

Study of Dermatophytes and incidence of different clinical types of Tinea in skin OPD

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ABSTRACT

Dermatophytes (keratinophilic fungi) are very common cutaneous mycoses. Materials and methods: The study was done to determine the proportion and distribution of dermatophytes among patients coming to a tertiary care hospital with clinically suspected ringworm lesions, taking into account different parameters like age, sex, occupation of the patient, socio-economic status and nature of the skin condition. One hundred clinically diagnosed cases of dermatophytosis were studied. Results: Based on direct microscopy and culture, it was concluded that the most common clinical type was Tinea corporis and the most common dermatophyte was *Trichophyton rubrum*. Infections were more common in manual labourers and males. Conclusion: They are very common infections and proper samples collection and processing are keys to diagnosis.

Keywords: dermatophytes, SDA, *Trichophyton*, *Microsporum*

Dermatophytes (keratinophilic fungi) are the most common cutaneous fungal infections seen in humans affecting skin, hairs and nails with a considerable morbidity [1]. Superficial infection caused by a dermatophyte is known as dermatophytosis or ringworm. Dermatophytic infection of skin is often called as "ringworm". This term is a misnomer because worms are not involved [2]. "Tinea", the Latin name for worm, describes the serpentine appearance of the skin lesions [3]. The gross appearance of the lesion is an outer ring of active, progressing infection, with central healing [4]. Infection may proceed more deeply from superficial involvement, and a variety of pathologic changes can occur depending on the fungus, the site of infection, and the immune status of the host [5]. The principal etiologic agents of dermatomycoses are the genera *Trichophyton*, *Microsporum* and *Epidermophyton* [4].

Species of the genus *Trichophyton* are capable of invading the hair, skin and nails; *Microsporum* species involve only the hair and skin; and *Epidermophyton* species involve the skin and nails [6]. According to habitat pattern, geophilic organisms originate in the soil and only sporadically infect humans. Zoophilic species are usually found in animals, but can infect humans also. Anthropophilic species have adapted to humans as host [2,7]. In 1910, Sabouraud, the Father of Modern Medical Mycology classified dermatophytoses as Tinea capitis (Ringworm of scalp), Tinea faciei (face), Tinea barbae (beard), Tinea manuum (wrist), Tinea corporis (Ringworm of trunk), Tinea cruris (Ringworm of groin), Tinea pedis (Ringworm of foot) [4,8]. Tinea capitis is predominantly seen in pre-pubertal children. Tinea cruris occurs only in adults especially the males and not in children. Tinea pedis and Tinea unguium are more common among adults [8,9].

Geographical distribution of dermatophytes is variable and this is reflected in differing patterns of dermatophyte seen in different parts of world. In Asia, *Trichophyton rubrum* and *Trichophyton mentagrophytes* are most commonly isolated. Infection is influenced by a number of factors like socio-economic conditions, hygiene and climate (hot and humid climate favours infection), type of population, climatic conditions, individual's susceptibility, and lifestyle, migration of people, cultural practices, socio-economic conditions and drug therapy. Jharkhand is a socially, economically and educationally backward area of India. The climate in Jharkhand (with a latitude of 23 45' N and a longitude of 85 30' E) also remains hot and humid for major part of year which are favourable for growth of dermatophytic fungus. Hence we planned this study.

MATERIAL AND METHODS

This study was conducted in the Department of Microbiology, of the institute during the period 2012-2015. The study group consisted of cases that were clinically diagnosed with dermatophytosis of different types with specific dermatological complaints attending the OPD of Skin and VD of the institute. A total of one hundred (100) clinically diagnosed, randomly selected cases of dermatophytosis (skin, hair and nail infection), of all age groups and of both sexes were included.

A detailed history of selected cases was taken regarding name, age, occupation, pets and address. After the detailed history, clinical examination of patients was made in well-lighted room, which included: The sites of lesion, Number of lesions and types, presence of inflammatory margins etc. Specimen was obtained when the patient had been off both topical and systemic antifungal drugs for two to four weeks under aseptic precautions.

