

# Recurrent dermatophytosis: A rising problem in Sikkim, a Himalayan state of India

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## ABSTRACT


Changing pattern of dermatophytic infection among people of Sikkim over the past few years and its recurrence rate has brought a need to do a study on clinical pattern and its recurrence from this part of the country. The objectives of this study are to discern the clinical patterns of dermatophytosis, identification of the isolated fungi to its species level and to see the pattern of its recurrence. The study was carried out from January 2015 to May 2016. A total of 192 samples were collected from the patients with clinical findings of dermatophytic infection. Required history of the patients was taken, followed by clinical examination of the lesions and sample collection. The samples were processed for mycological study till species identification and a follow up of patients were done to assess its recurrence pattern. The age distribution of the patients was from 2 to 80 years. The mean and median age was 30.33 and 33 years respectively. The male female ratio was 1.8:1. Dermatophytosis was noted more commonly in students ( $n = 64$ , 33.33%) and jawans ( $n = 44$ , 22.92%). Maximum occurrence was noted from April to July ( $n = 106$ , 55.20%) and was seen mainly in young Hindu males. Tinea corporis ( $n = 104$ , 54.16%) was the most common clinical manifestation followed by tinea unguium ( $n = 30$ , 15.63%). *T. mentagrophyte* (40%) was the most common species followed by *T. schoenleinii* (33.3%), *T. tonsurans* (16.6%) and *T. rubrum* (6.6%). The recurrence rate was seen most commonly in clinical cases of tinea faciei 100%, followed by tinea pedis 80% and tinea unguium 46.6%. Overall clinical cure rate was 58.3% and recurrence rate was 34.3%. In the isolated species of dermatophytes, the recurrence rate was 73.68% and that of non-dermatophytes it was 28.07%. Dermatophytosis is an important health problem with high recurrence in Sikkim with difference in the etiological agent from other parts of India.

**KEY WORDS:** Dermatophytes, Dermatophytosis, Sikkim

## INTRODUCTION

Dermatophytosis, commonly referred as “ringworm,” is superficial infection of keratinized tissue caused by organisms of three genera of closely related fungi known as dermatophytes.<sup>[1]</sup> Dermatophytes are classified into *Epidermophyton*, *Microsporum*, and *Trichophyton*. Dermatophytes are keratinophilic fungi which are capable of invading the keratinous tissue of living animals.<sup>[2]</sup> They are characterized by their ability to invade the superficial layers of epidermis, particularly the stratum corneum and the high keratin concentration containing appendages, the hair and nails of a living host. Infection is generally cutaneous and restricted to the nonliving cornified layers because of the inability of fungi to penetrate the deeper tissues or organs of immunocompetent hosts.<sup>[3,4]</sup>

Reactions to a dermatophytic infection may range from mild to severe as a consequence of host's reactions to the metabolic products of the fungus, virulence of the infecting strain or species, anatomic location of the infection, and local environmental factors. Dermatophytosis

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is one of the most common diseases in human beings. Although it does not cause mortality, it causes high morbidity and worsens the quality of patients' life. The prevalence of this disease is governed by environmental conditions such as high humidity, personal hygiene, and individual's susceptibility from place to place. The seasonal variation in dermatophytosis is also noted with increased number of cases reported in summer months. Today, we are facing an onslaught of chronic and recurrent dermatophytosis in volumes never encountered previously.

Although some studies on clinico-mycological aspects of dermatophytosis have been reported from different parts of India, no

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report from Sikkim is available. The present study aims to evaluate patients with dermatophytosis in relation to clinical manifestations, recurrences, seasonal variation, and species identification.

## MATERIALS AND METHODS

A prospective hospital-based study was conducted in the Department of Microbiology, Sikkim Manipal Institute of Medical Sciences, and Department of Dermatology, Central Referral Hospital (CRH), Tadong. Patients of all age and both the sexes visiting the Dermatology Outpatient Department (OPD) of CRH during the study period with signs and symptoms of dermatophytosis were included. Patients with underlying conditions such as diabetes, patient under immunosuppressive therapy, patients who are HIV positive, and patients who refused to give consent were excluded in the study.

