

Clinico-Mycological Study of dermatophytosis in a Tertiary Care Hospital

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ABSTRACT

BACKGROUND

Dermatophytes are fungi that infects the skin, hair and nails. They are hyaline septate moulds with more than hundred species described. Of these, 42 species are considered as valid and less than half are associated with human diseases. Dermatophytoses are infections produced by these and are common in tropical and subtropical areas of the country with high humidity.¹ We wanted to speciate dermatophytes using phenotypic methods, analyze the risk factors, and study their clinical correlation.

METHODS

The study was conducted in a tertiary care hospital in South India over a period of one year. All newly suspected cases of dermatophytosis attending Dermatology Outpatient Department were selected for the study. Thus, a total of 113 patients were enrolled in the study. Samples from these patients were subjected to direct microscopy and culture was done on Sabouraud Dextrose Agar with antibiotics. Potato dextrose agar was used for enhancement of pigment production. Culture confirmation and speciation were done by tease mount, slide culture and supplemental tests like urease test and hair perforation test.

RESULTS

The present study was carried out on 113 clinically diagnosed cases of dermatophytoses. Maximum number of cases occurred in the 11-20 years age group and slight female preponderance was noted. Tinea corporis was the most common type of dermatophytosis, 68 cases (60.2%) followed by mixed type (tinea corporis + tinea cruris) 14 cases (12.4%) and tinea cruris 13 cases (11.5%). Overall positivity by culture was 39% and by direct microscopy 96%. *Trichophyton rubrum* was the most predominant species - 18 isolates (38.3%) and most of them were isolated from tinea corporis. *Trichophyton verrucosum* (25.5%) and *Trichophyton mentagrophyte* (21.3%) were also obtained as major isolates.

CONCLUSIONS

This study highlighted that tinea corporis is the commonest clinical type. *Trichophyton rubrum* is the most predominant species. But we got a fairly good number of *Trichophyton verrucosum* and *Trichophyton mentagrophyte* also. Exposure to predisposing factors were present in almost half of the cases. Dermatophytosis is a trivial disease and antifungal agents are the drugs of choice for treatment but identification of predisposing factors and avoidance of these can decrease the incidence of the disease to some extent.

KEY WORDS

Dermatophytosis, Dermatophytes, Tinea, Trichophyton, Lactophenol Cotton Blue (LPCB)

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BACKGROUND

Dermatophytes are filamentous fungi which infect the keratinized layers of skin, hair and nails. They are more prevalent in tropical and sub-tropical areas². These fungi belong to three main anamorphic genera: *Epidermophyton*, *Microsporum* and *Trichophyton*. Disease produced by these fungi is known as dermatophytosis, characterized by dermal inflammatory response with severe itching. It is also known as 'ring worm' infection or 'tinea'. The name ringworm comes from the worm like appearance of the lesions with irregular inflammatory borders. Tinea is a Latin word for "ring worm". Infection is named according to the body site after the word tinea.¹ The prevalent species of dermatophyte varies considerably in different geographical areas of the country.^{2,3} Based on their ecological characteristics, dermatophytes are divided into geophilic, zoophilic and anthropophilic species. Heat and moisture play an important role in promoting the growth of these Fungi.⁴ Other factors such as Diabetes mellitus, immunocompromised states, use of steroids, poor hygiene, exposure to cattle rearing areas, taking bath in untidy ponds and dams, overcrowding etc. also promote increased incidence.^{4,5,6,7,8} The first scientific proof of the dermatophytosis was provided by Remak in 1845. Since then many have surveyed and isolated the dermatophytes in different countries.^{6,7,8,9} In India Dr. Powell first reported the case of dermatophytosis from Upper Assam³ in 1900.

The infection is common in coastal areas like Kerala and remains a public health problem. So far, not much data is available about the pattern of the disease in and around the central part of Kerala. Hence this study was being undertaken to know the disease pattern in patients attending the Government medical College, Thrissur.

METHODS

This study was conducted in a tertiary care center in South India over a period of one year after getting approval by the Institutional Ethics Committee. A total of 113 clinically suspected cases of dermatophytoses attending Dermatology Outpatient Department were taken for the study. All age groups of both sexes were included in the study. Samples were taken after getting written consent from the patient or from the parent in case of children. A detailed history including age, sex, occupation, duration, and site of infection were taken. Patients who were already on treatment were excluded from the study.

