

A Clinicomycological Study of Cutaneous Mycoses in Sawai Man Singh Hospital of Jaipur, North India

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Abstract

Background: Because of the widespread prevalence of the various cutaneous mycoses in a tropical country like India, it is important to know their patterns of etiology and clinical presentations. **Aim:** The present study was conducted in order to identify the clinical pattern of various cutaneous mycoses and the common etiological agents affecting the study populations admitted in SMS Hospital, Jaipur, in North India. **Materials and Methods:** Skin scrapings and hair and nail samples of 160 patients with clinical suspicion of dermatophytosis were collected and subjected to direct microscopy and were cultured in Sabouraud's dextrose agar. Fungal species were identified by macroscopic and microscopic examination. Data were presented as simple descriptive statistics (SPSS, Version 17.0 (Chicago IL, USA). Epi Info Version 3.5.1 (CDC, Atlanta, Georgia, USA). **Results:** Among the 160 clinically suspected patients of cutaneous mycoses, 60 (37.5%) were confirmed by culture. Dermatophytes and non-dermatophytes (NDM) were isolated from 66.6% (40/60) and 33.3% (20/60) of the positive cultures, respectively. *Tinea capitis* (50%) 30/60 was the most frequent clinical pattern and genus *Trichophyton violaceum* 32.5% (13/40) was the most common isolate in dermatophytosis-positive samples. Among the patients positive for NDM by culture, *Tinea unguium* 35% (7/20) was the most common clinical presentation and *Aspergillus* species 40% (8/20) were the most common etiological agents isolated. **Conclusion:** Although dermatophytes have been isolated from the cases of cutaneous mycoses all over the world with various frequencies, the role of NDM in the different cutaneous infections other than those of nail infections need to be evaluated.

Keywords: Dermatophytes, Dermatophytosis, Skin infections, Tinea, *Trichophyton*

Introduction

Dermatophytosis constitutes a group of cutaneous fungal infections of keratinized tissues. The disease is caused by fungi belonging to the genera *Trichophyton*, *Microsporum* and *Epidermophyton*.^[1] Dermatophyte infections are one of the earliest known fungal infections of mankind and are very common throughout the world. Although dermatophytosis does not cause mortality, they cause morbidity and pose a major public health problem, especially in tropical countries like India, due to the hot and humid climate. No race in any geographical location is totally free from dermatophytosis.^[2] The risk factors include socioeconomic

conditions like overcrowding, poverty and poor personal hygiene. The prevalence of these fungi tends to vary with time and geographical locations. In India, previous reports suggest that it is more common in southern and eastern regions than in the northern regions of the country.^[3-5]

Non-dermatophytes (NDM), although commonly considered as contaminants, have been reported to colonize damaged tissues and cause secondary tissue destruction.^[4] Their role in causing cutaneous infections is not proven, and a primary pathogenic role of NDM is controversial at best. But, these are increasingly implicated in causing primary invasion of the nail in onychomycosis.

A correct knowledge of the etiological agents of cutaneous mycoses is therefore important to initiate appropriate treatment, and is also essential for epidemiological purposes. Thus, the present study was undertaken to shed light on the clinical manifestations of cutaneous mycoses and their etiology among patients admitted to the Dermatology Outpatient Department of SMS Hospital, Jaipur, in North India.

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Materials and Methods

This study was carried out on 160 clinically suspected cases of cutaneous mycoses admitted in the outpatient clinic of the dermatology department of SMS Hospital in North India. After taking informed consent, the demographic data, detailed history and suspected clinical diagnosis of the patient were recorded. Clinical materials like skin scraping, nail clipping and infected hair were collected from the patient, depending on the infection site, and subjected to the following mycological examinations. Permission to undertake the study was taken from the SMS Hospital authorities.

Microscopic examination

The involved area of the body was disinfected with 70% alcohol. Skin scrapings were collected from the lesions, particularly at the advancing border of the infections, using a blunt sterile scalpel/ tweezers in a sterile Petri dish. In the case of nail infections, clippings and scrapings were taken from friable or discolored areas of hypokeratic nails. In hair infections, 10-12 hair with intact shaft were collected in a dry container (sterile Petri dish), and the active border of the lesions were scraped when present. All the scrapings were placed in a few drops of 10% potassium hydroxide solution with 40% di-methyl sulphoxide on a clear glass slide. Then, a cover slip was placed over the preparation and the slides were observed under the microscope immediately for the presence of unstained refractile fungal elements.