Nail clippings: The affected nail was cleaned with 70% alcohol [10]. Nail clippings of the infected part and scrapings from beneath the nail margin were collected in a sterile petridish [6].

Skin: The skin scrapings were taken from the active margins of the lesions. Lesions were disinfected with 70% alcohol [10,11] and then scraped from centre to edge, using blunt margin of a sterile scalpel blade. Suppurative lesions were sampled with a swab. The materials were then sent to the laboratory in sterile petridishes [12].

Hairs: The affected parts were cleaned with 70% alcohol. The infected hairs were removed by plucking with the roots intact using epilating forceps, scales were scraped off from the advancing border of the lesions.

Specimens were allowed to dry to avoid multiplication of bacterial and fungal spores. All the collected samples were then divided into two parts: one for (i) **direct microscopy** and the other for (ii) **culture**.

DIRECT MICROSCOPY: Potassium hydroxide (KOH) preparation: First, 20% KOH were added to all the samples and then a cover slip was placed on it and kept for 30 minutes. Slides were observed first under 10X objective and then 40X power immediately for the presence of typical fungal elements such as branching or unbranching hyaline septate hyphae and arthroconidia. The ectothrix type of infection was noted when arthroconidia appeared as mosaic sheath around hair or as chains on the surface of hair shaft and the cuticle of the hair remained intact. In the endothrix type, hyphae formed arthrospores within the hair shaft, which was severely weakened and cuticle was destroyed. The arthrospores were observed in chains filling inside shortened hair stubs.

Table 1: Distribution of clinical types in present study

Clinical types of dermatophytosis	Cases	Percentage
Tinea corporis	40	40
Tinea cruris	25	25
Tinea pedis	10	10
Tinea faciei	8	8
Tinea corporis with Tinea cruris	6	6
Tinea manuum	4	4
Tinea capitis	3	3
Tinea unguum	2	2
Tinea barbae	2	2
Total	100	100

CULTURE METHODS: Sabouraud's dextrose agar medium with antibiotics: Each sample was inoculated in tube of Sabouraud's Dextrose Agar with chloramphenicol (0.05%) and cycloheximide (0.5%) and incubated at 30°C in a BOD incubator for 4 weeks. Another part of the sample was inoculated in the Dermatophyte test medium (DTM) and incubated at 25°C. The culture tubes were examined after every two days, for a period of 4 weeks for the presence of growth. The growth was relatively slow

and usually observed after 6 days. Culture was reported as negative only after about 4 weeks of incubation. In DTM, growth of dermatophytes was associated with change of colour of medium to deep red within 3-6 days. Sample was declared as negative, if no change was seen upto 2 weeks.

Fungal isolate was identified based on: a) Colonial morphology on the culture medium and pigmentation b) Growth rate c) Microscopic morphology in LCB stain d) Slide culture e) Urease test. Slide culture (Riddell's Method) was done for all the samples.

Urease test: *T. mentagrophytes* demonstrated urease activity usually within seven days. *T. rubrum* and *T. verucosum* isolates were negative for urease test. The test was considered negative if there was no colour change, from straw to deep red colour within 7 days at 23-30°C.

RESULTS

Out of the 100 clinically suspected cases, 68 cases turned out to be dermatophytoses, which showed growth of different dermatophytes on culture. Remaining 32 were either contaminants, or fungi other than dermatophytes or showed no positive finding either in KOH preparation or

culture. Out of total 100 cases, 73 were males and 27 were females. Results are shown in tables 1 to 9.

