

Epidemiological and diagnostic study of onychomycosis

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Abstract

Objective: The present study aimed to identify the epidemiological factors, determinants and diagnostic methods of onychomycosis which helps in preventing morbidity.

Materials and Methods: An epidemiological and diagnostic study of patients with clinically diagnosed onychomycosis attending the DVL was undertaken and Samples were collected from the diseased nails for microscopy, culture and histopathological staining.

Results: The prevalence of onychomycosis was 1.06% of total outpatient attendance, mostly in men than women, with ratio 1.12:1 with age group 51-60 years. Housewives and agriculturists constituted 32% and 16% respectively. 48% of the patients had ≤ 1 year disease duration. Moisture (42%), tight footwear (6%), trauma (15%), excessive sweating (4%) and warmth (2%) predisposed to onychomycosis. Concurrent superficial fungal infection of skin was noted in 17% cases, in particular tinea corporis predisposing to the development of onychomycosis. Out of 100 patients, 10 were found to have diabetes. Distal lateral subungual onychomycosis (65%) was the most common pattern of onychomycosis followed by total dystrophic onychomycosis (15%), Mixed onychomycosis (12%), Proximal subungual onychomycosis (6%), Endonyx (1%) and Superficial Onychomycosis (1%). Among 100 cases, 86 showed positivity to any one of the three diagnostic methods. KOH mount demonstrated fungal elements in 55% of patients. The culture positivity rate was 41%. Histopathological PAS staining showed positivity in 71% patients. The sensitivity of KOH mount, culture and HP/PAS was 63.22%, 47.13% and 81.61% respectively. There was a very significant difference between culture and PAS staining ($p=0.0001$). Among the 41 culture positive cases, 15 cases (36.59%) positivity with *Trichophyton rubrum*, followed by 19.51% positivity with *Candida albicans*, 17.07% positivity with *Trichophyton mentagrophytes*, 12.19% positivity with *Aspergillus niger*, 4.87% each positivity with *Aspergillus flavus* and *Epidermophyton floccosum* and 2.43% each positivity with *Fusarium* and *Trichophyton violaceum*.

Conclusions: If there is any delay in the diagnosis of onychomycosis, it can lead to total nail dystrophy which may not allow the nail to regain its normal architecture in spite of adequate treatment. Results indicate that the combination of PAS and KOH were superior. By knowing various epidemiological profiles of onychomycosis, various clinical forms of onychomycosis and the role of different species to prevent morbidity.

Keywords: Onychomycosis, KOH mount, PAS staining, Fungal culture.

Introduction

Onychomycosis is the most common nail disease and accounts for approximately 40% of all onychopathies. It is a fungal infection of the toenails or fingernails that may affect any component of the Nail unit including the matrix, bed or plate. Though it is not a life threatening condition, but it can still cause significant physical and psychological morbidity due to its chronicity. Patients may fear that they will transmit their infection to family members and friends. This fear leads to diminished self-esteem and the avoidance of close relationships. It is predisposed by multiple factors like age, sex, occupation and associated comorbidities. Its treatment response mainly depends on etiological factors. If it is not treated early there is a chance of complete disfigurement of nail.

The prevalence of onychomycosis ranges from 0.2%-2.8% in India.¹ The symptomatic disease can be a source of embarrassment and potential cause of morbidity.

Healthy nails are a thing of beauty, while unhealthy and deformed nails give a psychological set back. Recent studies show that onychomycosis can have significant negative effects on patient's emotional,

social and occupational functioning. Sometimes it prevents them from carrying out work related tasks such as writing or typing.

Toenails are about 25 times more likely to be infected than fingernails, due to prolonged exposure of the toenails to fungal pathogens, by sporting activities and occlusive foot wear. The longest toe, either the first or the second, which bears the pressure and trauma is particularly susceptible to invasion, although multiple nails are typically infected.²

It is more common in farmers, housewives, forestry workers and miners owing to their greater rates of contact with pathogenic fungi present in soil, water and animals.²

Among the three groups of fungi associated with onychomycosis, dermatophytes are the principal pathogens. Among these dermatophytes, *Trichophyton rubrum* and *Trichophyton mentagrophytes* are the dominant species involved.

Six clinical types of primary onychomycosis have been described. They include Distal lateral subungual onychomycosis (DLSO), Superficial onychomycosis (SO), Endonyx onychomycosis (EO), Proximal subungual onychomycosis (PSO), Total dystrophic

onychomycosis (TDO) and Mixed pattern onychomycosis (MO).

