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Original Research Article

Etiological Profile, Clinicomycological Correlation and Risk Association in Onychomycosis

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Abstract

Background: Onychomycosis may manifest itself in various forms, notably onychodystrophy, onycholysis, subungual hyperkeratosis, or nail-plate discoloration.

Aims & Objectives: The study was conducted to formulate baseline data for various fungal etiological agents, its clinical correlation and to understand the risk factors associated, in patients with suspected onychomycosis.

Materials and Methods: 113 clinically suspected cases of onychomycosis were subjected to mycological studies& diagnosis was confirmed with help of direct microscopy and culture.

Results: Overall isolation rate of onychomycosis in suspected cases was 75%.

Most common age affected was 21-40 years (53.9%) and males (60.1%) were more affected than females (39.8%).Majority of suspected patients were farmers (24.7%), followed by students (19.4%). Housewives contributed to 16.8% of cases.

Disease was limited to fingers in 78.7% cases, followed by toes which amounted to 18.5% of the cases. 2.6% had both, fingers as well as toes affected. Distal Lateral Subungual Onychomycosis was the most prevalent clinical pattern found in 68.1% participants. This was followed by Proximal subungual Onychomycosis, Total Onychomycosis, Candidial onychomycosis, Superficial White Onychomycosis in 9.7%, 7.9%, 7% and 7% participants respectively. None of the participants were having endonyx. Associated risk factors included trauma (36.2%) which is also a part of etiological process, followed by use of occlusive footwear (15.9%), diabetes (13.2%). Positive family history was given by 2.6% cases. Predominant organism turned out to be *T. mentagrophytes* with 45.7%followed by *T .rubrum* with 40.6%. Dermatophytes, thus being the leading causative agent with 86.4% of the total agents isolated. Nondermatophytes contributed to 3.3%and yeasts were causative agents in 10.1% cases.

Conclusion: The results show that relying only on the clinical manifestation in the diagnosis of onychomycosis is often misleading. The present study tries to highlight the need for microbiological confirmation in case of onychomycosis.

Keywords: Fungal infection, nail infection, Onychomycosis, Etiological agents, Dermatophytes.

Introduction

Onychomycosis, responsible for up to 50% of all nail diseases and 30% of all fungal infections [1] is a chronic fungal infection of nails [2]. It derives its name from Greek word "onyx" meaning nail and "mykes" meaning fungus.

Onychomycosis is seen in various forms, and the clinical types recognized by a recent classification scheme includes distal and lateral subungual onychomycosis, superficial onychomycosis, proximal subungual onychomycosis, endonyx onychomycosis and total dystrophic onychomycosis [3]

This disease is more frequent among men than women and it increases with age [4]. Dermatophytes account for most (90%) cases of onychomycosis of the toenails &at least 50% of fingernail infections [5]. Most often causative agent is from dermatophytes of genus Trichophyton, mainly *Trichophyton rubrum* and *Trichophyton mentagrophytes* being the main culprits. One other important organism includes Candida [6]. Nondermatophytes are less common causative agent in general population causing 1.5% to 6% of cases of onychomycosis, establishing the ability of dermatophytes to infect skin and its appendages.

There are many risk factors that are associated with onychomycosis such as diabetes, nail trauma, smoking, peripheral vascular disease and immunosupression e.g. Human Immunodeficiency Virus infection [7]. Compared to other superficial and cutaneous mycosis, onychomycosis is intractable, persistent and poses serious concern to the clinicians as it represents a chronic course of recurrent superficial fungal skin infections, besides causing considerable disfigurement [8]. Even though not life threatening, it is a cosmetic problem and also serves as a chronic reservoir producing repeated infections.

Many of the skin conditions mimic onychomycosis, like psoriasis, chronic onycholysis, lichen planus etc leading to clinical confusion. Here laboratory diagnosis plays a vital role in final confirmation and starting of therapy.

Treatment of onychomycosis is becoming a challenging task as the nail fungus usually doesn't live on the nail surface but within the nail bed where there is a rich blood supply, which encourages growth of the fungus. Nail bed uses the nail as a protective shield, so that many topical medicines do not reach the affected area. In addition, most of the systemic drugs have poor affinity to the nail bed tissues. Even with currently available treatments, rate of complete elimination of the disease is not satisfactory [9] Categorization of onychomycosis clinically as well as mycologically definitely improves patient care.