Specimen Collection

Lesions were cleaned with 70% alcohol to remove the dirt and contaminating bacteria. In case of skin lesions active border of the lesion was selected and scrapings were taken with the blunt end of a sterilized scalpel. In affected nails, clippings along with the subungual debris were collected with a nail cutter. Hairs were collected by plucking them with the forceps so that the roots were preserved intact. Samples were collected in a sterile paper - folded, labelled and then transported to microbiology lab at room temperature and no specific transport media used. Further processing was carried out under mycology section. Materials were divided into two

parts one for wet mount examination (10% KOH for skin lesions and 40% KOH for nail and hair) and the other for culture. Wet mount (KOH): In a positive preparation the fungi appeared as septate branching hyphal elements, highly refractile among the epidermal scales.

Culture

Specimens were inoculated into Sabouraud's Dextrose Agar (Accumix) with antibiotics - chloramphenicol (50 mg/L) and cycloheximide (500 mg/L). The tubes were incubated at room temperature for 4 weeks. If there was no growth after 4 weeks the culture was considered as negative. In culture positive cases, colony morphology was recorded weekly including topography (flat, raised, folded or cerebriform), texture (granular, velvety or cottony) and pigmentation (obverse or reverse, diffusible or non-diffusible). After observing the colony morphology, they were speciated by the standard procedures.^{1,2,10} Lactophenol cotton blue mount (LPCB) were taken from the culture to study the morphology of the hyphae (Septate or not, its diameter), conidia and their arrangement. Slide culture was done for those isolates that could not be identified by LPCB mount. The slides were examined under low power and then under high power. The arrangement of hyphae and conidia were noted. Adhesive cellophane tape preparation done for those isolates which were plated for better sporulation. Aerial hyphae adhere to the tape and examined by placing on a drop of LPCB on a slide. Microscopic examination done for the presence of conidia i.e. microconidia and macroconidia - its number, size, shape and thickness were noted. In case of macroconidia number of cells or compartments were also recorded. Presence of accessory structures such as chlamydospores and pectinate hyphae were also observed. Species were identified by urease test to differentiate between *Trichophyton rubrum* (urease negative) and *Trichophyton mentagrophyte* (urease positive) and hair perforation test to differentiate between *Trichophyton mentagrophyte* and *Trichophyton rubrum*. Wedge shaped perforation was formed for *Trichophyton mentagrophyte* whereas the absence of perforation was typical for *Trichophyton rubrum*. Pigment production by Dermatophyte species was enhanced (e.g.: red pigment by *Trichophyton rubrum*) by growing them on Potato dextrose agar and cornmeal agar (HiMedia) with 1% dextrose. Growth in Lowenstein- Jensen medium (HiMedia) was noted for *Trichophyton soudanense*.

Statistical Analysis

The analyzed results were expressed as percentage for the description of the patterns of distribution of various clinical types of dermatophytoses and various species of dermatophytes. Association between the clinical types of dermatophytoses and the species isolated were also studied. Chi square distribution was used to test the qualitative distribution. Predictive value (p value) of less than 0.05 was considered as a significant association between the variables tested.

RESULTS

The present study for isolation, identification and clinicomycological study of dermatophytes was done on 113

clinically diagnosed cases of dermatophytosis. Out of these 113 samples: 94 (83.1%) were skin scrapings, 7 (6.2%) were nail clippings and 2 (1.77%) were hair stubs. Out of 113 clinically diagnosed cases of dermatophytoses, males accounted for 48.7% (55 cases) and females 51.3% (58 cases). The male female ratio was 0.95:1 The maximum number of cases were found in the age group of 11-20 yrs. 29 cases (25.7%) followed by 31-40 yrs. 21 cases (18.6%) [see table 1]

Age Group (Yrs.)	Number of Cases	Percentage (%)
0 to 10	14	12.4
11-20	29	25.7
21-30	15	13.3
31-40	21	18.6
41-50	18	15.9
51-60	7	6.2
61-70	8	7.1
71-80	1	1.1

Table 1. Distribution of Patients with Dermatophytoses According to Age

Of the total 113 patients, 57 cases (50.3%) had no exposure to predisposing factors whereas 56 (49.7%) had history of some predisposing factors. 32 (28.3) cases had history of using occlusive dressings, 10 (8.8%) had history of taking bath in ponds/dams, 6 (5.3%) had contact with affected family members, 5 cases (4.4%) had contact with pet animals and 4 (3.5%) cases had cattle rearing in house [see table 2].