At present, we mainly rely on clinical examination and a combination of direct microscopy by KOH mount and fungal culture to arrive at a diagnosis. Reported false negative rates are relatively high but it may score over culture in its ability to detect fungal elements.³

Fungal cultures are neither highly sensitive nor specific. Histopathological examination by PAS staining of the nail specimen is the single most sensitive test of all the available diagnostic tests. In more than 85-90% of cases, PAS staining is sufficient for the diagnosis – confirmation or exclusion of onychomycosis. Enrichment of the specimen using KOH treated nail clippings stained with PAS may further enhance the sensitivity of histopathology⁴. When treating onychomycosis, it is imperative to consider several factors before selecting a therapy: fingernail or toenail disease, severity of the onychomycosis, number of nails affected, causative organism, concomitant drugs and patient/physician preference. Identification of the specific causative organism is important because some organisms are less likely to respond to certain antifungal agents.

Materials and Methods

The current study was conducted after taking the approval of Ethics committee and informed written consent from the patients, who were attending the outpatient department of Dermatology, Venereology and Leprology, Narayana Medical College Hospital for a period of one year from January 2014 to December 2014.

All the clinically suspected cases of onychomycosis with features of onychodystrophy, onycholysis, subungual hyperkeratosis and discolouration of the nail were included as source of data.

A comprehensive history was recorded in patients with reference to the age, sex, address, occupation, onset of disease, number of nails involved, clinical type, duration and any associated skin or systemic disease or history of various treatments etc.

Inclusion Criteria: All clinical types of onychomycosis irrespective of age, sex and immune status with above mentioned nail changes of either finger or toenail or both.

Exclusion Criteria: Individuals with psoriasis, lichen planus or other nail dystrophies. Patients who have used any topical application or have taken antifungal drug in the past 6 months.

Specimen collection: Samples were collected from the most diseased nail, after thorough cleansing of the nail area with 70% alcohol to remove contaminants. For distal and lateral subungual onychomycosis, the abnormal nail was clipped at proximal end of discolouration along with attached subungual debris; the outermost debris was discarded. For proximal

subungual onychomycosis, the surface of the proximal end of nail plate is pared down with a No.15 surgical blade and the white debris was collected with a sharp curette from the deep portion of the plate and the proximal nailbed. For superficial onychomycosis and endonyx onychomycosis, a tangential biopsy of nail plate is taken with a no.15 scalpel and the white debris directly underneath was also collected. The obtained nail sample and subungual debris was divided into three parts and subjected to KOH, culture and PAS staining. The sample for culture was collected in sterile container and sent to microbiology laboratory and the sample for PAS staining was placed in 10% formalin solution and sent to pathology.

Direct Microscopy using 20% KOH: The sample of nail was placed in few drops of 20% potassium hydroxide (KOH) on a clean glass slide, then cover slip was placed over the preparation and the slide was gently heated by passing over the flame 3-4 times. They were kept for 1 to 2 hours at room temperature in a moist chamber. After this, the specimen was examined first under the low power view (10x) of the microscope and then under the high power (40x) to look for the presence of hyphae or arthrospores and the results were noted.

Culture: The specimen collected was divided into two parts and these portions were inoculated onto two test tubes irrespective of demonstration of fungal elements on KOH mount. One test tube contained Sabouraud's dextrose agar with 0.05% chloramphenicol and gentamicin and the other contained Sabouraud's dextrose agar with 0.5% cycloheximide, 0.05% chloramphenicol and gentamicin incubated at 28°C for up to 4 weeks. Cultures were read initially at 24 to 48 hours for nondermatophytes and then on periodically for up to 4 weeks for dermatophytes. If no growth was found after 4 weeks, it was taken as negative for the growth of fungi and discarded. Repeat cultures were performed in cases where culture was negative for dermatophytes, but positive for nondermatophytic moulds or yeasts to rule out the possibility of contamination. The criteria used to report nondermatophytic moulds or yeasts as pathogens were direct microscopy positivity and isolation of the same fungus in three consecutive samples at intervals of seven days each. Fungal isolate was identified based on colony morphology, reverse pigmentation, growth rate, microscopy using Lacto Phenol Cotton Blue (LPCB) stain, slide culture and special tests like hair perforation test, urease test, germ tube test, sugar fermentation and assimilation tests on to Sabouraud's Dextrose agar.

PAS staining: Before processing, the nail clippings were placed in 20% KOH to soften the nail for 2 hours, it was then dehydrated, embedded in paraffin for 6 hours, cut with a microtome into thin slices of 4 µm and stained afterwards with periodic acid Schiff stain. If only subungual tissue was collected, it was processed as normal skin tissue and then stained.

