Clinical and mycological profile of dermatophytic infections among patients attending a tertiary care hospital

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Abstract

Introduction: Superficial mycotic infections affect skin, hair and nail and they are caused by dermatophytic and non-dermatophytic species. Most of the infections are due to dermatophytes, which belong to either of the three genus namely *Trichophyton, Microsporum* and *Epidermophyton*. Studies estimating the prevalence and pattern of species distribution among the dermatophytes in superficial mycoses are limited. Hence we have aimed to conduct a study to analyze the clinical and mycological profile of dermatophytes among patients attending dermatology OPD in a tertiary care hospital.

Materials and Methods: This cross sectional study conducted over a period of one year in 217 patients attending a tertiary care hospital. Depending on the site of lesion, skin scrapings, nail clips or hair specimen was collected and transported to laboratory with precautions. Direct microscopic examination was done; 10% KOH was used for skin and hair specimens; 20% KOH used for nail specimen. Fungal culture was done in Sabouraud`s dextrose agar and Dermatophyte test medium and species identification done based on macroscopic and microscopic features.

Results: Microscopic KOH examination showed features suggestive of dermatophytic infections were seen in 98/217 samples (45.16%). By fungal culture, out of 217, 80 samples (36.8%) showed significant growth with characteristic findings suggestive of dermatophytes.

Conclusion: Even though clinical diagnosis of superficial mycotic infections shows promising results, it is must to do microscopic KOH examination of the clinical material and advisory to do fungal culture whenever resources are available. This aids in improved knowledge about the prevalence of species and epidemiology of dermatophytic infections.

Introduction

Superficial mycosis is one among the most common fungal infections that affect children, elderly and mostly immunocompromised persons worldwide.(1) Skin, hair and nail infections constitute superficial mycosis and it is mostly caused by group of fungi belonging to dermatophytes. Dermatophytes are the keratinophilic fungi that invade keratinized structures and can cause chronic lesions.(2) These are subdivided into three genera based on their macroscopic and microscopic features as *Trichophyton, Microsporum* and *Epidermophyton*.(3) Literature says around 20 to 25% of the world`s population is being affected by dermatophytic infections.(4,5) Clinically based on the site of involvement, dermatophytic infections are coined as Tinea or ringworm infections namely Tinea pedis, Tinea cruris, Tinea corporis, Tinea barbae and many more.(6) Occurrence of dermatophytic infections has a geographical prediction with more prevalence in tropical countries like India.(7) There is a heterogeneity in the prevalence and lack of treatment seeking behavior occurs in skin infections unless until it becomes a serious condition or due to cosmetic reasons.(8) Studies that estimate the prevalence of dermatophytes upto species level are not so widely available which limits the real detection of burden of dermatophytic infections in our country. Hence we aimed at studying the clinical and mycological profile of dermatophytic infections in patients attending dermatology clinic in a tertiary care hospital.

Materials and Method

This cross sectional study was conducted in Department of Microbiology in samples obtained from Dermatology clinic in a tertiary care teaching hospital. Around 217 patients over a period of one year, who attended the clinic with complaints of skin, hair and nail infections with clinically diagnosed dermatophytosis were included for the study. The patients who were already started on antifungal treatment were excluded. After obtaining informed consent from the patient, detailed history pertaining to the clinical condition was collected. Depending on the site of lesion, skin scrapings, nail clips or hair specimen was collected and transported to laboratory with precautions.

Sample collection:

1. **Skin scraping**: The affected lesion was swabbed thoroughly with 70% alcohol, allowed to dry. Active edge of the lesion was then scrapped with a flame sterilized blunt scalpel held at right angle to the skin surface.
2. **Nail clipping**: Affected nail was swabbed with 70% alcohol, dried and nail was clipped from free edge with base fully included.
3. **Hair specimen**: Affected hair was epilated or with blunt scalpel the scaling is collected. Basal portion is must for better isolation

Direct microscopic examination: Skin scrapings and hair specimen were visualized directly at the time of sample collection. Keratin material of nail needs to be

dissolved and it took hours to maximum of one day to visualize by microscopy. 10% KOH was used for skin and hair specimens; 20% KOH used for nail specimen. A large drop of KOH solution was placed with a Pasteur pipette on a clean grease free glass slide. A part of the specimen was then transferred into the KOH on the slide and a clean cover slip was gently placed over the mixture without any visible air bubbles. Slide was kept at room temperature for 20 minutes (skin/hair) or overnight (nail). Observation was done under microscope and looked for hyphae, septation, branching, thickness, arthroconidia and for any other morphological features. All the samples were then subjected to mycological culture irrespective of microscopic findings.

Fungal culture: Specimens were inoculated in duplicate sets into slopes of a) Sabourauds dextrose agar (SDA) with chloramphenicol b) Sabourauds dextrose agar (SDA) with chloramphenicol and cycloheximide c) one set of Dermatophyte test medium (DTM) containing chloramphenicol and cycloheximide. DTM slopes were incubated at 25degC.One of the SDA slopes was incubated at 37degC, another one at 25deg C and examined daily in the first week, and twice a week thereafter for any fungal growth. Slopes not showing growth for 4 weeks were considered negative for growth. When growth is seen, identification was done on the basis of colony characteristics as well as microscopic morphology in Lacto phenol cotton blue mount.