Culturing

The culture was performed with two different sets of antibiotics incorporated in Sabouraud's dextrose agar

media in McCartney bottles, one with chloramphenicol (16 µg/mL) plus gentamicin (5 µg/mL) and the other with cycloheximide (500 mg/L).^[6] The culture bottles were incubated at 25°C and 37°C, and the growth was observed twice a week. All culture media and antibiotics were obtained from Hi-Media Laboratories, Mumbai, India. They were discarded only after 6 weeks in the absence of growth. The mycological identification was based on gross examination of macroscopic features of the colony, which included duration of growth, surface morphology and pigment production as well as microscopic examination in lacto phenol cotton blue stain. Special tests like urease production test, slide culture techniques (for filamentous fungi), germ tube test and spore production on corn meal agar (for yeast isolates) were performed wherever required.^[6] Data were presented as simple descriptive statistics (SPSS, Version 17.0 (Chicago II, USA). Epi Info Version 3.5.1 (CDC, Atlanta, Georgia, USA).

Results

Of the 160 samples processed, 100 (62.5%) showed fungal elements on KOH preparation, while 60 (37.5%) samples were confirmed by culture. Dermatophytes were isolated from 66.7% (40/60) culture-positive cases, while NDM were isolated from the remaining 33.3% (20/60) positive samples. The ratio of males to females was 3:1 in positive clinical cases. About 75% (45/60) of the fungal agents were isolated in the age group of 5-10 years [Table 1]. Tinea capitis 50% (30/60) was the most frequent clinical pattern noted in the present study group, followed by Tinea unguium 18.3% (11/60), and others [Table 2].

The most common dermatophyte isolated was *Trichophyton violaceum* 12.5% (13/40), followed by *Microsporum audouinii* and *Trichophyton tonsurans* 15% (6/40). *Trichophyton violaceum* 43.3% (13/30) was the most common species isolated from cases of Tinea capitis. *Epidermophyton floccosum*, one each, was isolated from Tinea corporis and Tinea cruris. In Tinea unguium, NDM 63.6% (7/11) were the most commonly isolated, followed by 9.1% (1/11) each of *Trichophyton rubrum*, *Trichophyton violaceum*, *Epidermophyton flocculosum* and *Microsporum nanum*. In Tinea corporis, NDM and dermatophytes were isolated with comparable frequencies.

Table 1: Distribution of dermatophytes in relation to age and sex of the patient

Fungus isolated	Boys	Girls	0-4 years	5-10 years	>11 years
<i>Trichophyton</i> sp	20	7	3	23	1
<i>Microsporum</i> sp	6	4	2	8	0
<i>Epidermophyton</i> sp	2	1	0	3	0
Non-dermatophytes*	17	3	3	11	6
Total	45	15	8	45	7

*Non-dermatophytes: Includes, *Aspergillus* sp, *Alternaria* sp, *Scopulariopsis* sp, *Fusarium* sp, *Candida* sp

Table 2: Numerical distribution of clinical pattern and strain isolate

Clinical pattern	Total	<i>T. vi</i>	<i>T. r</i>	<i>T. m</i>	<i>T. t</i>	<i>E. fl</i>	<i>M. a</i>	<i>M. n</i>	ND
Tinea capitis	30	13	2	2	5	0	5	0	3
Tinea cruris	5	-	1	1	-	1	-	-	2
Tinea corporis	4	-	1	-	-	1	-	-	2
Tinea corporis+Tinea cruris	5	-	1	-	-	-	-	-	4
Tinea unguium	11	-	1	-	1	1	-	1	7
Tinea pedis	2	-	-	-	-	-	-	-	2
Tinea manum	2	-	-	-	-	-	-	2	-
Tinea faciei	1	-	-	-	-	-	-	1	-
Total		13	5	3	6	3	6	4	20

**T. r*: *Trichophyton rubrum*, **T. vi*: *Trichophyton violaceum*, **T. m*: *Trichophyton mentagraphyte*, **T. t*: *Trichophyton tonsurans*, **M. n*: *Microsporum nanum*, **M. a*: *Microsporum audouinii*, **E. fl*: *Epidermophyton floccosum*, *ND: Nondermatophytes

Among NDM, *Aspergillus* sp. 40% (8/20) was the most common isolate, followed by *Fusarium* sp. 20% (4/20), *Candida* sp. 15% (3/20), *Scopulariopsis* sp. 15% (3/20) and *Alternaria* sp. 10% (2/20) [Table 3]. The NDM were most commonly isolated from *Tinea pedis* 100% (2/2) and *Tinea corporis* + *Tinea cruris* infection 80% (4/5) cases as well as from *Tinea unguis* 63.6% (7/11) cases, although they were also isolated from the scalp scrapings and other types of skin samples. NDM molds were considered significant only if they were isolated repeatedly (> 2 times) in pure culture and with a positive KOH finding.