Predisposing Factors	Number	%
No exposure	57	50.3
Occlusive dressings	32	28.3
Frequent bath in stagnant waters - (Ponds & dams)	10	8.8
Affected family members	6	5.3
Contact with dog/cat	5	4.4
Cattle rearing in house	4	3.5
Total	113	100

Table 2. Predisposing Factors of Dermatophytoses

Tinea corporis was found to be the commonest clinical type with 68 cases (60.2%) followed by mixed type (Tinea corporis + tinea cruris) 14 (12.4%), tinea cruris 13 (11.5%), tinea unguium 7 (6.2%), tinea pedis 4 (3.5%), tinea faciei 4 (3.5%), tinea capitis 2 (1.8%) and tinea manuum 1 (0.9%). [see table 3]

Sl. No.	Clinical Types	No. of Cases	Percentage (%)
1	Tinea corporis	68	60.2
2	Tinea corporis+ Tinea cruris	14	12.4
3	Tinea cruris	13	11.5
4	Tinea unguium	7	6.2
5	Tinea pedis	4	3.5
6	Tinea faciei	4	3.5
7	Tinea capitis	2	1.8
8	Tinea manuum	1	0.9

Table 3. Clinical Types of Dermatophytoses

Out of 113 cases of dermatophytoses, 96 (85%) cases were positive by direct microscopy and 17 (15%) were negative. Among the 96 wet mount positive cases only 43 were culture positives, remaining 53 did not grow in culture. Of the 17 wet mount negative cases 4 samples yielded dermatophyte isolates. Remaining 13 cases were negative by direct smear as well as culture. Sensitivity of microscopy was 91.5% and specificity was 19.7%.

Of the total 113 cases 47 (41.6%) were culture positive as mentioned above. *Trichophyton rubrum* was the commonest

isolate 18 (38.3%). Other isolates were *Trichophyton verrucosum* 12 (25.5%), *Trichophyton mentagrophyte* 10(21.3%), *Trichophyton tonsurans* 3 (6.4%), *Trichophyton soudanense* 2 (4.3%) and *Microsporum gypseum* 2 (see table 4)

Species	Number of isolates	Percentage (%)
<i>T. rubrum</i>	18	38.3
<i>T. verrucosum</i>	12	25.5
<i>T. mentagrophytes</i>	10	21.3
<i>T. tonsurans</i>	3	6.4
<i>T. soudanense</i>	2	4.3
<i>M. gypseum</i>	2	4.3

Table 4. Species of Dermatophytes Isolated

Of the total 18 isolates of *Trichophyton rubrum* 10 (55.6%) were from tinea corporis, 3 (16.7%) from tinea cruris, 2 (11.1%) from mixed type and 1 (5.6%) from tinea pedis, tinea faciei and tinea unguium each. *Trichophyton verrucosum* was the second common type i.e., 12 isolates (25.5%) 8 from tinea corporis, 3 from mixed type and 1 from tinea cruris. *Trichophyton mentagrophytes* were 10 (21.3%). Of these 5 (50%) were from tinea corporis, 3 (30%) from mixed type and 1 (10%) from tinea cruris and tinea faciei each. *Trichophyton tonsurans* was the fourth type 3 isolates 6.4%, 1 from tinea corporis and 2 from tinea unguium lesions. 2 (4.3%) isolates of *Trichophyton soudanense* obtained from 2 cases of tinea cruris and *Microsporum gypseum* obtained from 2 (4.3%) cases, 1 from tinea corporis and tinea capitis each. (see table 5)

	<i>T. rubrum</i>	<i>T. verrucosum</i>	<i>T. mentagrophyte</i>	<i>T. tonsurans</i>	<i>T. soudanense</i>	<i>M. gypseum</i>	Total
Tinea corporis	10 (55.6%)	8 (66.7%)	5 (50.0%)	1 (33.3%)	-	1 (50%)	25
Tinea cruris	3 (16.7%)	1 (8.3%)	1 (10%)	-	2 (100%)	-	7
T. corporis +T. cruris	2 (11.1%)	3 (25.0%)	3 (30%)	-	-	-	8
Tinea pedis	1 (5.6%)	-	-	-	-	-	1
Tinea manuum	-	-	-	-	-	-	0
Tinea faciei	1 (5.6%)	-	1 (10%)	-	-	-	2
Tinea capitis	-	-	-	-	-	1 (50%)	1
Tinea unguium	1 (5.6%)	-	-	2 (66.7%)	-	-	3
Total	18	12	10	3	2	2	47
Percentage	38.3%	25.5%	21.3%	6.4%	4.3%	4.3%	100%

Table 5. Distribution of Various Species of Dermatophytes in Different Clinical Types

Association was tested by Chi square test. P value calculated as 0.002, indicating that there is significant association between clinical types and species isolated.