Therefore, the present study was carried out to find out the various etiological agents responsible for onychomycosis, to identify various risk factors associated with onychomycosis and categorize the patients clinically.

Material & Methods:

Inclusion Criteria: The present study was a prospective, observational. The study population consisted of all the clinically suspected cases of onychomycosis who visited the Dermatology Outpatient department.

Exclusion Criteria: Patients who were undergoing treatment with systemic or topical anti-fungal agents within the 4 weeks preceding the study period were excluded.

A written, informed consent of the suspected cases were taken and complete history was recorded with special emphasis on history of trauma, occupation & associated co-morbid diseases. Relevant past medical history was also taken.

General examination of all finger and toe nails was done. Diagnosis was made through amalgamation of clinical presentation, potassium hydroxide preparations and culture of nail samples [10]

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

For nail clipping pre-sterilized nail clipper was used, while for nail scraping sterile scalpel blade no.15 was used.

In suspected DLSO: Nail bed scraping was done after nail clipping as concentration of viable fungi is the highest in the nail bed [11]

In suspected PSO: Upper nail plate of the proximal nail was debrided, and the underlying debris was collected.

In suspected SWO: Superficial aspect of the nail was scraped[6]

In suspected TO: Nail clippings were taken.

In suspected CO: Material from the proximal and lateral nail edges was used.

Processing of Samples: All specimens were subjected to Direct microscopy with 40% KOH and culture.

KOH mount: Sample was mixed in 40% KOH and this preparation was then kept at room temperature for a period of 24 hours [12] .Fungal hyphae, arthrospores, yeasts and pseudohyphal forms were looked for the next day under low and high power of microscope.

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

1. Two slopes of Sabouraud Dextrose Agar with antibiotic (Chloramphenicol) were inoculated. One was kept at room temperature (25^0C) for dermatophytes and molds. The other slope was incubated 37^0C for yeasts.

2. One plate of Dermatophyte Test Medium (DTM) was inoculated for primary isolation of dermatophytes from the specimen and later incubated at 25^0C.

3. Un-inoculated sterile 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 act as sterility controls.

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

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

Because of difficulty in discerning pathogens from contaminants, following guidelines were used to label the isolate as pathogen:

1) If a dermatophyte was isolated on culture, it was a pathogen regardless of KOH result 2) If a non-dermatophyte mould (NDM) or yeast was culture positive, it was significant only if direct microscopy was positive along with culture OR Direct microscopy was negative but isolated repeatedly on culture [13,14]

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

Slide culture: So as to gain a better morphological view, growths were subjected to slide culture using Cornmeal agar block when LPCB was inconclusive [15]. The growth pattern, morphology of hyphae, conidia and spores for suspected dermatophytes and non-dermatophytes was observed using LPCB mount of slide culture.

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 candida albicans*

d) Incubation at 42^0C to differentiate between *C. albicans* & *C. dubliniensis* as both are germ tube positive.

e) CHRO Magar 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)