Species identification:

a. **Lacto phenol cotton blue mount:** A drop of LPCB was placed on a clean dry glass slide. Using a straight mycological loop, a tiny portion of the colony was removed from the slope and placed on the stain, dissected into tiny pieces with a teasing needle. A sterile cover slip placed over it and visualized for morphological features for identification of the species.

b. **In vitro hair perforation test:** By using test organism, lawn culture was made in potato dextrose agar (PDA) plate. Prepubescent blonde child sterile hairs were placed onto the lawn. To accelerate the reaction, 5-6 drops of 1% yeast extract were added. It was incubated at 30°C for upto 28days and examined. Some amount of the hairs were taken and placed in a clean glass slide and add a drop of lacto phenol-cotton blue, then place a sterile coverslip over it. The test was considered positive when cone-shaped perforations were appreciated along the long axis of the hair. Interpretation: Positive test seen in *Trichophyton mentagrophytes* and *Microsporum canis*; Negative test in *Trichophyton rubrum* and *Microsporum equinum*.

c. Urease test: Christensen`s urease agar slant was inoculated with test organism and incubated at 30°C for 7 days. The production of bright pink color indicates positive reaction, which is seen in *Trichophyton mentagrophytes.*

Statistical analysis: Data was analyzed using statistical package for social sciences (SPSS) software where descriptive tables were generated to demonstrate the findings. Chi square test was used to compare the proportions; Cohen's kappa coefficient was used to quantify the degree of agreement between the KOH positive and Culture positive cases. Validity of the KOH test was also performed to identify its sensitivity and specificity in accordance with the culture test. P value less than 0.05 was considered as statistical significance.

Results

Among 217 clinically diagnosed dermatophytosis patients, samples collected and processed.

Age distribution was studied which revealed that 21 -30 years were the most commonly affected group with 24.88% among the study population, followed by 31-40 years with 21.1%. p value was not significant with age group and clinical presentation. Gender distribution revealed that 133 males (61.2%) contributed to study population and 84 (38.7%) were females. Male to female ratio is 1.58:1.**(Table1)**

Table 1: Distribution of Age and Gender among the study population (n = 217)

S. No	Age group	Male	Female	Total (%)
1	1-10	7	8	15(6.96)
2	11-20	20	13	33(15.2)
3	21-30	34	20	54(24.88)
4	31-40	28	18	46(21.1)
5	41-50	27	9	36(16.5)
6	51-60	12	12	24(11.05)
7	>60	5	4	9(4.14)
	(Chi Square) $X^2 = 5.692$ p value=0.46			

Risk factors associated with acquiring dermatophytic infections were analyzed by detailed history. Among study population, 44/217 (20.27%) were diabetics. 41/217 (18.89%) were having history of animal contacts and 35/217 (16.12%) were agricultural workers. 33/217 (15.2%) were having history of close family contacts, 25/217 (11.5%) were hostel dwellers and 7//217 (3.22%) were having hyperhidrosis.

Frequency distribution of clinical types of fungal lesions was studied. Tinea corporis was the major type with 44.23% among all followed by Tinea unguium 25.8%, Tinea cruris 11.5% and then others as shown in table 2. Skin scrapings were collected from 139 patients; nail clippings from 56 patients and epilated hair from 22 patients.**(Table 2)**

Table 2: Distribution of clinical type of dermatophytosis among study population (n=217)

S. No	Specimen	Clinical type	No. of cases	Percentage
1	Skin (n=139)	Tinea corporis	96	44.23%
		Tinea cruris	25	11.5%
		Tinea mannum	2	0.92%
		Tinea pedis	11	5.06%
		Tinea faciei	5	2.3%
2	Hair(n=22)	Tinea capitis	21	9.67%
		Tinea barbae	1	0.46%
3	Nail(n=56)	Tinea unguium	56	25.8%

Microscopic KOH examination showed features suggestive of dermatophytic infections were seen in 98/217 samples (45.16%) and 119/217 (54.83%) showed no findings by KOH examination.

By fungal culture, out of 217, 80 samples (36.8%) showed significant growth with characteristic findings suggestive of dermatophytes. Correlation was done between microscopic examination and fungal culture of the specimens, which revealed that 77.9% of the KOH positive samples were grown in culture and showed significant growth. (p value = 0.000*). Culture was considered as gold standard method and when KOH mount was compared with culture, Sensitivity of KOH mount is 96.25% and specificity is 84.67%. **(Table 3)**

Table 3: Correlation between KOH examination and fungal culture (n=217)

Correlation	10%KOH Positive	10%KOH Negative	Total
Culture Positive	77(78.57%)	3(2.52%)	80(36.86%)
Culture Negative	21(21.42%)	116(97.45%)	137(63.13%)
Total	98(45.16%)	119(54.83%)	217
	R=0.779 p value =0.000*		

p value < 0.05

Species identification was done which showed the following distribution; *Trichophyton* was the most common genus in 77 out of 80 (96.25%) culture isolates. There was one isolate in *Microsporum* and two isolates in *Epidermophyton*. Frequency distribution of individual species revealed that *Trichophyton rubrum*was the most common among isolates 63/80 (78.75%), followed by *Trichophyton mentagrophytes* 7/80 (8.75%), *Trichophyton verrucosum* 6/80 (7.5%), *Epider mophyton floccosum* 2/80 (2.5%), *Trichophyton tonsurans* 1/80 (1.25%) and *Microsporum gypseum* 1/80 (1.25%). Individual dermatophytic isolates associated with clinical conditions were analyzed and species distribution among different clinical specimens is depicted in **Table 4** and **Table 5** respectively.