Results were expressed as number and percentages as proportions. Sensitivity of each test was calculated. Chi Square test performed to analyse KOH versus and PAS staining. Categorical data was analysed using Mc Nemar's test using SPSS (version 16.0). P value of 0.05 or less was considered for statistical significance.

Results

Epidemiology: The mean age was 46.6 years with 16.26 years of standard deviation. The maximum and minimum ages of sample were 13 and 78 years respectively, with majority of patients belonging to the age groups of 51-60 years followed by 31-40 years, 23% and 22% respectively. Least number of sample belonged to ≤ 20 years age group (4%). The current study shows that the incidence among males is slightly higher than that of the females. Out of 100 cases, 53 were males and 47 were females contributing to a male: female ratio of 1.12:1. The current study showed that the distribution of the affected sample was relatively higher among the rural population when compared to the urban population. Out of the 100 cases, 52% was rural and 48% was urban, contributing to a ratio of 1.08:1.

In the present study housewives constituted the maximum number of cases (32%), followed by agriculturists (16%), unemployed (11%), others (11%), students (10%), businessmen and coolies (8% each) and computer operators (4%) respectively.

Disease Characteristics: Duration of the disease at the time of presentation varied from a minimum of 1 month to a maximum of 10 years. Most of the patients (48%) had complaints of ≤ 12 months duration, 24% had complaints of 13-24 months, 19% had complaints of 25-60 months and 9% had > 60 months duration. The mean duration of infection was 27.68 ± 29.68 months.

In the present study of 100 patients, 42% gave history of moisture, 15% trauma, 6% tight footwear, 4% excessive sweating, 2% warmth and remaining 31% patients have not given any history of aggravating factors.

In the current study only diabetes mellitus was the systemic illness observed for about 10 cases.

Among the 47 female patients, 19 patients had toenail involvement, 14 patients had fingernail involvement and 14 patients had involvement of both finger and toe nails. Among 53 male patients, 20 patients had fingernail involvement, 17 patients had toenail and 16 patients had both finger and toenails involved.

In the current study, out of 2000 nails observed 405 nails were affected by Onychomycosis. It shows that at an average 4.05 nails/individual were involved. Average number of nails infected in females at a rate of 4.38 (206/47) was more than males at a rate of 3.75 (199/53) per individual.

More number of toenails (212 nails) were involved compared to fingernails (193 nails). Total number of nails involved on right hand (111 nails) was more than left hand (82 nails). But it was almost equal in feet (Right foot 107 nails, Left foot 105 nails). Among toenails highest incidence was seen for great toe nail followed by 2nd toe nail. Among fingernails, thumb nail had higher incidence followed by middle and index fingers respectively (Table 1) (Fig. 1).

Onychomycosis Distribution: Out of 100 patients, 17 (17%) patients had coexisting Superficial Cutaneous Fungal infections constituted by 9% of tinea corporis, 3% of tinea pedis, 2% each of candidiasis and tinea cruris and 1% of tinea manuum.

Table 1. Onychomycosis distribution in individual nails

	Nail	Females	Males	Total	Percentage
Right Hand	Thumb fingernail	19	18	37	9.14%
	Index fingernail	11	8	19	4.69%
	Middle fingernail	15	12	27	6.67%
	Ring fingernail	11	5	16	3.95%
	Little fingernail	8	4	12	2.96%
Left Hand	Thumb fingernail	12	13	25	6.17%
	Index fingernail	9	8	17	4.20%
	Middle fingernail	8	9	17	4.20%
	Ring Fingernail	6	8	14	3.46%
	Little fingernail	2	7	9	2.22%
Right Foot	Great toe nail	29	25	54	13.33%
	2nd toe nail	12	11	23	5.68%
	3rd toe nail	7	7	14	3.46%
	4th toe nail	5	4	9	2.22%
	5th toe nail	4	3	7	1.73%
Left Foot	Great toe nail	27	27	54	13.33%
	2nd toe nail	12	12	24	5.93%
	3rd toe nail	6	7	13	3.21%
	4th toe nail	2	8	10	2.47%
	5th toe nail	1	3	4	0.99%
Total		206	199	405	100.00%

Onychomycosis Clinical Pattern: The most common nail changes noted in the present study were discolouration in 98% followed by onycholysis in 72%, subungual hyperkeratosis in 60%, nail dystrophy in 51%, crumbling in 43%, paronychia in 22% and other changes in 2%.