Table 2: Age wise distribution of dermatophytosis

Age in years	Number of Cases	Percentage
≤ 10	6	6
11-20	18	18
21-30	33	33
31-40	20	20
41-50	17	17
51-60	4	4
>60	2	2
TOTAL	100	100

Table 3: Socio-economic status of the study group

Socio-economic status	Number	Percentage
Low income group	63	63
Middle income group	25	25
High income group	12	12
Total	100	100

Table 4: Age wise distribution in relation to clinical types

Total No. (%)	6(6%)	18(18%)	33(33%)	18(18%)	17(17%)	4(4%)	2(2%)	100(100%)
Tinea barbae	-	-	1(50%)	1(50%)	-	-	-	2(2%)
Tinea corporis with cruris	-	1(16.67%)	2(33.33%)	-	2(33.33%)	1(16.67%)	-	6(6%)
Tinea manuum	-	1(25%)	1(25%)	1(25%)	1(25%)	-	-	4(4%)
Tinea unguium	-	-	-	1(50%)	-	1(50%)	-	2(2%)
Tinea faciei	1(12.5%)	1(12.5%)	2(25%)	1(12.5%)	3(37.5%)	-	-	8(8%)
Tinea capitis	3(100%)	-	-	-	-	-	-	3(3%)
Tinea pedis	-	2(20%)	3(30%)	2(20%)	3(30%)	-	-	10(10%)
Tinea cruris	-	7(28%)	10(40%)	6(24%)	1(4%)	1(4%)	-	25(25%)
Tinea corporis	2(5%)	6(15%)	14(35%)	8(20%)	7(17.5%)	1(2.5%)	2(5%)	40(40%)
Age in years	≤ 10	11-20	21-30	31-40	41-50	51-60	>60	Total

Table 5: Distribution of cases according to KOH mounts positivity and dermatophytes isolated in culture

KOH mount results	Dermatophytes found in culture No. (%)	Dermatophytes not found in culture No. (%)	Total No. (%)
KOH positive	61(61%)	15(15%)	76(76%)
KOH negative	7(7%)	17(17%)	24(24%)
Total No. (%)	68(68%)	32(32%)	100(100%)

Sensitivity: 89.71% and Specificity: 53.12%

Table 6: Sex wise distribution in relation to different clinical types of dermatophytosis

Clinical presentation	Males no. (%)	Females no. (%)	Total no. (%)	P-value
T. corporis	26(65%)	14(35%)	40(40%)	0.14156
T. cruris	20(80%)	5(20%)	25(25%)	0.36282
T. pedis	7(70%)	3(30%)	10(10%)	0.8181
T. faciei	5(62.5%)	3(37.5%)	8(8%)	0.48392
T. corporis with T. cruris	5(83.33%)	1(16.67%)	6(6%)	0.5552
T. manuum	3(75%)	1(25%)	4(4%)	0.92828
T. capitis	3(100%)	-	3(3%)	0.28462
T. unguium	2(100%)	-	2(2%)	0.3843
T. barbae	2(100%)	-	2(2%)	0.3843
Total	73(73%)	27(27%)	100(100%)	

P-values were calculated using Z test of significance [13], These results were not significantly different. ($p>0.05$).

Table 7: Study of clinical types in relation to occupation:

Clinical types	Manual workers	Household workers	Students	Professionals	Others	Total
Tinea corporis	17(42.5%)	11(27.5%)	5(12.5%)	4(10%)	3(7.5%)	40(40%)
Tinea cruris	12(48%)	3(12%)	5(20%)	3(12%)	2(8%)	25(25%)
Tinea pedis	3(30%)	3(30%)	2(20%)	-	2(20%)	10(10%)
Tinea capitis	-	-	2(66.67%)	-	1(33.33%)	3(3%)
Tinea faciei	3(37.5%)	2(25%)	2(25%)	-	1(12.5%)	8(8%)
Tinea unguium	2(100%)	-	-	-	-	2(2%)
Tinea manuum	3(75%)	1(25%)	-	-	-	4(4%)
Tinea corporis with cruris	2(33.33%)	1(16.67%)	2(33.33%)	-	1(16.67%)	6(6%)
Tinea barbae	2(100%)	-	-	-	-	2(2%)
Total	44(44%)	21(21%)	18(18%)	7(7%)	10(10%)	100(100%)

Table 8: Distribution of different species of dermatophytes according to different clinical findings

