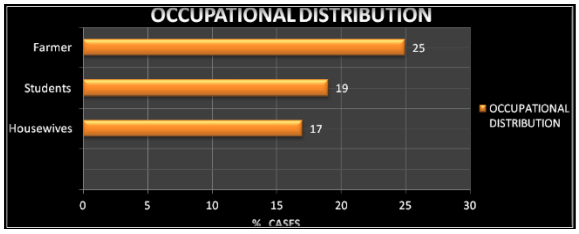


Figure 2:



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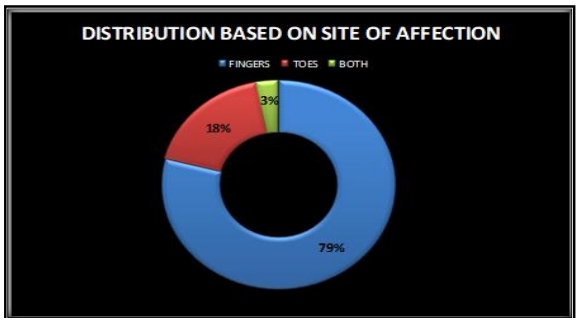

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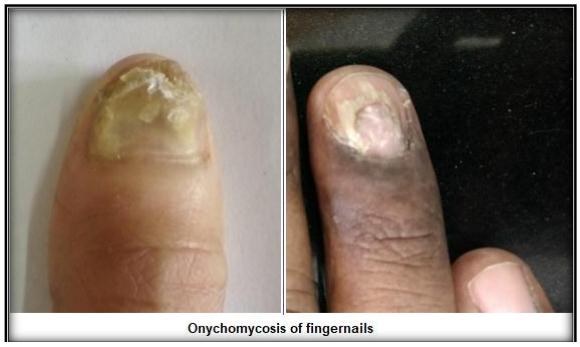
Onychomycosis of fingernails

Figure 5:

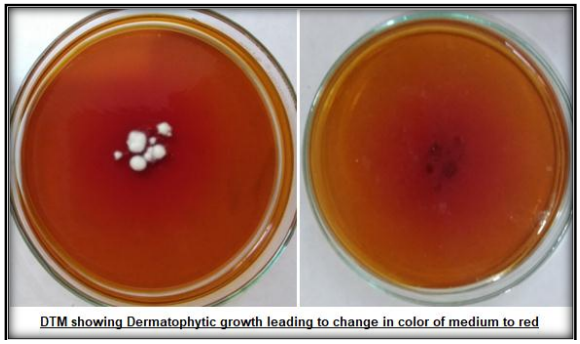
DTM showing Dermatophytic growth leading to change in color of medium to red

Figure 6:

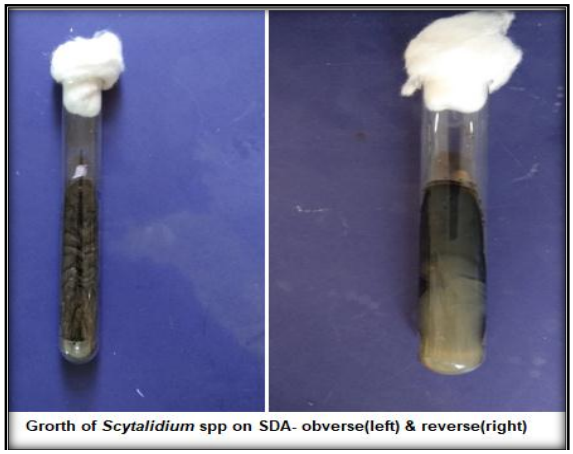
Grorth of *Scytalidium* spp on SDA- obverse(left) & reverse(right)

Figure 7:

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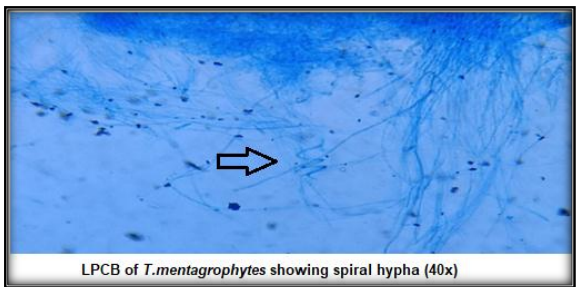

Figure 8:

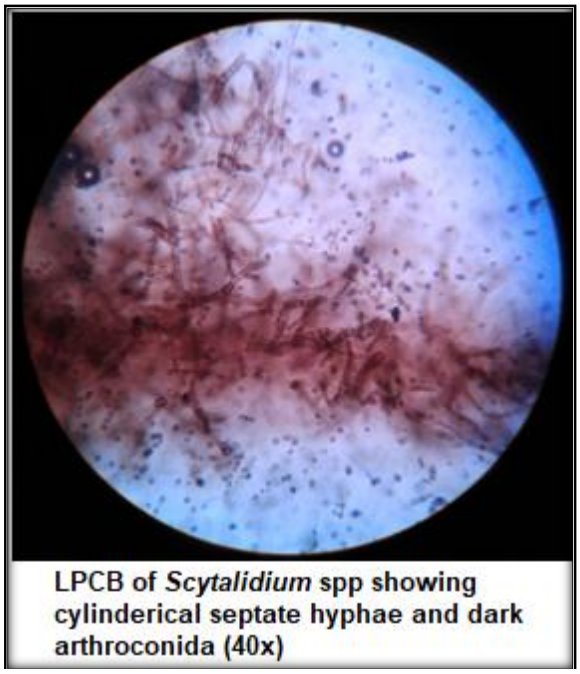

Figure 9:

DISCUSSION

Isolation: The total KOH positivity was 70% and total culture positivity was 52%. All the Six samples that were KOH negative, but culture positive, turned out to be dermatophytes, making the overall isolation rate of onychomycosis in this study to 75%.

Some of the samples were KOH positive but turned out to be culture negative. Presence of non viable fungus in the nail might be the reason.

Some samples were KOH negative but turned out to be culture positive. Low visibility of scattered and scanty fungal filaments on KOH mount may be the reason for this.

In our study 5 samples did not show growth on SDA, but grew on DTM, indicating the importance of inoculation on DTM routinely for all samples.

Positivity for onychomycosis was reported as 78.7% by Prabhav et al,[13] 2017similar to present study. Khalique et al, 1974, Aurangabad, Maharashtra had isolation rate of only 0.6%.[14]Reddy et al, 1977, Uttar Pradesh gave an isolation rate of 2.2%.[15]

This clearly shows a drastic increasing trend in India at alarming rate. The difference in such isolation rates observed in various studies can be caused due to a various factors like age, sex, occupation, residing area etc and also to the fact that patients affected by onychomycosis may or may not seek medical attention.

Age: Onychomycosis can occur at any age but is most commonly seen during 40-60 years of age and is unusual before puberty.[7] All age groups were seen to be affected in present study, from 9 years to 65 years but highest numbers of cases were from the age group of 21-40 years of age.

In a study done by Ashokan et al, 2017, Chennai, majority cases lied in age group of 20-40 years (47%), similar to our study.[16] There were also studies that had cases affecting elderly and children. In a study done by Jeelani et al, 2016, Kashmir, 64% cases belonged to age group of less than 18 years.[17]

The distribution of onychomycosis in our study population is consistent in that, onychomycosis is the disease of the adults and is quite uncommon in children. Less number of children suffer from this disease. The reason for the low prevalence of onychomycosis in children may be attributed to rapid growth rate of nail plate leading to elimination of fungi, difference in structure of nail plate.[18]

Onychomycosis has a tendency to have increasing prevalence with age. Age related factors such as lower peripheral circulation, inactivity and inability to cut the nails and perform its proper care and hygiene thus causing a longer exposure to fungi may be factor. Less number of elderly cases in our study might be due to lower presentation to the hospital either due to ignorance, or may be due to painless asymptomatic nature of the condition in majority of the cases and dependency upon others for medical and social help. On the other hand increased cases in young adults may be because they are more often exposed to occupation related trauma due to the outdoor activities. Self consciousness to the cosmetic aspect than the elderly age groups might also be a reason to approach clinicians on time.

Sex: This disease is more frequent among men than women19. In our study, onychomycosis was found more common in males (60.1%) than in females (39.8%).