The study population consisted of 192 patients with clinical sign and symptoms of dermatophytosis attending the Dermatology OPD during the period of sample collection from January 2015 to December 2015. Required history of the patients was taken, followed by clinical examination of the lesions. The location, shape, color, surface, and texture of the lesion were examined. Follow-up was carried out after 3 months of initiation of the treatment for each patient to check whether the lesion has been clinically cured or not.

A total of 192 samples (156 skin scrapings, 30 nail clips, and 6 plucked hair strands) were collected from the patients with clinical findings of dermatophytic infection. Skin scrapings were collected from the edges of the lesions; infected hairs were plucked, and nail scraping, clippings, and subungual debris were collected. The specimens were collected on sterilized paper envelope. Paper envelopes were folded and clipped with paper clips. Patient's name, identification number, source of material, and date of collection were labeled on it and were transported immediately to the laboratory.

All the specimens were subjected to direct examination (20% or 40% KOH mount) and culture on Sabouraud dextrose agar (SDA), SDA with cycloheximide (500 µg/ml) and chloramphenicol (40 µg/ml), and dermatophyte test medium (DTM). The culture media were kept at 28°C for 3 weeks with regular examination for growth of fungi. Identification was performed on the basis of colony characteristics as well as microscopic morphology in lactophenol cotton blue mount. Slide culture, urease test, and hair perforation test were done when necessary.<sup>[5]</sup>

### Identification of fungus

#### KOH (20%/40%) mount

The slides were examined microscopically using ×10 and ×40 objectives and looked for branching hyphae, type of branching, the color, septation, and thickness of hyphae.<sup>[5]</sup>

#### Isolation of dermatophytes (Sabouraud dextrose agar, Sabouraud dextrose agar with antibiotics, and dermatophyte test medium)

The colonies on the slant were examined for their

topography (elevation, margin, and folding), texture on the surface (cottony, wooly, velvety, powdery, ceribriform, granular, or glabrous), and pigmentation on the surface and reverse.<sup>[5]</sup>

The confirmation of species was done by microscopic examination (lactophenol cotton blue), urease test, slide culture, and *in vitro* hair perforation test.

#### Lactophenol cotton blue

The appearance and arrangement of the macroconidia and/or microconidia were noted. Characteristics of the macroconidia and microconidia such as size, shape, number of septation, characters of their walls, and the way they were borne on the hyphae were helpful in identification.<sup>[5]</sup>

#### Urease test

This test was done to differentiate the dermatophyte such as *Trichophyton mentagrophytes* species, which hydrolyzes urea and the color of the medium becomes pink red from the species such as *Trichophyton rubrum* which does not hydrolyze urea.<sup>[5]</sup>

#### Slide culture

One centimeter square agar block from SDA was cut aseptically and was transferred onto the slide placed over a sterile Petri plate. A small amount of colony was transferred to the four sides of the agar block with the sterile needle and covered with coverslip. One milliliter of sterile water was added to the Petri dish to avoid drying of agar block and the whole set up was placed in a canister and incubated in the dark for 10–14 days till mature conidia or spores were observed.<sup>[5]</sup>

### Observation

The slide was observed under microscope (×40) for the microconidia, macroconidia, and their arrangements.

#### In vitro hair perforation test

This test was utilized to differentiate between *T. mentagrophytes* which gives positive test result and *T. rubrum* which gives negative result, and they are difficult to differentiate based on morphological characteristics.<sup>[5]</sup>

Follow-up was done after 3 months of initiation of treatment for each patient to check whether the lesion has been clinically cured or not.

Recurrent dermatophytosis refers to the reoccurrence of the dermatophytic infection within few weeks, after completion of treatment and cured are the one with clinical disappearance of the lesion.

### Statistical analysis

The obtained final result of the study was analyzed statistically by calculating Chi-square test and the association was studied between different variables. In the Fisher's Chi-square table, the calculated Chi-square value was compared with the highest obtainable by chance at the desired degrees of freedom given in the table under different probabilities such as 0.05, 0.02, and 0.01.<sup>[6]</sup>

## RESULTS

In this study, of 192 cases, 124 (64.58%) were male and 68 (35.41%) were female ( $df = 4$ ,  $P < 0.001$ ). Maximum cases, i.e., 106 (55.21%), were seen in the young age group of 16–30 years followed by the age group of 31–45 years, i.e., 54 (28.12%). The disease was most commonly seen in students (33.33%) followed by the semiprofessionals (22.92%) which comprised mostly of jawans. Maximum number of the cases (106/192) were seen between mid of spring and mid of summer (April–July), which was found to be significantly high compared to other two-quarters ( $df = 2$ ,  $P < 0.001$ ). The disease was predominantly seen in Hindu religion (62.52%), followed by Buddhist (26.04%). Tinea corporis (54.16%) was figured at the top followed by tinea unguium (15.63%). All the clinical manifestations were seen more commonly in males, except tinea unguium.