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

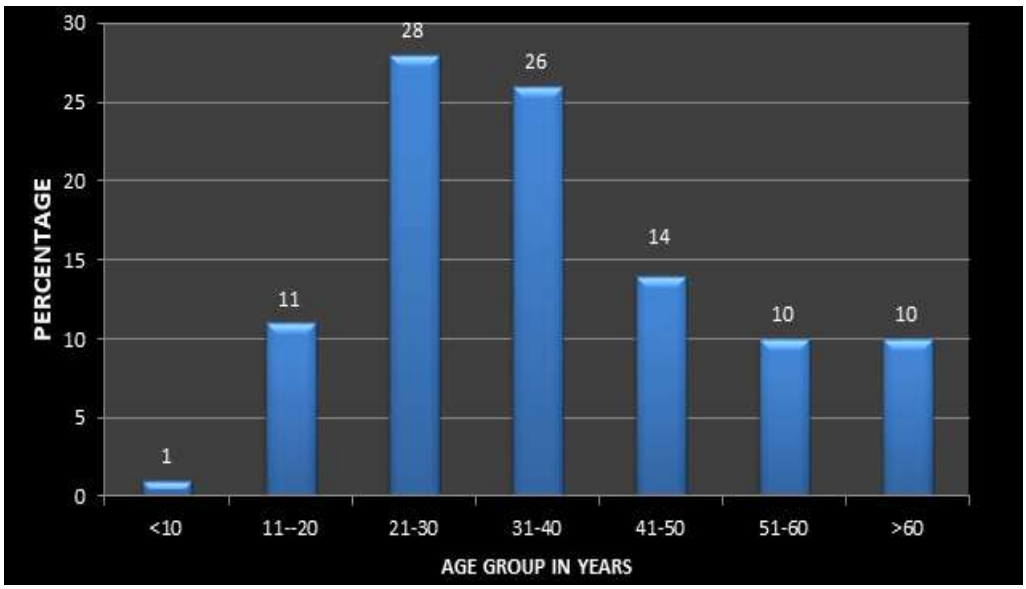


Figure 1: Shows age distribution of onychomycosis cases

Sex: Male preponderance was seen, with male to female ratio of 1.5:1

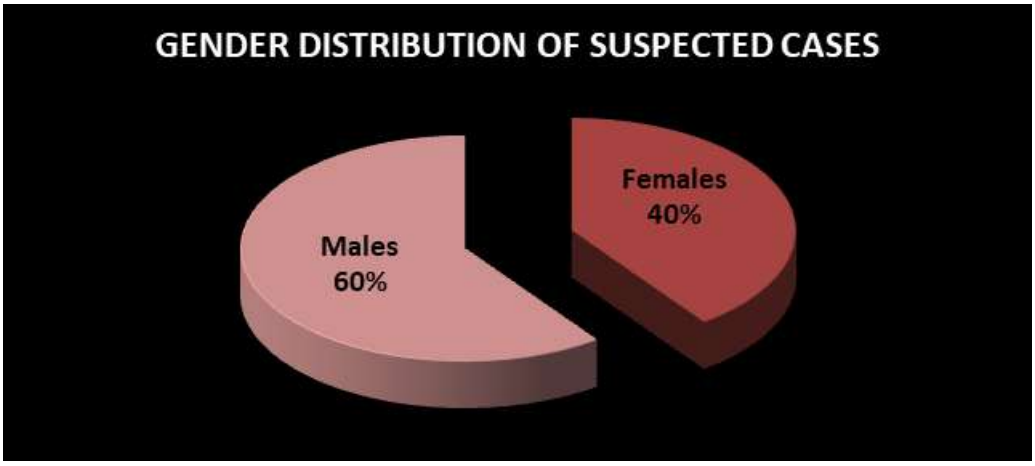


Figure 2: Shows gender distribution of suspected cases

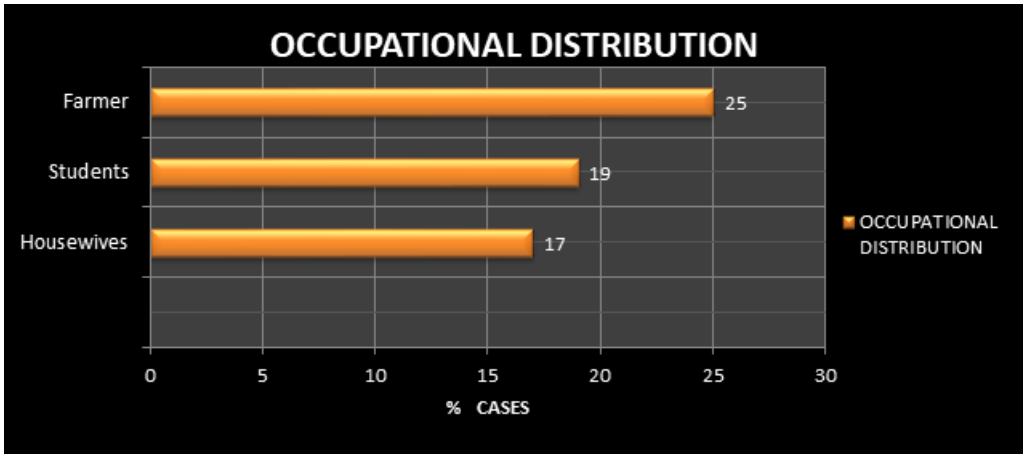


Figure 3: Occupational distribution

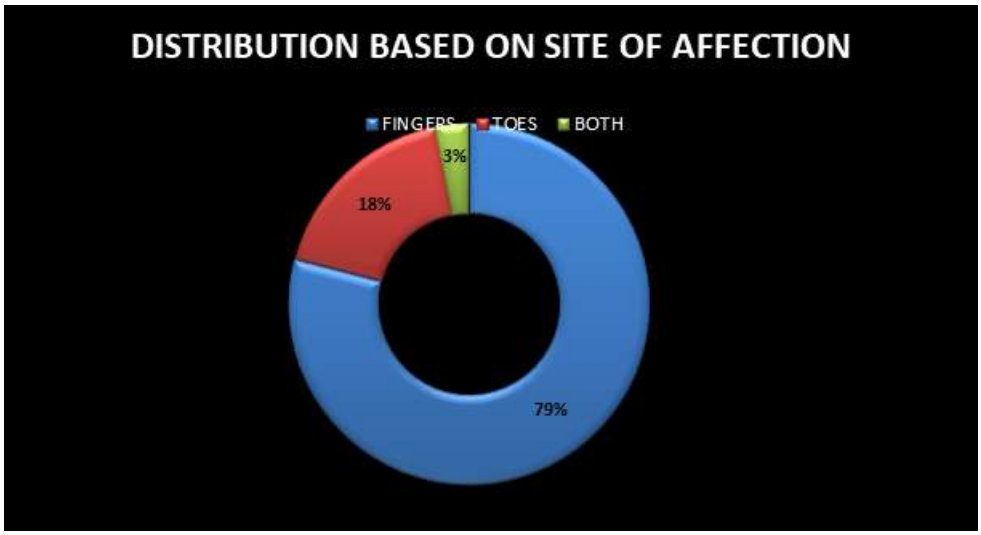


Figure 4: Site affected

Table 1: The observation regarding pattern of Nail involvement-

Sr. No	Type	Cases (%)
1	Distal Lateral Subungual Onychomycosis (DLSO)	77 (68.1%)
2	Proximal subungual onychomycosis (PSO)	11 (9.7%)
3	Total Onychomycosis (TO)	09 (7.9%)

4	Candidial onychomycosis (CO)	08 (7%)
5	Superficial White Onychomycosis (SWO)	08 (7%)
6	Endonyx	00

Table 2: Pathogen isolated in various patterns

Organism	DLSO (n-77)	PSO (n-11)	CO (n-8)	SWO (n-8)	TO (n-9)	Total (N-113)
T. mentagrophytes	25	1	0	1	0	27