In the current study the most common clinical pattern observed was DLSO (65%) followed by TDO (15%), MO (12%), PSO (6%) and 1% each of Endonyx and SO. DLSO type of nail involvement was seen in toenails in 27% followed by fingernails (19%) and both finger and toenails (19%). TDO type of nail

involvement was mostly seen in both finger and toenails in 6% followed by 5% in fingernails and 4% in toenails. In MO type of nail involvement fingernails (5%) were most commonly involved followed by both finger and toenails (4%) and toenails (3%). In PSO type of nail involvement fingernails (4%) were most commonly involved followed by toenails and both finger and toenails (1% each). SO type of fingernail involvement was seen in one patient. EO type of toenail involvement was seen in one patient (Table 2 & Table 3).

Table 2: Clinical pattern

Clinicaltype	Fingernail	Toenail	Both	Total	Percentage
DLSO	19	27	19	65	65%
Endonyx	0	1	0	1	1%
MO	5	3	4	12	12%
PSO	4	1	1	6	6%
SO	1	0	0	1	1%
TDO	5	4	6	15	15%
Total	34	36	30	100	100%

The most common clinical pattern observed in males was 34 cases of DLSO (64.15%) followed by 8 cases of MO (15.05%), 6 cases of TDO(11.32%), 4 cases of PSO (7.54%) and one case of EO (1.88%).

In females the most common pattern observed was 31 cases of DLSO (65.95%) followed by 9 cases of TDO (19.14%), 4 cases of MO (8.51%), 2 cases of PSO (4.25%) and one case of SO (2.12%).

Table 3. Clinical pattern of onychomycosis in males and females

	Female	Male	Total
DLSO	31	34	63
	65.95%	64.15%	63.00%
EO	0	1	1
	0.00%	1.88%	1.00%
MO	4	8	12
	8.51%	15.05%	12.00%
PSO	2	4	5
	4.25%	7.54%	4.00%
SO	1	0	1
	2.12%	0.00%	1.00%
TDO	9	6	15
	19.14%	11.32%	15.00%
Total	47 (100%)	53 (100%)	100 (100%)

Culture and Staining Technique: It was difficult to comment upon the specificity of these tests, since we did not consider any single test as the gold standard.

The current study shows that the sensitivity by PAS stain (81.61%) was more than KOH mount (63.22%) and culture (47.13%) (Table 4 & Table 5).

Table 4. Details of test results

Total positive	KOH mount	Mycologic culture	HP/PAS#	No. of patients
All tests positive	+	+	+	32
Two tests positive	+	+	-	6
	+	-	+	10
	-	+	+	1
One test positive	+	-	-	7
	-	+	-	2
	-	-	+	28
All tests negative	-	-	-	14
Total	55	41	71	100

#Histopathologic examination using PAS staining

Table 5: Positivity and sensitivity of diagnostic methods

Test	Positive (Total No. of samples = 100)	Sensitivity* %
KOH mount	55 (55%)	63.22
Culture	41 (41%)	47.13
HP/PAS#	71 (71%)	81.61

*Calculated using samples showing at least one laboratory test positive (i.e., 86 samples as the denominator).

Histopathologic examination using PAS Staining.

In this study, PAS was positive in 71 patients (71%) and culture was positive in 41 (41%) patients. 38 false negative cultures were obtained. There were 8 specimens that were PAS negative and culture positive.

The difference between two methods for diagnosing onychomycosis was extremely statistically significant, with a P value of 0.0001 calculated with Mc Nemar's test with the continuity correction (Table 6).

Table 6: KOH versus passtaining

	PAS +ve	PAS -ve	Total
KOH+ve	42	13	55
KOH -ve	29	16	45
Total	71	29	100

Chi Square =5.357 p=0.0206 Significant

Out of the 100 patients, direct microscopy with KOH mount showed positive results in 55 patients (55%) versus 71 (71%) patients with PAS +ve. 29 false negative KOH were obtained. 13 cases showed PAS negativity and KOH positivity. Statistically there was significant difference between the two methods with a P value of 0.0206 calculated with Mc Nemar's test with the continuity correction.

Among the 8 organisms isolated the most common type was *Trichophyton rubrum* (36.58%) followed by *Candida albicans* (19.51%), *Trichophyton mentagrophytes* (17.07%), *Aspergillus niger* (12.19%), *Aspergillus flavus* (4.87%), *Epidermophyton floccosum* (4.87%), *Fusarium* (2.43%) and *Trichophyton violaceum* (2.43%) (Fig. 3).