Around 64.4% of patients gave a history of similar illness, single or multiple episodes, within the last 2 years. A significant difference was noted between the occurrence of new cases and the occurrence of cases with previous episode of similar lesions ( $df = 1$ ,  $P < 0.01$ ). The study revealed a mycological positivity of 55.21% on direct microscopic examination (KOH mount). Culture was positive in 63.54% (122/192) cases, of which 31.25% were dermatophytes and 32.29% were nondermatophytes [Table 1].

A total of 60 (31.25%) dermatophytes isolated. *T. mentagrophytes* was the most common species ( $n = 24$ , 40%), followed by *Trichophyton schoenleinii* ( $n = 20$ , 33.33%), and *Trichophyton tonsurans* ( $n = 10$ , 16.66%). *T. rubrum* ( $n = 4$ , 6.66%) was the least commonly isolated *Trichophyton* species. Two isolates of *Epidermophyton floccosum* (3.33%) and no *Microsporum* species were isolated.

Follow-up showed recurrent dermatophytosis in 34.33% (66/192) cases. Recurrence was seen mainly in tinea faciei (100%), tinea pedis (80%), and tinea unguium (46.6%). The overall cure rate was 58.33% and the recurrence rate was 34.38% ( $df = 1$ ,  $P < 0.001$ ) [Tables 2 and 3].

In the isolated species of dermatophytes, the recurrence rate was 73.68% (42/57), and that of nondermatophytes, it was 28.07% (16/57). Recurrence rate was very high in all the species of dermatophytes ( $\geq 50\%$ ).

## DISCUSSION

In this study, most of the patients were males, i.e., 64.58%. Different studies have reported different sex-specific prevalence: more in males,<sup>[7-11]</sup> more in females,<sup>[12]</sup> and equal in both the sexes.<sup>[13]</sup> The maximum numbers of cases were seen in the young age interval; 16–30 years - 55.2% and 31–45 years - 28.1%. Similar finding was noted in other studies too: 21–30 years,<sup>[8,11,14,15]</sup> 31–40 years,<sup>[9,12]</sup> 22–45 years,<sup>[10]</sup> and 21–50 years.<sup>[7]</sup> Higher incidence in young males could be due to the fact that males are physically more active, which predispose to increase sweating. The disease was noted more commonly in students and jawans (33.33% and 22.91%, respectively). Other studies reported the most common occurrence among farmers and office workers<sup>[7]</sup> and homemakers.<sup>[9]</sup>

Maximum number of the cases (106/192) were seen between mid of spring and mid of summer (April–July) compared to other two-quarters, which could be due to favorable temperature (35°C) and humidity (90%) for growth of fungus during these months. A study from Meghalaya also reported maximum number of cases between April and September.<sup>[8]</sup> The disease was predominantly seen in Hindu religion (62.5%), followed by Buddhist (26%). This could be due to the number of patients coming for treatments in the hospital were predominantly Hindus. Significant difference ( $df = 1$ ,  $P < 0.001$ ) was noted between the most populated religions, Hindu and Buddhist, of the state. Although all the clinical manifestations were seen more commonly in males, except tinea unguium, significant difference was noted only in tinea corporis ( $df = 1$ ,  $P < 0.001$ ). Like in other studies,<sup>[7,10,15,16]</sup> tinea corporis (54.1%) was figured at the top, but there are studies showing tinea pedis,<sup>[8]</sup> tinea unguium,<sup>[9,11]</sup> and tinea capitis,<sup>[17]</sup> as the most common clinical manifestations of dermatophytosis.