Clinical types	Dermatophyte Isolates, No. (%)					Total
	T. mentagrophytes	T. rubrum	T. tonsurans	M. gypseum	E. floccosum	
T. corporis	7(25.93%)	16(59.26%)	1(3.7%)	1(3.7%)	2(7.41%)	27(67.5%)
T. cruris	3(18.75%)	11(68.75%)	-	-	2(12.5%)	16(64%)
T. pedis	4(57.14%)	3(42.86%)	-	-	-	7(70%)
T. faciei	-	5(100%)	-	-	-	5(62.5%)
T. corporis with cruris	2(40%)	3(60%)	-	-	-	5(83.33%)
T. manuum	-	3(100%)	-	-	-	3(75%)
T. capitis	-	1(50%)	1(50%)	-	-	2(66.67%)
T. unguium	1(50%)	1(50%)	-	-	-	2(100%)
T. barbae	1(100%)	-	-	-	-	1(50%)
Total	18(26.47%)	43(63.24%)	2(2.94%)	1(1.47%)	4(5.88%)	68(68%)

DISCUSSION

In present study, *T. corporis* was the commonest type of dermatophytosis (40%). This finding is comparable with other studies done by Ellabib et al (45.9%) [14], Bindu et al. (54.6%) [15], Singh et al [11], Sen et al. (48%) [16]. Jain et al. (37%) [17] and Venkatesan et al (64.8%) [10]. *Tinea cruris* was found to be the second most common clinical type (25%). This finding was comparable with studies done by Siddappa K [18], Mishra M [19], Sen SS [16] and Peerapur BV [20]. *T. pedis* was seen in 10% cases. This finding was comparable with Chimelli PAV (9.9%) [21], Ellabib MS (8.1%) [14], Singh S (11.53%) [11] and Huda MM (7%) [22]. *T. faciei* was seen in 8% cases, and *T. corporis* with *cruris* was seen in 6% cases, which is comparable with studies done by Karmakar S [23]. *T. manuum* was seen in 4% cases, and is comparable with studies done by Huda MM, Chimelli PAS and Siddappa K who reported *tinea manuum* in 3%, 1.9% and 1.53% cases respectively [18,21,22].

In our study, dermatophytosis was found to be more common in the age group of 21-30 years (33%) followed by 31-40 years (20%), which corroborates with other studies [16,19,24,25]. In *T. cruris*, the most common age group affected was of 21- 30 years (40%), which matches with previous studies [16,18-20]. In *T. capitis*, all the 3 cases (100%) were seen in age group of ≤ 10 years. This study was comparable with that of studies done by Vena et al. (81.8%) [26], Kumar et al (78%) [27], Siddappa et al (77.78%) [18], Reddy BSN (73.5%) [28] and Kalla et al (85.5%) [29]. High occurrence of *T. capitis* in ≤ 10 years of age may be due to lack of secretion of fungistatic sebum by scalp before puberty. Adult sebum has fungistatic action.

In our study, males (73%) were more commonly affected than females (27%), which is comparable with previous studies [11,15,16,18,22,30,31]. In this study, males (65%) were more commonly affected with *T. corporis* than females (35%). This was comparable with that of other studies [10,11,14-16]. In our study, males (80%) were more commonly affected with *T. cruris* than females (20%), which was comparable with that of studies done by Sen et al., Siddappa et al., Mishra et al. and Peerapur [16,18-20]. males (70%) were more commonly affected with *T. pedis*, than females (30%) as shown by Vena et al. [26], and Rizvani et al [32] also.

In our study, all the cases of *T. capitis* were seen in males (100%). This study was comparable with that of studies done by Siddappa et al. [18], Kumar [27], and Kalla et al [29] whereas Reddy BSN [28] and Jha NB [33] reported higher incidence among females. Also, all the cases of *T. unguium* were seen in males (100%) as shown by other studies done by Grover *et al.* (M: F ratio of 1.6:1), Kaur *et al.* (M: F ratio of 1.09:1), and Vijaya et al. However, Bhokari et al, Madhuri et al, and Cordeiro et al reported that females were more commonly affected than males, with male to female ratio being 1:2.6, 1:1.08, 0.31:1 and 0.69:1 respectively [24,31,34-37].