T. rubrum	21	3	0	0	0	24
1. niger	0	0	0	0	1	1
Scytalidium spp	0	0	0	0	1	1
C. albicans	0	0	6	0	0	6
Total	**46**	**4**	**6**	**1**	**2**	**59**

Table 3: Associated symptoms

Sr. No	Symptoms	% cases
1	Discoloration	90.2 %
2	Onycholysis	70.7 %
3	Subungual hyperkeratosis	69 %
4	Thickening	44.2 %
5	Pain	15.9 %
6	Paronychia	15.9 %

Table 4: Associated Risk Factors-

Sr. No	Risk factors	Cases (%)
1	Trauma	41(36.2%)
2	Occlusive foot wear	18(15.9%)
3	Diabetes	15(13.2%)
4	Family history	03 (2.6%)
5	Peripheral Vascular Disease (PVD)	00

Table 5: Risk factors observed in various occupations

Sr No	Risk factors	Suspected cases (%)	Farmers cases (%)	Housewives cases (%)	Students cases (%)	Others cases (%)
1	Trauma	41(36.2)	18(43.9)	10(24.3)	3(7.3)	10(24.3)
2	Diabetes	15(13.2)	3(20)	4(26.6)	0	8(53.3)
3	Occlusive footwear	18(15.9)	4(22.2)	0	14(77)	0
4	Family History	3(2.6)	0	0	0	3(100)

Pathogen Isolated

Table 6: Following were the predominant pathogens.