Higher number of males were affected in a study by Ratna et al,2015, Andhra Pradesh (62%) which is similar to our study.[20] A study by Alvarez et al, 2014, Columbia had female majority contradictory to our study.[21]

This male preponderance may be due to their outdoor activities due to types of occupation which is found less in females in Indian set up and also because of under reporting.[22] Also, some authors have postulated that the differences in hormonal levels leads to different capacities to inhibit the growth of the dermatophytes.[23]

Occupation: In study of ours, farmers contributed to maximum number of suspected cases (24.7%) followed by students with 19.4% and housewives with 16.8%. Gupta et al, 2006, Shimla conducted a study in which 20% of affected cases were farmers. which is similar to ours, followed by office workers,

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housewives and students with 20%, 10% and 11.5% respectively.[24]

Farmers have an increased risk of developing trauma due to working in fields and so are housewives who are involved in household jobs. Also students have a risk of developing onychomycosis because of the increased extracurricular and physical activities and due to occlusive footwear as well, hence contributing to major chunk

Site: In general, toenails tend to be more affected.[25] Slower growth rate and reduced blood supply of toenails, and being closer to soil makes it more susceptible than fingernails for Onychomycosis. A study by Gupta et al, 2001, Canada more cases of onychomycosis of toenails (22.7%) were seen,[26] But, in present study 78.7% had predisposition to fingernails and 18.5% to toenails, while 2.6% showed involvement of both fingernails as well as toenails. Thakur, 2015, Uttar Pradesh, observed maximum cases of 80% of fingernails in her study,[27] Reddy et al, 2012, Karnataka had mostly cases with affected fingernails (55%).[28]

The reason for less toenail cases in study of ours might be because of the increased chances of occupation related trauma to fingernails, as majority of the cases in our study were farmers. Also, infection of fingers is more likely than the toenail infection to arouse cosmetic concern, thus driving patients to seek medical attention.

Pathogen: In present study, Dermatophytes were the leading causative agent with 86.4% of the total agents isolated, and the most common organism turned out to be T.mentagrophytes with 45.7% followed by T.rubrum with 40.6%. Yenisehirli G et al, 2009, also demonstrated study with higher isolation of dermatophytes with T.mentagrophytes in maximum cases (58.3%),[29] A study by Adhikari et al, 2009, Sikkim also had a contrasting result with T.tonsurans as the main agent (44%).[9]

In our study Candidial onychomycosis was found to be in 6 cases (10.1%) amongst all pathogens. All turned out to be C.albicans. Study by Narain et al, 2014, Uttar Pradesh showed Candidial onychomycosis to affect 10% of the cases similar to our study.[30]

In our study all the 6 cases (100%) of Candidial onychomycosis were females. This is in agreement with the study conducted by Koussidou et al, 2002, Greece which had majority females with Candida as the causative agent.[31]

Most of these Candidial onychomycosis patients were housewives. They are predisposed to various minor trauma during various household responsibilities of kitchen work be it cutting, peeling or washing clothes and dishes. This makes them exposed to moist environment and thus prone to injury thus helping the pathogenic fungi to enter. All the six patients were seen to be engaged in various domestic activities that had involvement in wet work and all of them gave a history of trauma. This suggesting that healthy nail doesn't get infected frequently by fungus and demonstrates role of trauma as an important factor. Also, Candidial onychomycosis, being symptomatic, leads to discoloration, disfigurement, pain and thus these females sought treatment earlier.

Non dermatophytes were found out to be 3.3% in our study.

Study conducted by Fragner et al, 1966, obtained rate of 6.3% of non dermatophytes similar to us.[32] Hashemi et al, 2009, Tehran had 19.9% non dermatophytic isolation which is higher than present study.[33]

By comparing our study with various other studies we could conclude that there keeps on occurring epidemiological and mycological changes in the characteristics of onychomycosis. Since the beginning dermatophytes were the most frequently implicated causative agents and non dermatophytes and yeasts were considered to be contaminants. They are now increasingly recognized as etiological pathogens in fingernail infections. The epidemiology of Onychomycosis has multifactorial influences and the presence or absence of particular fungi depends on the age and other factors like association with other diseases and pattern of lifestyle. The similarity and contrast with regards to the most common etiological agents in our study compared to other studies can be because of the above mentioned factors. Since nothing can predict change in the microbiological environment, it is therefore imperative to be aware of such changing patterns as well as the causative fungi so as to make adequate and proper strategies.[34]

CONCLUSION

Once fungus is established in nails, they provide a constant source of infection to other parts of the body[34], causing chronicity. The variability of results in our study show that diagnosis of onychomycosis based solely on clinical features is not sufficient. Here laboratory diagnosis plays a vital role.

The diagnosed cases in our Institute were treated successfully with either Topical drugs like Ciclopirox or systemic drugs like Fluconazole, Itraconazole.

Selection of suitable antifungal agent was possible only if the underlying pathogen is correctly identified. Thus equal contribution from clinicians and microbiologists and their joining hands is the need of the hour.

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