In this study, dermatophytosis was most common in the low income group with 63 cases (63%) which was followed by middle income group with 25 cases (25%) and high income group with 12 cases (12%). This observation is almost similar to the other observations which report that 69.2% of affected people are from low income group and 23.2% from middle income group [38]. Sivakumar N et al. reported that 74.7% of affected people were from low income group and 18.68% from middle income group [12]. This may be due to poor hygienic conditions, overcrowding, sharing unwashed clothes and also due to malnutrition. In the present study, dermatophytosis was most commonly seen in manual workers with 44 cases (44%), which included agricultural workers and manual labourers, followed by household workers, students, others with 10 cases (10%) and professionals with 7 cases (7%). The above findings are comparable with other observations [30,39].

This could be due to increased physical activity and increased opportunity for exposure in manual workers. Farmers are engaged in handling with hay, soil and clay in the field, walk barefooted and work with unprotected hands (without using gloves) and hence more exposed to these dermatophytic infections. In case of household workers, there is increased wet working premises due to household chores (like mopping of floor, handling of mud and garbage), hence more exposed.

In our study, out of 100 clinically diagnosed cases, 83 were positive for fungi, either by KOH and / or culture. 61 cases (61%) were positive by both microscopy and culture. 15 cases (15%) were positive by microscopy and negative by culture. 7 cases (7%) were negative by microscopy but culture positive. 17 cases (17%) were negative both by microscopy and culture. These findings are comparable

with other studies done by Karmakar et al. [23], Bindu et al. [15] and Nada et al [40]. This variation could be due to presence of commensal non-pathogenic fungi or non-viability of fungal elements in some cases. Though KOH can give false negative results in 5-15% cases, but this technique is a great aid for prompt detection of dermatophytes in the clinical sample. The data in the present study was analysed and it was found that the sensitivity of the KOH mount technique was 89.71%, but the specificity was 53.12%. *T. rubrum* was the commonest etiological agent in majority of clinical types with 43 cases (63.24%) followed by *T. mentagrophytes* with 18 cases (26.47%), which is comparable to other studies done by Bindu et al. [15], Ranganathan et al. [38], Singh et al. [11] and Jain et al. [17]. In study by Ranganathan S et al. [38], *T. rubrum* was the etiological agent in 52.2% cases followed by *T. mentagrophytes* with 29.35% cases. *E. floccosum* was the third etiological agent of dermatophytosis to be isolated in 4 cases (5.88%), which is similar to previous studies done by Bindu et al. [15], Ranganathan et al. [38], Venkatesan et al. [10], Sahai et al. [25] and Kannan et al [41].

In Tinea unguium, both *T. rubrum* and *T. mentagrophytes* were isolated in 1 case each (50%). The most frequent etiological agent of Tinea unguium (80-90%) are *T. rubrum* and *T. mentagrophytes*. Mathur et al. [42] reported equal isolation rates of 11.1% for both *T. rubrum* and *T. mentagrophytes*, whereas Veer P et al. [39] reported *T. rubrum* in 57.64% cases followed by *T. mentagrophytes* in 42.3% cases of onychomycosis. The geographical distribution of different dermatophyte species in different regions, as well as the behaviour of people who get exposed to these agents in those regions may be the reason behind the heterogeneity of the findings in various studies.

CONCLUSION

Dermatophytoses are very common form of superficial mycosis in our country, due to hot and humid climate in association with poor hygienic conditions. By and large, *Trichophyton* spp. is the commonest etiological agent of dermatophytosis. Although this infection responds to conventional antifungals, dermatophytosis has a tendency to recur at the same or different site. Hence a correct diagnosis is important to initiate an appropriate treatment and also for epidemiological purposes.

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