Species	Number of isolates	Percentage
Dermatophytes (*T. mentagrophytes, T. rubrum)*	**51**	**86.4**
Nondermatophytes (*A. niger, Scytalidium spp)*	**2**	**3.3**
Yeasts (*C. albicans)*	**6**	**10.1**
TOTAL	**59**	**100**

Discussion:

Age:

Onychomycosis can occur at any age but is most commonly seen during 40-60 years of age and is unusual before puberty [12]. All age groups were seen to be affected in present study, from 9 years to65 years but highest number of cases were from the age group of 21-30 years(28.3%) followed by the age group of 31-40 years having 25.6% Thus 53.9%cases were between age group of 21-40 years of age in our study. [16-18]

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 [19] 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 [20]

The distribution of onychomycosis in our study population is consistent with the view 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 with subsequent elimination of fungi, difference in structure of nail plate and lack of cumulative trauma [21]

Onychomycosis has a tendency to have increasing prevalence with age. This is due to some 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 nail fungi. Additionally association with various co morbidities such as diabetes, lower immunity also plays a vital role. Lower number of suspected elderly cases in our study might be due to lower presentation to the hospital either due to ignorance, or may be due to comparatively asymptomatic nature of the condition in majority of the cases and dependency upon others for medical and social help. Also, increased cases in young adults might be because they are more often exposed to occupation related trauma due to the outdoor activities and hence predisposing them to greater extent to onychomycosis due to increased exposure of nails to fungi. Also, another reason adding to it may be over self-consciousness to the cosmetic aspect than the elderly age groups which makes them to approach clinicians on time.

Sex:

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 [22] A study by Alvarez et al, 2014, Columbia had female majority contradictory to our study [23]

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 underreporting [24]. Also, some authors have postulated that the differences in hormonal levels leads to different capacities to inhibit the growth of the dermatophytes [25]

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%,while 38.9% cases belonged to various other fields like labor, clerical, welding etc. 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, housewives and students with 20%, 10% and 11.5% respectively [26]

In our study farmers, students and housewives occupied major chunk of total cases. This may be due to the fact that 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 lack of foot hygiene, increased extracurricular and physical activities and due to occlusive footwear.

Most of 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, colonize and damage the tissue.

Risk Factors:

In our study majority cases gave the history of trauma. (36.2%). Svejgaard et al, 2004, Denmark had 17% cases with a history of trauma comparable to our study [27]. In study by Kaur et al, 2008, Delhi 5% cases gave history of trauma [28]

15.9% gave a history of wearing occlusive footwear in present study. In a study by Kumar et al, Kempegowda ,2013, 9% cases gave history of wearing occlusive footwear [29]

Diabetes history was found in 13.2% cases in our study. Dogra et al, 2002, Chandigarh, shows the prevalence of onychomycosis in diabetic patients were about 17% like our study [30]. A study by Gulcan et al, 2011, showed rate of 25%135Agarwallaet al, Nepal had 44% diabetic cases in their study [31]

In our study only 3 cases (2.6%) gave a positive family history.1% cases with family history was found in study by Yadav et al, 2015,Delhi [32] A very high rate of family history (26%) was seen in the study by Gupta et al, 2007, Himachal Pradesh [33]

Trauma as a main risk factor in our study may be due to the fact that, in our study as majority population was formed by farmers and housewives. Also, all the six patients of CO 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 cannot be infected by fungus and demonstrates role of trauma as an important factor.

Majority of the cases in our study were students and farmers who gave history of wearing occlusive footwear. This might be because; footwear leads to crowding of toes or may prevent adequate air circulation. This can prevent evaporation of excess moisture thus compounding to the problem.

Site:

In general, toenails tend to be more affected [34]. A study by Gupta et al, 2001,Canada more cases of onychomycosis of toenails (22.7%) were seen [35] 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 [36]

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.

Type:

DLSO was the most common pattern found in this study (68.1%) followed by PSO, TO, CO and SWO with 9.7%, 7.9%, 7% and 7% respectively. We did not get any Endonyx cases.