Discussion

In the present study, fungal agents were isolated from 37.5% of the patients' samples. This incidence is comparable to two other studies.^[5,6] The incidence is lower as compared with a study done in Chennai.^[5] The lower incidence may be due to geographical and climatic variations, as Chennai receives higher rainfall and is more humid as compared with Rajasthan; thus, higher are the chances of acquiring fungal infections. Another study in north east India^[7] has reported 8.06% positivity, which was quite low because of the inability of patients to reach the hospital from far flung remote areas in north east India.

The majority of patients were from rural areas in and around Jaipur. Majority (75%) of the patients and parents of infected children were involved in farming thus exposing them to environmental agents. The average family size was six members per family, and, in most (75%, i.e., 120/160) of the cases, the whole family resided in a single room. Among the *Tinea capitis* cases (50%, i.e., 30/60), the most common risk factor was the coinfection among siblings (83.3%, i.e., 25/30). The normal level of hygiene was not maintained as the bathing frequency was not more than twice a week (83.3%, i.e., 50/60) in diagnosed cutaneous mycoses cases because of lack of awareness as well as limitation of water supply. The practice of frequent shaving of scalps as well as doing it with common blades and sharing of combs allowed the spread of infection at a higher rate in *Tinea capitis* cases specifically. Majority (83.3%, i.e., 25/30) of children showing infection of *Tinea capitis* went to overcrowded government schools (which merely contained two rooms for a strength of 100 students). Patients (56.2%,

i.e., 90/160) have pointed to the use of some traditional and local therapies for themselves or their domestic animals, such as use of burned car oil, mixture of sulfur and yoghurt and local pistacia (*pistacia-khinjuk*) and oiling of hair repeatedly to deal with the infection initially.

Most of the dermatophytes infections were found in the age group of 5-10 years, as reported by other studies.^[8] Although another study in the same area reported a higher incidence in the age group of 31-40 years,^[9] the difference in age predilection depended on the most common clinical pattern seen in the study group. The age predilection in *Tinea capitis* patients is believed to result from the fungistatic properties of fatty acids of short and medium chains in post-pubertal sebum.^[10] It may also be assumed that a higher incidence of tinea infection of, especially, the capitis variety in school going children and among siblings would be due to increased contact, resulting in increased transmission between them and overcrowding in classrooms, lack of awareness and apathy to personal hygiene, sharing of personal items, exposure to soil and even animals on playgrounds.

In the present study [Table 1], we observed a male to female ratio of 3:1 among the culture positives for dermatophytes, which correlates with other studies.^[5,8,11] This male preponderance may be explained by the fact that males tend to have more outdoor activities than their female counterparts, and are less concerned about personal hygiene and appearance.

In the present study, KOH examination was positive for fungal agents in 62.5% of the cases, and the culture was positive in 37.5% of the cases, while 22% were positive by KOH and culture both. However, recent studies conducted in north India^[5,7] have reported 72.5% KOH positive, 58.3% culture positive and 90% KOH positive and 60% culture-positive cases. As confirmation of dermatomycoses is made on the basis of culture, the positive predictive value and negative predictive value for KOH examination was 10% and 16.6%, respectively. The isolation rate in the present study was low because the patients were clinically suspected cases and not confirmed cases of dermatophytosis, and presence of fast growing NDM moulds may have inhibited the growth of dermatophytes, which would have resulted in culture negativity.

Table 3: Numerical distribution of clinical pattern and strain isolate among non-dermatophytes

Clinical pattern	Total	A n	A t	A f	C a	C g	<i>Fusarium</i>	<i>Scopulariopsis</i>	<i>Alternaria</i>
<i>Tinea capitis</i>	3	1				1			1
<i>Tinea cruris</i>	2		1		1				
<i>Tinea corporis</i>	2			1	1				
<i>Tinea corporis</i> + <i>Tinea cruris</i>	4	2					1	1	
<i>Tinea unguis</i>	7	2	1				2	1	1
<i>Tinea pedis</i>	2						1	1	
<i>Tinea manum</i>									
<i>Tinea faciei</i>									
Total	20	5	2	1	2	1	4	3	2