DISCUSSION

Studies on dermatophytoses in India have received much attention in recent years because of the increasing incidence of the mycotic infections worldwide. Dermatophytoses can occur at any age. In the present study the occurrence of disease was more in the age group of 10-20 years (25.7%). Vineetha Met al⁹ in 2018 reported maximum number of cases of dermatophytoses in the age group of 10-20 years. But various other workers reported maximum number of cases in 20-30 years of age group.^{7,9,11,12} The higher incidence in

young age may be due to increased physical activity, use of cosmetics, prolonged studying pattern and hormonal pattern. In our study 21 cases (18.6%) occurred in 30-40 years and most of them were housewives. This was due to their involvement in household activities most of the times. In the present study incidence of Infection was almost equal in both sexes with a slight preponderance in females. M: F ratio was 0.95:1.

Vineetha M et al⁹ in 2018 conducted a mycological study on dermatophytoses in Kottayam and found that the incidence was more in females in the first episode with a male female ratio 1:1.1. But studies conducted by KAK Surendran et al in 2011 and Hosthota A et al in 2018 revealed male preponderance with the male female ratio of 1.63:1 and 2.06:1 respectively. Of the total 113 cases, 56 had history of predisposing factors such as occlusive dressing pattern, use of cosmetics and continuous studying pattern which may create the favourable environment such as increased moisture or exposure to cattle rearing areas where chance of getting zoophilic dermatophytes is high.^{5,9,11,13} 68.3% of patients belonged to middle class. This was in accordance with the study by Agarwal US⁷ in 2014 in which 60% belonged to middle class. But various other studies revealed that the incidence was more in low socio-economic groups.^{4,5,14} This change is attributed to changing lifestyles mainly.

Out of 113 cases, 96 cases (85%) were positive on KOH mount. Similar higher positivity was reported in the study of Agarwal US⁷ (84.67%). Culture positivity was observed in 47 cases (41.6%). Overall positivity was 40% in the study by Noronha et al. Out of 47 positives, 43 (91.5%) were positive by KOH & remaining 4 (8.5%) were negative. In the study by K a K Surendran, out of the total 39 culture positives, 35 (89.7%) were KOH positive and the remaining 4 (10.3%) were KOH negative. Sensitivity of microscopy was 91.5%. Specificity of microscopy was 19.7%.

Predictive value of positive microscopy was 44.8%. Predictive value of negative microscopy was 76.5%. Among the 47 dermatophyte species isolated *Trichophyton rubrum* was the most common agent 18 (38.3%) causing dermatophytosis. Other workers who reported *Trichophyton rubrum* as predominant isolate in their study were KAK Surendran in 2014 (67.5%), Sumit Kumar⁸ in 2014 (65.09%), Agarwal US⁷ in 2014, Vineetha M⁹ in 2018 and Abhineetha Hosthota in 2018.

The common occurrence of *Trichophyton rubrum* in various parts of the country may be due to its greater adaptability to survive in varying climatic conditions and warm and humid climate as in central part of Kerala. Next common were *Trichophyton verrucosum* 12 (25.5%) and *Trichophyton mentagrophyte* 10 (21.3%). Higher number of isolates of *Trichophyton verrucosum* was also reported by Belurkar DD (21.6%) and Noronha T M (8.3%). In this study we got a fairly good number of *Trichophyton verrucosum* species. An important fact is that 3.5% of patients in our study were having cattle rearing in houses and 8.8% were having the habit of taking bath in common places such as ponds, dams etc. where the herd animals frequently come in contact.