Around 60.4% of patients gave a history of similar illness, single or multiple episodes, within the last 2 year. High occurrence of recurrence could be due to the persistence of risk factors such as damp house, physical activities leading to sweating, continuous wearing of closed shoes, and sharing of clothes and linens. Fungus survives in moist and damp environment and can infect the same or new person again and again. Further, high rate of relapse or recurrence in case of unguium could be due to inability of drugs to reach the site<sup>[18]</sup> as well as due to wet occupations.<sup>[9]</sup>

**Table 1: KOH and Culture positive**

Disease	KOH positive (%)	Culture positive			KOH Positive culture negative (%)	Culture Positive KOH negative (%)	Positive by both (%)
		Dermatophytes (%)	Non-dermatophytes (%)	Total (%)			
T. corporis (n=104)	60 (56.6)	30 (50)	28 (45.1)	58 (55.7)	12 (66.6)	18 (42.8)	44 (51.1)
T. cruris (n=28)	08 (7.5)	8 (13.3)	4 (6.4)	12 (42.8)	4 (22.2)	6 (14.2)	8 (9.3)
T. pedis (n=20)	12 (11.3)	12 (20)	6 (9.6)	18 (90)	0	6 (14.2)	12 (13.9)
T. unguium (n=30)	22 (20.7)	8 (13.3)	18 (29)	26 (86.6)	2 (11.1)	8 (19)	18 (20.9)
T. faciei (n=02)	2 (1.8)	2 (3.3)	0	2 (100)	0	0	2 (2.3)
T. capitis (n=06)	2 (1.8)	0	06 (9.6)	6 (100)	0	4 (9.5)	2 (2.3)
T. manuum (n=02)	0	0	0	0	0	0	0
Total (n=192)	106 (55.21)	60 (31.25)	62 (32.29)	122 (63.54)	18 (9.38)	42 (21.86)	86 (44.79)

**Table 2: Result of follow-up**

Disease	Recurrence rate (%)	Cure rate(%)	Lost to follow up (%)
Tinea corporis (n=104)	28 (26.92)	70 (67.31)	6 (5.7)
Tinea cruris (n=28)	6 (21.43)	16 (57.14)	6 (21.43)
Tinea unguium (n=30)	14 (46.66)	16 (53.33)	0
Tinea pedis (n=20)	16 (80)	4 (20)	0
Tinea capitis (n=06)	0	4 (66.66)	2 (33.3)
Tinea faciei (n=02)	2 (100)	0	0
Tinea manuum (n=02)	0	2 (100)	0
Total (n=192)	66 (34.38)	112 (58.33)	14 (7.33)

**Table 3: Recurrence rate and cure rate in relation to isolated fungi**

Organism	Recurrence rate (%)	Cure rate (%)	Lost to follow-up (%)
<i>Trichophyton mentagrophytes</i> (n=24)	18 (75)	4 (16.66)	2 (8.33)
<i>Trichophyton schoenleinii</i> (n=20)	14 (70)	5 (25)	1 (5)
<i>Trichophyton tonsurans</i> (n=10)	6 (60)	4 (40)	0
<i>Trichophyton rubrum</i> (n=4)	2 (50)	2 (50)	0
<i>Epidermophyton floccosum</i> (n=2)	2 (100)	0	0
<i>Candida</i> spp. (n=22)	4 (18.18)	17 (77.27)	1 (4.54)
<i>Aspergillus</i> spp. (n=40)	12 (30)	24 (60)	4 (10)
No growth (n=72)	8 (11.11)	58 (80.55)	6 (8.33)
Total (n=192)	66 (34.33)	119 (61.98)	14 (7.29)