Among the females, the most common organism isolated was *Trichophyton rubrum* (33.33%) followed by *Candida albicans* (23.80%), *Trichophyton mentagrophytes* (23.80%), *Aspergillus niger* (14.28%) and *Fusarium* (4.76%).

Among the males the common organism was *Trichophyton rubrum* (40%) followed by *Candida albicans* (15%), 10% each of *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Aspergillus niger* and *Aspergillus flavus* and 5% of *Trichophyton violaceum* (Fig. 2).



Fig. 1: A. Distal lateral subungual onychomycosis affecting toenails B. Proximal onychomycosis C. Total dystrophic onychomycosis affecting both finger and toenails D. Endonyx onychomycosis E. Superficial onychomycosis associated with distal and lateral subungual onychomycosis F. Co-existence of both tinea corporis and onychomycosis in a female patient

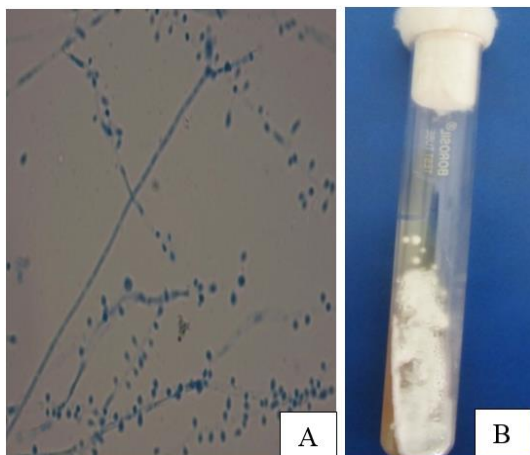


Fig. 2. A & B. Trichophyton rubrum macroscopic and microscopic appearance of colony

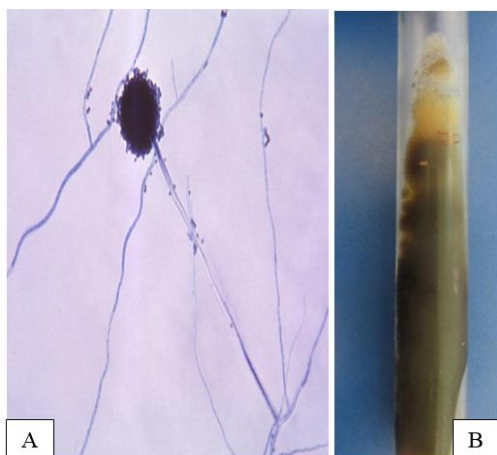


Fig. 3: A & B. Aspergillus niger macroscopic and microscopic appearance of the colony

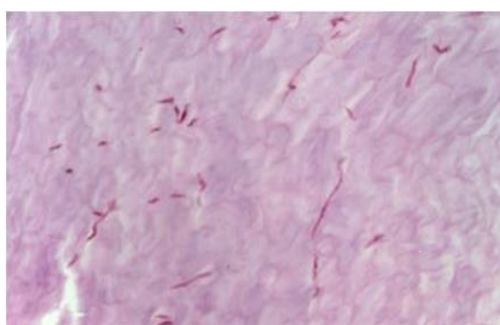


Fig. 4: Pas staining of nailplate showing fungal hyphae

Discussion

Onychomycosis is a fungal infection of nails caused by dermatophytes, yeast or nondermatophyte moulds. Increasing incidence of onychomycosis is being viewed as more than a mere cosmetic problem. In spite of improved personal hygiene and living environment, onychomycosis continues to spread and persist, contributing to significant physical and psychological morbidity.

The current study shows the prevalence of onychomycosis to be 1.06% of total outpatient attendance. In 2002 Madhuri et al¹ in their study reported 0.94% of prevalence rate. In 1970 Mulay et al⁵ in Delhi reported the prevalence as 1.2% and Kaur et al⁶ in Chandigarh found that the prevalence of onychomycosis was 1.7%. Maheshwari et al⁷ in 1982 reported 1.4% prevalence from Kerala. Karmakar et al⁸ in year 1995 from Rajasthan reported prevalence of onychomycosis as 2.8% followed by Kaur et al⁹ in the year 2007 who reported a prevalence of 45% in Delhi. These findings were comparable with the findings observed in the present study.

The present study showed that the mean age was 46.6 years with 16.26 years of standard deviation. This finding was similar to Mohammad and Seyed¹⁰ study reported that majority of subjects were under 49 years of age.

The current study shows that the incidence among males was slightly higher than that of the females. Out of 100 cases 53 were males and 47 were females contributing to a male: female ratio of 1.12:1.

