



Original Research Article

Dermatophytosis: Clinico-Mycolological Study on Patients Attending the Department of Dermatology Rims Hospital, Imphal, Manipur

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ABSTRACT

A total of 95 clinically diagnosed cases of dermatophytosis over a period of two years attending the department of Dermatology, RIMS, Hospital, Imphal, Manipur were studied mycologically in the Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur . Out of the total cases, by direct microscopy in KOH wet mount examination showed 39(41.05%) cases were positive, the rest 56 (58.94%) cases were negative. In the culture, 55 (57.89%) cases were culture positive and the rest 40 (42.10%) cases were culture negative. Among the dermatophytes isolated, the commonest was *Trichophyton rubrum*(60.00%) followed by *T. menta grophytes*(32.72%), *T.violaceum* and *Epidermophyton floccosum*(3.63%) each. The commonest clinical type was tinea corporis(36.84%) followed by tinea cruris(24.21%), tinea unguium (15.78%), tinea pedis (6.31%), tinea manuum and tinea faciei (5.26%)each and least of tinea capitis and tinea barbae with 3.15% each. Males were more commonly affected than females. The maximum numbers of cases were in the age group of 21-30 years and the least was in the age group of below 10 years. Maximum numbers of cases were detected during the hot and rainy season.

Keywords

Dermatophytosis,
Dermatophytes,
RIMS Hospital,
Manipur

Introduction

The term dermatophytosis is used to describe infections of the skin, hair and nails due to a group of related filamentous fungi, the dermatophytes which belong to three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*. The dermatophytes are termed geophilic, zoophilic or anthropophilic depending upon whether

their normal habitat is the soil, an animal or man.

Dermatophytosis is among the most common dermatological conditions in industrialized countries. Being superficial, dermatophyte infections have been recognized since antiquity. However,

because many cases are never brought to medical attention, reliable incidence and prevalence figures are difficult to obtain. The US population, at large, has been estimated 10- 20 % life time risk of acquiring a dermatophyte infection. Furthermore, dermatophytosis has been estimated to account for around four million physician visits in the United States each year between 1995 and 2004(Malcolm and Warnock,2012).

Dermatophytosis is a contagious, host to host transmissible disease of humans and animals. Infection with an anthropophilic dermatophyte is acquired by direct or indirect contact with an infected individual. Indirect transfer may occur via the floors of common bathing facilities, or on shoes, clothing, brushes, towels, bedding and other fomites.

In Tropical countries including India, the dermatophytosis are more prevalent, especially because of hot and humid conditions, low socio-economic ,poor hygiene, overcrowding and lack of affordable treatment. The first case of dermatophytosis was recorded in India from Assam (Powell, 1900). In recent decades, the indiscriminate use of broad spectrum antibiotics, corticosteroids and other immunosuppressant have caused an alarming increase of fungal infections besides the expanding number of immunocompromised patients.

The prevalence of specific dermatophyte species differs from one geographic location to another and has varied over time, due to factors such as changing social conditions and standards of hygiene, movement of populations and the introduction of new treatments.

The clinical forms are based on the site of involvement. A single species is able to

cause more than one type of clinical infections. Conversely, a single clinical form such as tinea corporis may be caused by more than one dermatophyte species.

Although the dermatophytosis is not debilitating or life threatening, it can be persistent and troublesome for which large amounts are expended annually in their treatment. So, laboratory investigations are essential before treatment for correct diagnosis, management for optimal therapy and to minimize cost and side effects.

In addition, isolation and identification of the aetiological agents can assist in controlling infections due to household pests or other domesticated animals where there is an ongoing source of inoculum.

The present research was undertaken in the Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur which is a premier referral Hospital of Manipur with an attempt to find out the pattern of dermatophytosis, their aetiological agents, the relationship of the diseases with age and sex and to compare the findings available with other studies.