Table 4: Clinico mycological profile of dermatophytes (n=80)

Clinical type	*T.rubrum*	*T.menta grophytes*	*T.verrucosum*	*T.tonsurans*	*E.floccosum*	*M.gypseum*
Tinea corporis	32(40%)	4(5%)	1(1.25%)	-	-	-
Tinea cruris	12(15%)	1(1.25%)	-	-	1(1.25%)	-
Tinea pedis	7(8.75%)	-	-	-	-	-
Tinea barbae	1(1.25%)	-	-	-	-	-
Tinea faciei	4(5%)	-	-	-	-	-
Tinea manuum	1(1.25%)	-	1(1.25%)	-	-	-
Tinea unguium	5(6.25%)	2(2.5%)	4(5%)	_	1(1.25%)	_
Tinea capitis	1(1.25%)	-	-	1(1.25%)	-	1(1.25%)

Table 5: Distribution of dermatophytic isolates in clinical specimens (n=80)

Specimen	Isolates	No. of positives
Skin	*Trichophytonrubrum*	57(71.25%)
	Trichophytonmentagrophytes	5(6.25%)
	Trichophytonverrucosum	2(2.5%)
	Epidermophytonfloccosum	1(1.25%)
Hair	*Trichophytonrubrum*	1(1.25%)
	Trichophytontonsurans	1(1.25%)
	Microsporumgypseum	1(1.25%)
	Trichophytonrubrum	5(6.3%)
Nail	*Trichophytonverrucosum*	4(5%)
	Trichophytonmentagrophytes	2(2.5%)
	Epidermophyton floccosum	1(1.25%)

Discussion

Among 217 specimens processed and analyzed based on its clinical and mycological background, age, gender, clinical risk factors and clinical types, mycological profile was studied in detailed in above study.

In this study, age group most commonly involved is among 21 to 30 years and there is a male preponderance among the study population, which is supported by other studies conducted in various places.[9–12] This is mainly attributed to more exposure to outdoor activities in those age group and male gender with more chance to acquire infection during occupational activities, increased sweating due to work and contacts with those infected.[11] Among the risk factors studied for acquiring dermatophytic infection, 20.27% showed history of diabetes. Diabetics have increased risk of acquiring fungal infections and many studies have been done to estimate the prevalence and epidemiology of dermatophytes among diabetes population.[10,13–15] Certain host factors like skin pH, temperature of the body and increased hydration as in case of excessive perspiretion has a direct preponderance to chance of acquiring dermatophytic infections.[16] In our study, only 3.22% had history of hyperhidrosis. Among different species classified in dermatophytes, certain among them are zoophilic, some anthropophilic and some geophilic. In our study, 18.89% had history of contacts with animals and 16.12% were agricultural workers, which suggested that they have increased chance of acquiring zoophilic and geophilic dermatophytic infections.[17]

Clinical types of dermatophytic lesions showed Tinea corporis as the majority among all with 44.23% that is similar like all studies conducted in estimation of prevalence of dermatophytic lesions.[5,8,18] Other types are less prevalent which shows that exposure of body to risk factors in tropical countries leads to more chance of Tinea corporis followed by other areas.[19,20] Among the fungal species identified, *Trichophyton rubrum* was the majority showing 78.75%,which is similar to other studies. [5,9,11,19] *Epidermophyton floccosum* was seen in a case of Tinea cruris and Tinea unguuim and *Microsporum gypseum* was isolated in Tinea capitis. There is an increased prevalence of *Microsporum* species as estimated in other countries, but in our study *Trichophyton* is the leading isolate.[21,22]

Although clinical diagnosis plays a major role in identifying superficial mycotic infections, direct microscopic examination, which is a bedside procedure and culture identification confirms the etiology and species identification.[12] This improves the management of infection. In our study, sensitivity of KOH mount is 96.25% and specificity is 84.67%, which signifies that at least microscopic examination of specimens should be done to find the etiology in resource poor settings. This is supported by study conducted by Dass *et al*., which states that sensitivity and specificity of KOH mount as 83.02% and 70.1%.[23]

Conclusion

As dermatophytic infections are more common in tropical countries like India, estimating the prevalence and pattern distribution of species in respective locality needs to be done. Al though clinical diagnosis helps in identification of the condition and treatment, proper microscopic examination and fungal culture helps in confirmation of diagnosis and proper management.

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