This finding was almost similar to Adhikari et al¹¹ study with a male to female ratio of 1.125:1. This finding was also in agreement with S. Neupane et al,¹² who reported a higher incidence in males compared to females.

In the current study the higher prevalence in males may be due to increased outdoor physical activity and increased opportunity for infection. In the present study, 52 patients were living in rural areas, while 48 patients were residing in urban areas, in a ratio of 1.08:1. This finding is however not in agreement with that of Madhuri et al.¹

Out of 100 patients in the present study, 32% were housewives followed by 16% being agriculturists and 10% being students. These findings were almost similar to those of Kaur et al¹³ and Bokhari et al.¹⁴

Kaur et al¹² found most common occupation groups were housewives, agriculturists, labourers, industrial workers, clerical jobs, students and most of the patients were involved in domestic activities (33.33%), the most common being cooking followed by tailoring. This observation is also in agreement with other reports by Bokhari et al.¹⁴

S. Neupane et al¹² in their study observed that most of the patients were students (31.3%) followed by housewives (28%) and occupational involvement in wet work was seen in 41.8%. In current study the higher incidence in housewives and agriculturists probably reflects prolonged contact with water. The higher incidence among the students may be due to wearing of occlusive shoes throughout the day leading to moisture sweat retention and thus contributing to the role of moisture and microtrauma in the causation of onychomycosis. In the present study the mean duration of infection was 27.68±29.68 months, with 48% patients suffering with complaints of onychomycosis for ≤12

months duration. Yadav P et al¹⁵ showed a higher mean duration of toenail onychomycosis as 54.1 months.

Lower mean of disease duration in the current study could be probably explained by the predominance of health conscious population seeking early dermatological intervention or social stigma.

Moisture being the main aggravating factor onychomycosis in this area can be explained in the study population as majority of them were housewives and agriculturists with a history of prolonged contact with water predisposing them to onychomycosis.

In the present study 42% shown history of Moisture, 15% trauma, 6% tight footwear, 4% excessive sweating, 2% warmth and remaining 31% patients have not given any history of aggravating factors.

Out of the 100 patients involved in the present study, 10% were found to have diabetes mellitus. Amongst the diabetic population finger nail involvement was more common. Mean duration of onychomycosis in diabetic patients was 17 ± 17.0 months, which was less than the general population, i.e., 27.68 ± 29.68 months. The less duration of onychomycosis in these patients may be due to increased health consciousness and may be due to early diagnosis and referral by physicians treating diabetes.

Garg et al¹⁶ in their study reported that onychomycosis was associated with diabetes in 4.4%.

In the current study, toenail involvement (36%) was found to be slightly more compared to fingernail (34%) or both finger and toenail involvement.

This observation was in concordance with Raghavendra et al¹⁷ which shows that involvement of 38% of fingernails alone, 33.33% of toenails alone and 28.66% of both.

In the current study, out of 2000 nails observed 405 nails were affected by Onychomycosis, it shows an average of 4.05 nails/ individual were involved. Average number of nails infected in females at an average of 4.38 (206/47) was more than males at an average of 3.75 (199/53) per individual.

More number of toenails were involved compared to fingernails. Among toenails highest incidence was seen for great toe nail followed by 2nd toe nail. Among fingernails, thumb nail had higher incidence followed by middle and index fingers respectively.

This was because in females more number of nails were exposed to trauma or infective agents during their regular household work.

In a study conducted by Gupta et al¹⁸ showed ≤ 5 nails involved in 57.6% of patients. Macit Ikit¹⁹ in his study observed that fingernails were affected more in females [M:F ratio of 27:73] and toenails in men [M:F ratio of 73:27].

In present study, total number of nails involved on right hand (111 nails) was more than left hand (82 nails). But it was almost equal in feet, Right foot (107nails) and Left foot (105 nails).

This suggests that right hand was more predisposed to factors causing onychomycosis than left hand like trauma, exposure to moisture and warmth.

The increased prevalence of toe nail infection could be the result of more trauma to the toenails and exposure to moisture.

Out of 100 patients, 17% patients had coexisting superficial fungal infections which constituted 9% Tinea corporis, 3% tinea pedis, 2% each of candidiasis and tinea cruris and 1% Tinea mannum. Neupane S et al¹² in their study observed that 58.2% of onychomycosis was accompanied by superficial fungal infection at other sites. Kaur et al¹³ found the presence of coexisting fungal infections in other parts of the body in 45% patients, the most frequent being tinea mannum.