Material and Methods

The Study was conducted in the Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur over a period of two years starting from 1st August, 2010 to 31st July, 2012. Ninety five clinically suspected cases of fungal infections were studied mycologically in various age groups of both sexes attending the department of Dermatology, RIMS Hospital. Detailed history regarding the age, sex, race, hygienic & socio-economic status, duration, type and site of lesions were recorded in the proforma. The specimens were collected aseptically and transported immediately to the laboratory and processed

at the earliest preferably within an hour. The specimens included are listed below:

Collection and transport of specimens

i) Skin: The collection of sample is best made by collecting epidermal scales from near the advancing edges of the rings. The lesion is lightly disinfected with alcohol in gauze and then scraped from center to edge, crossing the lesion margin, using a sterile scalpel blade or equivalent. If the lesions have vesicles or bullae, the tops of the vesicles or bullae were clipped and included in the sample with a swab when it is impractical to obtain scrapings. Other skin dermatophytoses, such as tinea pedis and tinea manuum, are scraped in such a way that the whole infected area is represented, since an advancing margin is often not evident.

ii) Hair: Hairs are best sample by plucking so that the root is included as the basal root portion of the hair is best suited for direct microscopy and culture.

iii) Nails: After light alcohol disinfection, by scraping the debris from beneath the distal end of the nail with a special and collecting scrapings from near the nail bed, where viable inoculum is most likely to be encountered. Close clipping of the whole end is as alternative methods. Superficial white onychomycosis is sampled by scraping materials from the white spots on the surface of the nail. Discarding the uppermost layer of material is recommended in order to reduce contamination of the inoculum.

Transport of specimens

The collected sample materials are best transported in dry, strong black paper folded in the manner of a herbarium packet. Black paper allows easy visualization of small skin

squamesh. The specimens are processed as soon as possible.

Microscopic examination and culture

Direct microscopy

Specimens of skin, nail & hair are digested & macerated. Specimens were taken on a clean grease free glass slide and mounted in 10-30% potassium hydroxide (Hay *et al*, 1998), according to the thickness of the specimens. Usually higher percentage of KOH preparation was added in case of hairs & nails. Higher the percentage, faster is the clearance. KOH dissolves the keratin and cellular materials but does not affect the fungi. The preparation was kept for 5-10 min (Roberts, 1994). Nails, if were not possible to make thin enough, then it was put in the incubator at 37°C for 1 hour or even more after making the wet mount. Then the preparation was covered by a cover slip and examined by light microscope, firstly under low power objective (X10) & then under high power (X40). While examining for fungal elements, the artifacts viz mosaic artifacts, crystals, fibers, bubble were constantly kept in mind.

In the present study, the isolates were inoculated on the following media:

1. Dermatophyte Test Media (DTM).
2. Sabouraud's Dextrose Agar (SDA) Medium with antibiotics.

Culture

1. **Dermatophyte test media (DTM)** : This selective media (Rebell and Taplin, 1970) is used to differentiate rapidly between dermatophytes and other fungi. Primary isolation of dermatophyte was made on DTM. Commercially available DTM was used

from TITAN Biotech Ltd, Bhiwadi, Rajasthan (TM083).

Procedure : Inoculation was done on the surface of the culture medium with specimens obtained and incubated in the room temperature. Colour change from yellow to red was observed in the plate if dermatophytes were present. Dermatophytes turn the medium red by raising the pH (alkaline) through metabolic activity while most fungi and bacteria do not.

2. Sabouraud dextrose agar with antibiotics: This selective medium was used for the isolation of significant fungal pathogens and to avoid bacterial and fungal contaminants (Chander, 2002). This selective medium is used for species identification as the species identification is not possible by DTM only.

Procedure:

The screened samples on DTM were inoculated on SDA and incubated at 25°C and 37°C and growth was observed daily up to a period of four weeks before discarding as negative (Washington *et al.*, 2006). The dermatophyte species were identified on the basis of cultural characteristics like growth rate, surface texture, and pigment production, microscopic examinations in LCB preparation, slide culture and biochemical tests whenever necessary.

Hair perforation test

This test was done by placing a hair on a filter paper placed on a sterile petri dish. The filter paper was soaked with sterile water previously. A portion of the colony to be studied was directly inoculated on the hair and incubated at room temperature for 10-14 days. From time to time hair was examined under microscope for presents of conical perforation of hair.

Urease test

This test was done to differentiate between *Trichophyton rubrum* (negative) and *Trichophyton mentagrophytes* (positive). The little portion of the colony was inoculated in Christensen's urea agar at room temperature and looked for colour change from straw to red for up to 7 days.

Results and Discussion

Over a period of two years, 95 cases of clinically diagnosed dermatophytosis were subjected to direct microscopy in KOH wet mount, culture in DTM and SDA with antibiotics. Primary isolation was done on DTM, and then finally the screened samples were subjected for culture in SDA which yielded the same results with those of DTM. Repeated cultures were performed to rule out contaminants.