♣A: *Aspergillus*, A n: *A. niger*, A t: *A. terreus*, A f: *A. fumigatus*, ♣C: *Candida* C a: *C. albicans*, C g: *C. glabrata*, ♣T: *Tinea*

Another possible explanation for the low prevalence of dermatophytosis observed is that the study only looked at those with detectable signs of fungal infection; this has the potential of missing healthy asymptomatic carriers. Persons with an asymptomatic carrier status of dermatophytes infection have been reported in the literature to be both the reservoir in the community and to constitute an almost similar prevalence to symptomatic cases.^[9,12]

Trichophyton violaceum was the most common *Trichophyton* species isolated in the present study, because the most common clinical pattern was Tinea capitis in the present study. This corroborates well with most other Indian studies done in Madras, Varanasi and Kashmir, who have either found 100% isolation of *Trichophyton violaceum* or found it as the predominant isolate,^[3,13,14] although another study conducted in south east Rajasthan has isolated *Trichophyton mentagraphyte*^[15] as the main fungal agent isolated from Tinea capitis cases. This difference in the isolation pattern of fungus from Tinea capitis cases could be explained to some extent on the basis of climatic difference (amount of rainfall received) in different places. Because immigrant people with different traditions and cultures comprise most of the population in this area and due to the proximity of the city and villages in this area, people migrate from villages to this area. This condition could affect the fungal fauna and the distribution of new species. Among other fungal species, *Microsporum audouinii* and *Trichophyton tonsurans* and *Trichophyton rubrum* were the more commonly isolated, which is in consonance with other studies done in western and south east Rajasthan, respectively.^[7,15]

NDM moulds included *Aspergillus*, *Fusarium* and *Candida* species [Table 3]. *Scopulariopsis* was also isolated from the study samples in 5% of the positive cultures. This is comparable with some other studies^[16,17]. A study done in north east India^[4] has reported NDM in 34% of the cases, and only 8.6% of these cases were grown in nail samples; the remaining were isolated from skin scrapings from various sites on the body. It is suggested that this subgroup may have a direct causative role if it fulfills the criteria of a pathogen that is isolated in pure culture and KOH test positive and absence of dermatophytes in the same culture. But, their primary pathogenic activity in cutaneous fungal infections cannot be proven with certainty yet. This pattern was also seen in some studies performed outside India.^[17]

The isolation of NDM was higher and that of dermatophytes was lower as compared with earlier studies^[3-5] in the same area due to careful isolation of these agents by repeated culturing. This might not be unrelated with the local environment and climatic conditions of the area studied, which probably favor the growth of other fungi over and above the dermatophytes.^[18] It is also not known whether there are substances produced by NDM in culture that would inhibit the growth of dermatophytes in coinfecting samples (6.7%, i.e., in 4/60 positive cases,

coinfection of NDM with dermatophytes was seen), or whether fast growing, NDM molds have overgrown dermatophytes that require more stringent conditions for their isolation compared with NDM.^[19] Also, use of traditional remedies whose mechanism of action are unknown, by some of the respondents to treat their lesions, may perhaps have altered the *in vitro* isolation of dermatophytes. Earlier, they were considered as contaminants, but now they are important etiological agents of dermatophytosis. However, their emergence as casual agents of dermatophytosis still needs serious evaluation.

As treatment is normally prescribed in empirical form, without any confirmation, it will be more appropriate to gather as much information about the causative fungal agent and its interaction with antibiotics for effective and rapid cure. The careful isolation of NDM helps to understand the possible reason for resistance in some cases thus dealing with it accordingly. It is evident that, in practice, therapeutic success depends on a set of variables that combine information about the agent, clinical aspects – type, location and duration of lesion - discipline and compliance to treatment, which play a relevant role and should be taken into account. Moreover, laboratory tests may help to distinguish a reinfection by the same agent and infection by a new agent, and provide evidence as to whether the fungus is responsible for treatment failure or whether it is a limitation of the antifungal agent.

Limitations of the study

1. The study population was small; thus, an extensive study involving a larger number of patients should be encouraged.
2. The antifungal susceptibility pattern of the agents would have made the study more clinically useful.

Conclusion

Dermatophytes remain the most common cause of the infections of keratinized tissues, but the evolving role of yeasts and NDM moulds as causal agents of cutaneous mycoses should receive due consideration. There is an urgent need to analyze the role of NDM in these cutaneous infections other than in nail infections.

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