The study revealed a mycological positivity of 55.21% on direct microscopic examination (KOH mount). Other studies had reported KOH mount positivity of 32.8%,<sup>[11]</sup> 38.2%,<sup>[8]</sup> 43%,<sup>[15]</sup> 50.5%,<sup>[10]</sup> 85%,<sup>[19]</sup> 91.3%,<sup>[9]</sup> and 94.1%<sup>[14]</sup> in clinically suspected cases. This wide range of KOH positivity report shows a lot of deviation from the standard in the clinical diagnosis, sample collection, processing and examination of the mount; this subjective bias can be minimized by developing and following standard operating procedure for each of the components. Culture was positive in 63.54% (122/192) cases, of which 31.25% were dermatophytes and 32.29% were nondermatophytes. Other studies reported culture positive (dermatophytes) in 29.3%,<sup>[8]</sup> 30.3%,<sup>[11]</sup> 36.6%,<sup>[7]</sup> 60%,<sup>[19]</sup> and 87.43%.<sup>[9]</sup> Fungal culture has the advantage of differentiating the causative agent to generic and species level. From the 122 culture-positive samples, 21.8% (42) of them showed no fungal elements on direct KOH mount, which could be because of fungus in an inactive sporulating phase difficult to be seen by microscopy but able to grow in appropriate media.<sup>[20,21]</sup> Of the culture-negative cases, 9.3% (18) showed fungal elements on KOH mount but failed to grow in culture. This could be due to nonviability of fungus before inoculation.<sup>[21]</sup> Similar finding was reported in a study conducted in Meghalaya.<sup>[8]</sup> For the diagnosis of fungal infection of skin, hair, and nail, either positive KOH mount or positive fungal culture, or both positive should be taken into consideration. KOH mount should not replace fungal culture and vice versa. Increase in the number of nondermatophytes (32.3%) as noted in our study had been reported in other studies too (24.4%).<sup>[10]</sup>

Among dermatophytes, genus *Trichophyton* was responsible for majority of cases, a finding which concurs with other studies.<sup>[7-11]</sup>

Predominant isolates were *T. mentagrophytes* (40%) followed by *T. schoenleinii* (33.3%). Most of the studies reported *T. rubrum* as the most common species followed by *T. mentagrophytes*,<sup>[8-11,15]</sup> whereas we observed *T. mentagrophytes* as the most common species and *T. rubrum* (6.6%) as the least common species in the genus *Trichophyton*. *T. tonsurans* was isolated in 16.6% cases, whereas other studies reported *T. tonsurans* in 26.3%<sup>[8]</sup> and 44.4%<sup>[14]</sup> cases. Another study from Gangtok, Sikkim, showed *T. tonsurans* as the most common isolate followed by *T. mentagrophytes*,<sup>[14]</sup> which along with our study suggest *T. mentagrophytes*, *T. schoenleinii*, and *T. tonsurans* are the most common species prevalent in Gangtok, Sikkim.

Similar to other studies,<sup>[9,11]</sup> *E. floccosum* was found to be associated with very few cases of dermatophytosis (3.3%); unlike other studies where *Microsporum* was reported from dermatophytosis 2.6%,<sup>[17]</sup> 6.4%,<sup>[10]</sup> 11.1%,<sup>[14]</sup> and 13.7%,<sup>[9]</sup> we did not observe any such association. The association between the species of dermatophytes and the clinical lesions was insignificant, whereas association between the species of dermatophytes and age interval as well as the association between the species of dermatophytes and religion was found to be significant. Among the culture-positive nondermatophytes, the most common was *Aspergillus* spp. (64.51%) followed by *Candida* spp. (35.48%). Lakshmanan *et al.* reported *Candida* spp. (60%), *Aspergillus* spp. (20%), *Fusarium* spp. (10%), *Alternaria* spp. (5%) and *Curvularia* spp. (5%) as nondermatophytes.<sup>[10]</sup>

Follow-up showed recurrent dermatophytosis in 34.38% (66/192) cases. These recurrences could be due to suboptimal doses or duration of therapy, poor drug penetration and distribution, patients' compliance, or absence of a protected or persistence focus of infection.<sup>[22,23]</sup> Recurrences was high in all the species of dermatophytes ( $\geq 50\%$ ). Significant difference in the cure rate and recurrences was noted in *T. mentagrophytes* (df = 1,  $P < 0.01$ ) and *T. schoenleinii* (df = 1,  $P < 0.05$ ). Unlike our study, other studies reported *T. rubrum* as the major cause of recurrences.<sup>[16,18]</sup> Recurrences were less in dermatomycosis (28.07%) as compared to dermatophytosis (73.68%). This shows that the dermatomycosis responds to antifungal therapy better than the dermatophytosis.

## CONCLUSION

Dermatophytosis is an important health problem with high recurrence in Sikkim with difference in the etiological agent from other parts of India, as *T. mentagrophytes*, *T. schoenleinii*, and *T. tonsurans* are the most common species. Dermatophytes showed higher rate of recurrences compared to the nondermatophytes.

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## Conflicts of interest

There are no conflicts of interest.

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