Study by Reddy et al, 2012, Karnataka showed DLSO having 78.3%followed by 16.6% patients with CO, 3.34% patients with PSO and 1.7% showing SWO [37]

EO was not found in the present study. This may be attributed to underreporting due to absence of signs of inflammation in the nail bed like onycholysis or subungual hyperkeratosis and presence of normal nail plate.

Symptoms:

In this study 90.2% of the cases showed the sign of discoloration, followed by onycholysis and subungual hyperkeratosis contributing to 70.7% and 69%respectively. Nail thickening, pain and paronychia followed with 44.2%, 15.9% and15.9% respectively.

Discoloration was the most common symptom (98%) in study by Yadav et al, 2015; Delhi which is similar to our study, followed by brittle nails (89%) Pain was reported by 33% cases [38] Kaur et al, 2008, Delhi also had discoloration as the main symptom (100%), followed by pain in 17% and paronychia in 12% of the cases [28]

Gupta et al, 2007,Himachal Pradesh also had similar observation with 92% cases with discoloration followed by subungual hyperkeratosis in 68.5%, onycholysis in 26.9%, dystrophy in 37.7%, and paronychia in 10.7% patients respectively26

Candida albicans is reported as the commonest cause of paronychial onychomycosis1 (10.2%) This is also seen in our study where all the C. albicans cases had a symptom of paronychia.

Pathogen:

In present study, dermatophytes were the leading causative agent with86.4% 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%) [39] A study by Adhikari et al, 2009, Sikkim also had a contrasting result with *T. tonsurans* as the main agent (44%) [10]

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 [40]

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. [41]

Chronic exposure to moisture and chemicals including detergents and breached local immunity due to trauma, as seen in housewives, farmers, and fishermen, contributes to Candidial onychomycosis accompanied by Candida paronychia42.Keratin in the nail substance is known to act as an excellent growth environment for virulent Candida strains42. Candidial onychomycosis (CO) lacks gross distortion and accumulated detritus and mainly affects fingernails [12].

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 nondermatophytes similar to us [43] Hashemi et al, 2009, Tehran had 19.9% non dermatophytic isolation [44]which is higher than present study. [45]

It is now well established fact that geographical distribution of the fungi may vary time to time. 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. Furthermore, the distribution of the

pathogens is not uniform per se, depending on several factors like geography of the particular region and the migration of the population. The similarity and contrast with regards to the most common etiological agents in our study compared to other studies can be because of the abovementioned 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.

Conclusion:

The study and research on onychomycosis is very vital as once the fungus is established in the nails, these infected nails act as a reservoir of the organism providing a constant source of infection to other parts of the body[28], thus leading to chronicity and furthermore hampering the quality of life. The variability of results in studies also show that diagnosis of onychomycosis based solely on clinical features is not sufficient and is often misleading.

To conclude, both, clinical and mycological examinations are important for establishing the definitive diagnosis and must be mandatory prior to the initiation of antifungal oral therapy. Selection of suitable antifungal agent is possible only if the underlying pathogen is correctly identified.

Thus equal contribution from clinicians and microbiologists and their joining hands for better treatment of onychomycosis is the need of the hour.

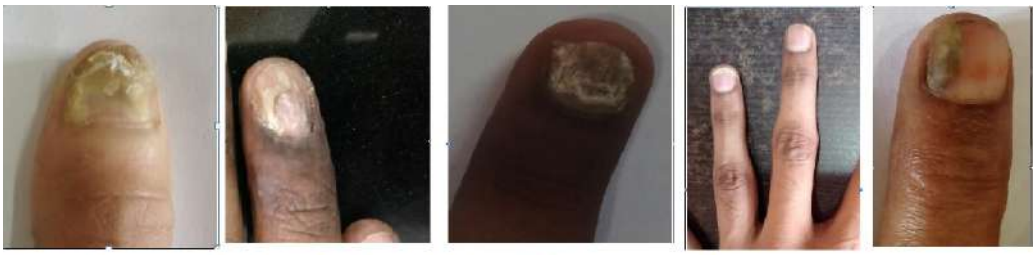


Photo 1- From Left to Right- DLSO, PSO, TO, SWO, CO

Photo 2- KOH mount showing hyphae and budding yeast cells

Photo 3- Lt to Rt- SDA showing dermatophytic growth, DTM with dermatophytic growth, SDA with non dermatophytic growth, SDA with candidial growth