Once fungus is established in nails, infected nails act as reservoir of organism providing a constant source of infection for other parts of the body as suggested by the presence of concurrent fungal infection.¹³

In current study the most common clinical changes noticed in nails were 98% discolouration followed by onycholysis 72%, subungual hyperkeratosis 60%, nail dystrophy 51%, crumblig 43%, paronychia 22% and others 2%.

Yadav P et al¹⁵ observed in their study discolouration contributing to 98% as the most common symptom. Kaur et al¹³ reported nail discolouration in 100% patients and pain in 17% patients. Garg et al¹⁶ in their study observed that nail discolouration was observed in 100% patients, subungual hyperkeratosis in 48%, onycholysis in 37% and paronychia in 12%. In the current study the most common clinical pattern observed was distal and lateral subungual onychomycosis (65%) followed by Total dystrophic onychomycosis (15%), Mixed onychomycosis (12%), Proximal subungual onychomycosis (6%) and 1% each of Endonyx onychomycosis and Superficial onychomycosis. DLSO type of nail involvement was seen in toenails in 27% followed by fingernails (19%) and both finger and toenails (19%). TDO type of nail involvement was mostly seen in both finger and toe nails in 6% followed by 5% in fingernails and 4% in toenails. In MO type of nail involvement fingernails (5%) were most commonly involved, followed by both finger and toenails (4%) and toenails (3%). In PSO type of nail involvement fingernails (4%) were most commonly involved followed by toenails and both finger and toenails (1each). SO type of fingernail involvement was seen in one patient. EO type of toenail involvement was seen in one patient. Among the 12 (12%) Cases of Mixed Onychomycosis, 10 were having DLSO+PSO followed by 2 cases of DLSO+SO. Similar reports were also reported by Dogra et al²⁰ in their study, that DLSO was the most common clinical presentation in 75% of patients and the most common fungus isolated was *Trichophyton rubrum* followed by *candida*.

In the present study 55% cases showed fungal spores and hyphae by direct microscopy on KOH mount. Among 55% cases, 38% cases were positive for fungal growth on culture media. Veer P et al²¹ in their study showed similar results. In certain studies KOH positivity rate varied from 35.6% to 88.6%.²² This was mainly due to inter observer variation.

In current study fungal growth was seen in 41% cases. These results were comparable with Madhuri et al,¹ Das NK et al²³ and Veer P et al.²¹

Certain studies showed culture positivity from 36% to 53.6%.²² This low positivity of culture compared to direct microscopy may be due to presence of dead fungus in the sample.

Shenoy M et al³ in their study observed KOH and culture positivity rates of 50% and 36% respectively. Kaur et al¹³ in their study observed that 34% had direct microscopy examination positivity and culture positivity in 45% of patients.

In present study dermatophyte moulds were isolated in 60.98% (25 out of 41), Nondermatophytes in 19.51% (8 out of 41) and candida albicans in 19.51% (8 out of 41). These results were comparable to Niranjana et al²⁴ and Das NK et al.²³

Niranjana et al²⁴ in his study says, most common etiological agent causing Onychomycosis to be dermatophytes (59.26%) followed by yeast (24.07%) and nondermatophytes (16.67%). Das NK et al²³ also showed most common etiological agent was dermatophytes (50%) followed by yeast (27.27%) and moulds (22.72%).

In contrast, Vijaya et al²⁵ and Adhikari et al¹¹ reported yeast and *Trichophyton tonsurans* as predominant fungi respectively. Madhuri et al also reported Candidial onychomycosis as the commonest type in their study. From this data it is evident that the commonest pathogen for onychomycosis is *Trichophyton rubrum* followed by *Trichophyton interdigitale*.

Trichophyton rubrum was detected as main pathogen for onychomycosis in this study, which is similar to studies in Turkey, India, United States, North and South Europe. The high prevalence of *T. rubrum* has been explained by its better adaptation to the hard keratin of nails.¹⁶

In the present study among nondermatophytes most common isolate was *Aspergillus niger* (62.5%) followed by (25%) *Aspergillus flavus* (12.5%) and *Fusarium*. In current study among the females the most common organism isolated was *Trichophyton rubrum* (33.33%) followed by *Candida albicans* (23.80%), *Trichophyton mentagrophytes* (23.80%), *Aspergillus niger* (14.28%) and *Fusarium* (4.76%). Among the males the common organism was *Trichophyton rubrum* (40%) followed by *Candida albicans* (15%), 10% each of *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Aspergillus niger* and *Aspergillus flavus* and 5% of *Trichophyton violaceum*.