The results are as follows :

Regarding the age and sex distribution as given in Table-1. The maximum number of cases was found in the age group of 21 - 30 years (n= 42; 44.21%). This was followed by the age group of 11 - 20 years (n=21; 22.10%), age group of 31 - 40 years (n=18; 18.94%), age group of 41 - 50 years (n= 8; 8.42%), 51 years and above (n=4; 2.10%). The least number of cases were detected in the age group of 0 - 10 years (n=2; 2.10%) as indicated in Table-1. Males were more commonly affected than females having 72 % and 28% respectively.

Out of 95 cases, on direct microscopy in KOH wet mount examination, 39 cases were KOH +ve (41.05%), rest 56 cases were KOH -ve (58.94%). Out of 95 cases, 55 cases were culture +ve (57.89%) and the rest 40 cases were negative (42.10%) as shown in Table-2.

Among the 55 culture +ve cases (Table-2), 34(61.81%) cases were positive on direct KOH examination while the rest 21(38.18%) cases were negative. And out of 40 (42.10%) culture negative cases, 5(12.50%) cases were positive on direct KOH wet mount examination and 35(87.50%) were KOH negative.

Regarding the different clinical types in the present study, tinea corporis (n=35; 36.84%) was the commonest condition seen followed by tinea cruris (n=23; 24.21%), tinea unguium (n=15; 15.78%), tinea pedis (n=6; 6.31%), five cases each of tinea manuum and tinea faciei (n=5; 5.26%) and three cases each of tinea capitis and tinea barbae (n=3; 3.15%) as shown in Table-3.

Of the different types of organisms isolated, *Trichophyton rubrum* was the predominant pathogen isolated (n=33; 60.00%) followed by *Trichophyton mentagrophyte* (n= 18; 32.72%), two cases each of *Epidermophyton floccosum* and *Trichophyton violaceum* (n=2; 3.63%) as shown in table.4. No case of *Microsporum* species was detected.

The maximum number of patients were detected during the hot and rainy seasons i.e., June to October of every year. During this season the temperature is hot and humid which favours the growth of fungi.

In Manipur, the meteorological data shows that temperature varies throughout the year with maximum temperature in the summer ($32\pm 1^{\circ}\text{C}$) and the minimum temperature in the winter ($4\pm 1^{\circ}\text{C}$), relative humidity of 75% and rainfall of 1467.5 mm per year which favours the growth of fungi. On the other hand, in recent decades, the indiscriminate use of antibiotics, corticosteroids and immunosuppressant drugs have caused an alarming increase in fungal infections, besides the expanding number of immunocompromised patients.

In the present study, two culture medias namely DTM and SDA with antibiotics were used. Primary isolation of dermatophytes was made on DTM as this medium is a good screening medium for the isolation of dermatophytes but not a specific indicator of a dermatophyte. SDA medium was specifically used for species identification.

The commonest age group affected was 21-30 years followed by 11-20 years and 31-40 years of age group. The least number of patients were from the age group below 10 years. Similar results were observed in the studies of Gujarathi *et al.*, 1996.

Males were more commonly affected than females. Similar findings regarding age and sex was reported by Gujarathi *et al.*, 1996; Mohan *et al.*, 1997 and Mohanty *et al.*, 1998.

As far as direct microscopy by KOH examination is concerned, 39 cases were positive (41.05%). Similar results were obtained by Murdia *et al.*, 1987; Pahwa *et al.*, 1992; Mitra *et al.*, 1997; Mohanty *et al.*, 1998 and Mohanty *et al.*, 1999.

In culture, 55 cases were positive (57.89%) in the present study. Similar findings have been reported by Pahwa, 1992 and Mitra *et al.*, 1997.

Regarding the different types of clinical presentation in the present study, the commonest presentation was tinea corporis. This clinical type accounted for 36.84 % of the total cases. Similar results were observed in the studies of Rani *et al.*, 1983; Sundaram *et al.*, 1986; Gujarathi *et al.*, 1996; Mohan *et al.*, 1997 and Singh, 1980.

The second common clinical type in the present study was tinea cruris contributing 24.21%. Similar reports have been reported by Murdia, 1987; Gujarathi *et al.*, 1996;

Tandon, 1996; Mohan *et al.*, 1997 and Mohanty *et al.*, 1998.