Aghamirian MR and Ghiasian S A ET al⁵ conducted an epidemiological study of dermatophytes as a cause of epizoonoses in dairy cattle and humans in Iran during 2006-

2007. It revealed that 33.1% of herdsmen developed fungal lesions suspected of dermatophytoses which on further examination, KOH and culture isolated *Trichophyton verrucosum* in 62.8%, similar to the isolate obtained from the affected cattle (92.6%). *Trichophyton mentagrophyte* was the second commonest isolate by Surendran et al (20%), Kumar et al⁸ (17.92%), and Hosthota A et al (20%) in 2018. In the present study *Trichophyton tonsurans* isolates were 3 (6.4%) of the total 47 isolates. Of these 1 (33.3%) was from tinea corporis and 2 (66.7%) from tinea unguium. *Trichophyton tonsurans* was the common isolate from tinea unguium in the study by BelurkarDD.⁴

Trichophyton soudanense species was obtained from 2 cases (4.3%) in this study and they were siblings, one was a 10-year-old boy and the other 7-year-old girl. Both were having tinea cruris type of lesions. *Trichophyton soudanense* is a common species causing tinea capitis¹. But there were no tinea capitis lesions when these patients came. According to the literature² it can involve other parts of the body also. *Microsporum gypseum* was obtained from 2 cases (4.3%) in the present study. One from tinea corporis and the other from tinea capitis. *Microsporum gypseum* is a common isolate causing tinea capitis mainly kerion type. Similar findings were obtained by Sumana V.⁶ In the present study the commonest isolate was *Trichophyton rubrum* 18 (38.3%) from all clinical isolates.

In tinea corporis 10 isolates (55.6%), in tinea cruris 3 isolates (16.7%), and in mixed type of infection (tinea corporis + tinea cruris) 2 isolates were *Trichophyton rubrum* from culture. 1 case of tinea pedis, tinea faciei and tinea unguium also yielded *Trichophyton rubrum*. Other studies also supported the present pattern. In the study done by Sumit Kumar and Srikara Mallya in 2014 commonest isolate was *Trichophyton rubrum* 69 (65.09%).³ In tinea corporis 34 isolates (61.82%), in tinea cruris 26 isolates (74.28%), in tinea unguium 3 isolates (60%), in tinea pedis 2 isolates (100%) in tinea capitis 1 isolate (20%) and in tinea manuum 1 isolate (50%) were *Trichophyton rubrum*. In tinea faciei, tinea pedis and in tinea pedis and only *Trichophyton rubrum* was isolated. In the study done by Hosthota A et al⁷ in 2018 *Trichophyton rubrum* was the main isolate in all clinical types.

Similar observation was found in the study by K a K Surendran ET al¹¹ and various other workers.⁹ In the present study tinea corporis was the predominant type accounting for 68 cases (60.2%) followed by the mixed type (Tinea corporis + tinea cruris - 14 cases, 12.4%) and tinea cruris 13 (11.5%) cases, almost equal incidence. Kumar ET al⁸ in 2014 and Vineetha M et al⁹ in 2018 observed tinea corporis as the commonest clinical type (47.6%) and (28.7%) respectively. KAK Surendran¹¹ reported higher incidence of tinea corporis followed by tinea cruris. Huda MM³ et al reported that mixed type was the most common type. Tinea cruris was the most common type (50%) in the study by Hosthota A.

Tinea unguium was found in 7 cases (6.2%) in this study. KAK Surendran also found similar occurrence pattern (8.1%). The incidence is more in females in our series. There were 4 (3.5%) cases of tinea pedis and tinea faciei. Similar lower incidence was reported by K a K Surendran (tinea pedis 22.7% and tinea faciei 1.3%). All the tinea pedis cases were in females. There were 2 cases of tinea capitis and both of these

were boys less than 10 yrs. of age. Both lesions were of kerion type. Many authors reported higher incidence of tinea capitis in children.^{6,8,15} Tinea manuum was noted in 1(1.1%) elderly male patient. Similar lower incidence was reported by K A K Surendran (3.3%). No tinea manuum cases were reported by Hosthota A et al.

CONCLUSIONS

Tinea corporis is the most common clinical type and *Trichophyton rubrum* is the most common causative agent. Unlike other studies we obtained a fairly good number of *Trichophyton verrucosum* and *Trichophyton mentagrophyte* species as well. This is probably due to exposure to cattle rearing areas and habits of taking bath in ponds and dams in this part of Thrissur, besides other common predisposing factors. Antifungal agents are the drugs of choice for dermatophytosis. But identification of predisposing factors and avoidance of these can prevent the occurrence of the disease to some extent.

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