In current study the only yeast isolated was *Candida albicans*, this result was concordance to results of Garg et al.¹⁶ *Candida* was most commonly affecting females compared to males, this finding was in comparison to the study of Niranjana HP et al.²⁴

Certain other studies also showed that *Candida albicans* was the most commonly isolated yeast, i.e., 84.6% and 83.3% in the studies of Niranjana et al²⁴ and Das NK et al²³ respectively. There is great variation in etiological fungus for onychomycosis. From the above studies it is evident that there is a continuous change in epidemiological and mycological characteristics of Onychomycosis in the same population with time. In this study 71 (71%) patients showed positive results with histopathologic PAS staining. Similar results have been reported by Lin et al⁴ Lawry et al²⁶ and Shenoy M et al³ with PAS positivity of 61%, 63% and 76% respectively. In contrast Alkhayat et al²⁷ and Machler et al²⁸ reported lower PAS positivity of 38% and 33% respectively.

In the current study, PAS was positive in 71 (71%) patients and culture in 41 (41%) patients. The finding of 38 false negative culture results (PAS positive but culture negative) suggests that the histologic evaluation of nail plate clippings increased the probability of diagnosing onychomycosis over that obtained by culture alone in certain clinical settings. Conversely there were 8 specimens that were PAS negative and culture positive. The two techniques therefore were seemingly complementary.

Statistically, there was significant difference between the two methods (P=0.0001, Using Mc Nemar's test). The false negativity could have been due to removal of an insufficient proximal sample of nail plate or to the fact that hyphae had not yet invaded the undersurface of the overlying nail plate from the nail bed. This finding is in concordance with Machler et al.²⁸

A nail incisional / punch biopsy is often painful and can cause permanent alteration of the nail plate. Hence in this study, nail specimens for PAS were obtained with nail clippers. The distal free edge of the nail plate, along with any attached subungual debris was clipped just distal to its attachment to the nail bed. Similar method of nail sampling were reported by Hussein et al²⁹ and Weinberg et al.³⁰ Out of the 100 patients, direct microscopy with KOH mount showed positive results in 55 patients (55%) versus 71 (71%) with histopathologic PAS staining.

The KOH mount had a sensitivity of 63.22% while PAS staining had a sensitivity of 81.61%. The finding of 29 false negative KOH results (PAS positive but KOH is negative) suggested that the histologic evaluation of nail plate clippings increased the probability of diagnosing onychomycosis over that obtained by KOH alone. Conversely there were 13 specimens that were PAS negative and KOH positive. Similar result shown by Manjunath et al.³ Statistically,

there was significant difference between the two methods ($p=0.0206$, Using Mc Nemar's test). Similar results have been reported in previous studies.^{26,30-32}

In contrast Machler et al²⁸ showed higher positivity of direct microscopy (75%) than PAS staining (33%) and culture (33%). In fact, false negative results are obtained in about 10% of the nail specimens under direct microscopic examination of KOH preparation, while the culture test suffers from a very high level of misleading results with a false negative results representing at least 20% of the cases and may often rise upto 35%.³³

The high false negative results with the possibility of false positive results of these standard diagnostic procedures are unacceptable; hence there is need for a diagnostic test with higher sensitivity and accuracy. There are multiple reports on histopathologic examination with PAS staining of nail clippings as a highly reliable diagnostic procedure for onychomycosis to facilitate an early intervention.^{3,26,30,31}

Conclusion

An early dermatologist's consultation noticed in the present study in majority of the sample points to an increased awareness or/and social stigma about appearance of nails. Main clinical signs of onychomycosis are discolouration and onycholysis. Right hand is more commonly involved due to its proneness to micro trauma secondary to manual dexterity. Distal lateral subungual onychomycosis is the commonest clinical type of nail involvement. Etiological factors causing onychomycosis in this area are similar to other parts of country. Dermatophytes remain the predominant cause of onychomycosis, with *T.rubrum* being the most common aetiological agent, but less commonly yeasts like *C. albicans* and nondermatophytic moulds like *Aspergillus* species can also cause nail infection. Hence an accurate diagnosis is needed, since the treatment is different for each group.

HP/PAS staining of the nail clippings has an additional advantage that the test results can be obtained in 1-2 days compared to culture. Less inconvenience has been encountered by patients, because in most of the cases nail clippings are sufficient compared to the nail biopsy. Main drawback of HP/PAS is its cost constraints than the culture and KOH mount. Despite of its high sensitivity, false positivity may occur with other inflammatory nail dermatoses.

Conflict of Interest: None

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