The third group of patients was those who suffered from tinea unguium (15.78%). The following study show the incidences of tinea unguium in India as reported by Mohan *et al.*, 1997 (15.02%), Mohanty *et al.*, 1998 (18%), Gokhale *et al.*, 1999 (20.63%) and Singh *et al.*, 2001(14.8%). All these showed the incidence of tinea unguium in the second or third position. In the international scenario, the incidence of tinea unguium is 20.1% in Japan by Kasai, 2001 and 34.95% in Parague by Kuklova, 2001. In this type the commonest causative organism is *Trichophyton rubrum* which has concomitant finding with that of Puri *et al.*, 1998.

Following tinea unguium, there was incidence of tinea pedis (6.31%). Incidences of tinea pedis in India has been reported by many workers like 7.72% by Mohan *et al.*, 1997, 18% by Mohanty *et al.*, 1998 and 11.6% by Singh *et al.*, 2001. On the other hand in the International scenario the incidence of tinea pedis is 15.6% in Perague by kuklova *et al.*, 2001 and 20% in Nepal by Agar Walla *et al.*, 2001.

The present study shows tinea faciei and tinea manum 5.26% each which tallies more or less to that of the studies conducted by Mohanty *et al.*, 1998, 3.8%, Mohan *et al.*, 1997 which showed incidence of tinea manum (5.66%). Three cases, 3.15% of tinea barbae and tinea capitis were found. Singh *et al.*, 2001 showed the incidence of tinea barbae (4%) while Murdia *et al.*, 1987 (3.75%) and Gujarathi *et al.*, 1996 (2.64). Studies of Mohan *et al.*, 1997 showed incidence of tinea capitis to be 1.25%.

Amongst the dermatophytes isolated, the commonest organism causing dermatophytoses in all is *Trichophyton rubrum* (60%) which is the responsible pathogen for tinea corporis, tinea cruris, tinea unguium, tinea capitis, tinea pedis, tinea manum, tinea faciei and tinea barbae. Similar studies were seen in the studies of Talwar *et al.*, 1980, Singh *et al.*, 1980, Gupta, 1993, Rani *et al.*, 1983, Huda *et al.*, 1996. In the international scenario, similar findings were reported from Japan by Kasai, 2001, Parague by Kuklova and Kuklova, 2001, Slovakia by Buchvald, 2002 and Singapore by Hiok-Hee Tan, 2005.

Table.1 Showing age and sex distribution

Age (years)	No. of cases	Male	Female
0 - 10	2	1	1
11-20	21	14	7
21-30	42	32	10
31-40	18	13	5
41-50	8	5	3
51 & above	4	3	1
Total	95	68	27

Table.2 Showing culture +ve, KOH +ve and KOH -ve cases

Culture status	Total	KOH +ve	KOH -ve
Culture +ve	55(57.89%)	34(61.81%)	21(38.18%)
Culture -ve	40(42.10%)	5(12.50%)	35(87.50%)
Total	95	39	56

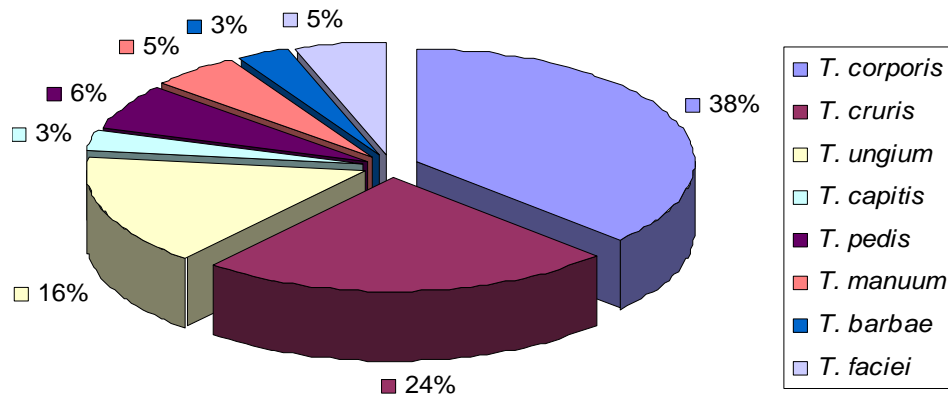
Table.3 Different clinical types and their KOH & culture status