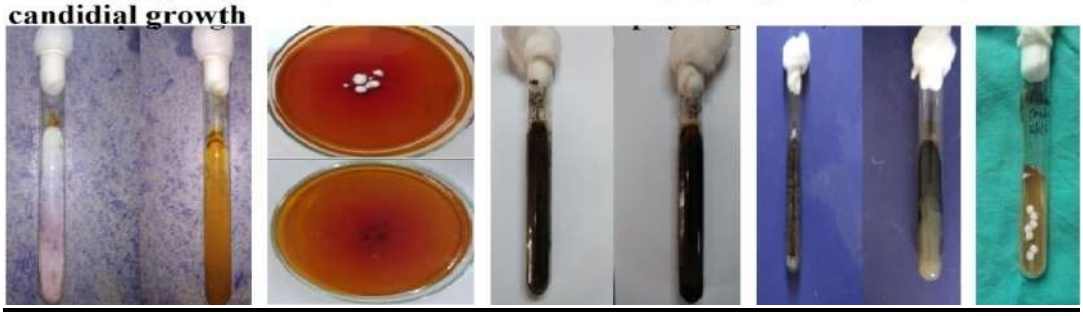


Photo 4-Lt to Rt- LPCB- Showing spiral hyphae of *T.mentagrophytes*, Grape like microconidia of *T.mentagrophytes*, tear shaped microconidia and pencil shaped macroconidia of *T.rubrum*

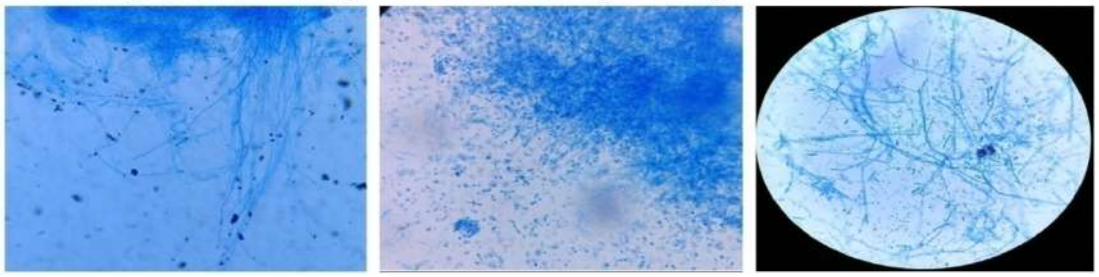
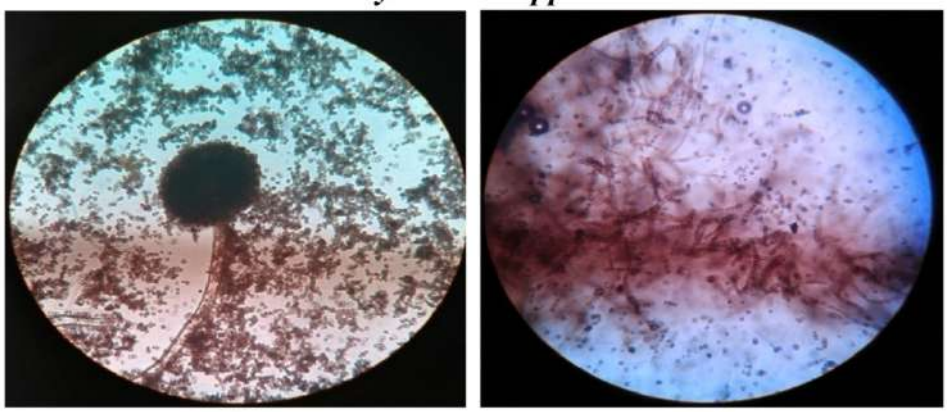


Photo 5- LPCB- showing *A.niger* with microconidia covering entire vesicle (Lt) and on (Rt) cylinderical septate hyphae and dark arthroconidia of *Scytalidium spp*



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