Sl.no.	Clinical type	Total no. of cases	Percentage	KOH +ve culture +ve	KOH -ve culture +ve	Total culture+ve
1	<i>T. corporis</i>	35	36.84	17	10	27
2	<i>T. cruris</i>	23	24.21	6	4	10
3	<i>T. unguium</i>	15	15.78	3	5	8
4	<i>T. capitis</i>	3	3.15	1	-	1
5	<i>T. pedis</i>	6	6.31	2	1	3
6	<i>T. manuum</i>	5	5.26	1	1	2
7	<i>T. barbae</i>	3	3.15	2	-	2
8	<i>T. faciei</i>	5	5.26	2	-	2
	Total	95		34	21	55

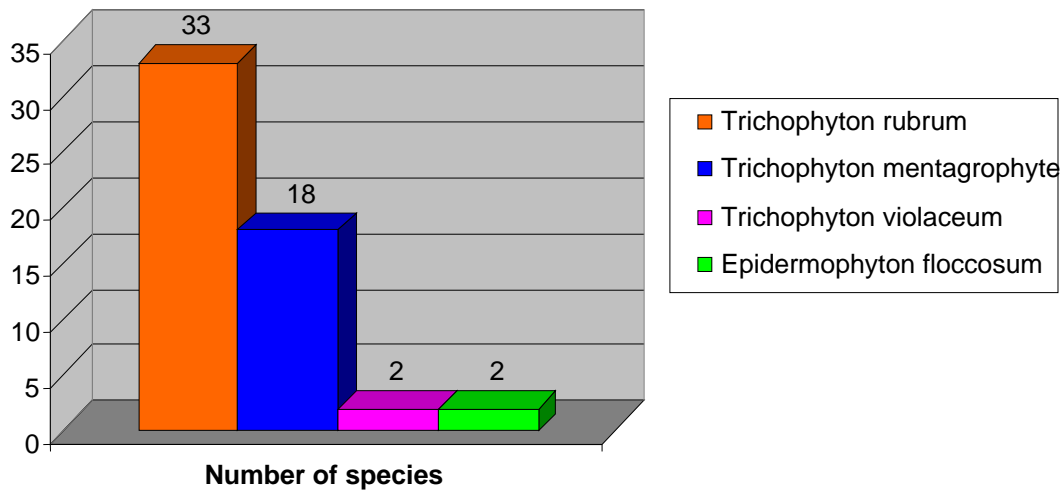
Table.4 Showing different species of fungal isolates

Sl.no	Name of species	Number of species	Percentage
1	<i>Trichophyton rubrum</i>	33	60.00
2	<i>Trichophyton mentagrophyte</i>	18	32.72
3	<i>Trichophyton violaceum</i>	2	3.63
4	<i>Epidermophyton floccosum</i>	2	3.63
	Total	55	

Pie diagram showing different types of clinical presentation



Bar graph showing different fungal isolates



The next common pathogen observed was *Trichophyton mentagrophytes* (32.72%) which is the responsible pathogen in cases of tinea corporis, tinea cruris and tinea unguium. Similar reports were made by Kumari, 1985; Murdia, 1987; Gujarathi *et al.*, 1996; Tendon, 1996; Mohan *et al.*, 1997 and Mohanty *et al.*, 1998.

Two cases of *Trichophyton violaceum* (3.63%) was isolated from a tinea corporis patient. Such similar low incidence was found in studies of Gupta, 1993.

Apart from these organisms, *Epidermophyton floccosum* was isolated from two patients (3.63%). This pathogen is responsible for tinea corporis and tinea cruris. Similar findings have been reported by Gujarathi *et al.*, 1996; Tendon, 1996; Mohan *et al.*, 1997 and Mohanty *et al.*, 1998.

No case of *Microsporum* species was detected. Mohanty *et al.*, (1998); Pahwa *et al.*, (1992); Murdia *et al.*, (1987) and Mitra *et al.*, (1997) also could not find *Microsporum* species in their studies.

Maximum number of dermatophytosis was seen to occur during the hot and rainy season where the temperature is hot and humid that encourages perspiration which favours the growth of fungi. Similar findings were observed in the studies of Mitra *et al.*, (1997). Most of the patients were not complicated.

In conclusion, the above findings reiterate that the majority of patients were males. The maximum number of cases was in the age group of 21-30 years. Among the dermatophytes isolated, *Trichophyton rubrum* remains the most prevalent pathogen isolated. The most common clinical presentation was tinea corporis followed by tinea cruris and the least one was tinea capitis and tinea barbae among the dermatophytes. No cases of *microsporum* species were detected. Maximum number of cases were not complicated.

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