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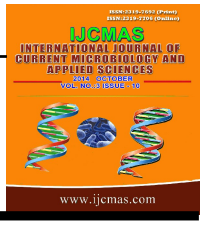


Original Research Article

Clinico-mycological profile of dermatophytosis in patients attending dermatology OPD in tertiary care hospital, India

Hemangi Ramesh Walke*, Anil A Gaikwad and Shrikant S Palekar

Department of Microbiology, S R T R G M C Ambajogai Dist Beed 416517, India
**Corresponding author*

A B S T R A C T

Clinically diagnosed 311 cases of dermatophytosis were subjected to mycological examination in our institute to know the pattern of Dermatophytes by direct microscopy using 10%-40% KOH depending on the type of sample processed and culture on Sabouraud Dextrose Agar with chloramphenicol and cycloheximide (SDCCA) and also in Dermatophytes Test Medium (DTM). Causative agents were identified macroscopically and microscopically from the growth obtained on DTM and SDCCA. Direct microscopy revealed fungal element in 239(76.84%) cases whereas 165(53.05%) were positive on culture.Commonest age group affected were between 21-30 years (33.40%). Incidence amongst male were 193 (62.10%) higher than females 118(37.90%) and male to female ratio being 1.64:1. Majority of cases belonged to low socioeconomic status 118 (87.78%), *Trichophyton mentagrophytes* in 37 cases (19.39%),Among different *Microsporum* species identified, *Microsporum gypseum* (9.70%) was the commonest isolate followed by *Microsporum canis* (2.42%) and Few rare species like *Microsporum ferrugenium* were also identified in this study. Among Genus *Epidermophyton*, *Epidermophyton floccosum* (11.52%) were isolated. DTM was found useful as a general screening medium for dermatophytes. SDCCA was found to be equally effective in isolating dermatophytes from clinical samples in our study.

Keywords

Dermatophytosis, *Tinea unguium, Trichophyton, Microsporum, Epidermophyton, Tinea capitis.*

Introduction

Although the fungi are worldwide today over 200,000 fungal species have been described, approximately 100 of which are able to cause human mycoses and out of them 41 are Dermatophytes (Liz, 2003). According to world Health Organization, the dermatophytes are defind as a group of molds that from three genera: *Epidermophyton, Trichophyton* and *Microsporum.* They comprise about 40 different species, and have common characteristics.

1. Close taxonomic relationships.
2. Keratinolytic properties.
3. Occurrence as etiologic agents of infectious disease of man and animals.

In many they invade hairs, nails and skin they found in the stratum corneum the keratinized outer layer and within the hair follical, in the nail folds and subungually in the nail bed. All these are extention of the

stratum corneum (Yousef, 1980). dermatophytosis commonly known as ringworm or tinea (DeiCas and Vernes , 1986). Though worldwide in distribution, it is more prevalent in Tropical and Subtropical region. Dermatophytes are more prevalent in India due to favourable climatic conditions liketemperature and humidity (Patwardhan and Dave, 1999). The etiological agents of dermatophytosis are classified in three anamorphic (asexual or imperfect) genera Trichophyton, Microsporum and Epidermophyton, of anamorphic class Hyphomycetes of the Deuteromycota (Fungi Imperfecti) (Weitzman and Summerbell, 1995). Dermatophytes are referred to anthrophilic,Zoophilic and geophilic depending on whether their usual reservoir in nature appears to be humans, animals or soil respectively (Vijayakumar *et al.*, 1990). the Dermatophytes are among the commonest infectious agent of man and no persons or geographic areas are free of them (Rippon, 1992). Dermatophytes are among the most prevalent infection. Millons of dollars are expended annually in their treatment (Emmons *et al.*, 1974). in the world today, Dermatophytosis is common in tropical

countries like India where heat and moisture play important role in promoting the growth of Dermatophytes (Betty *et al.*, 2002). Recently there has been increase in the incidences of fungal infection. This may be result of frequent usages of higher antibiotics, various condition like organ transplant, lymphomas, leukaemia's and HIV infectivity. In spite of all this because of benign nature of fungi these are not given much attention and usually diagnosis is established very late.

The present study was undertaken with the objectives like find out clinical pattern of dermatophytosis, isolation of various species of dermatophytes, determine the prevalence, co-relate the clinical pattern of dermatophytoses with the mycological study and the contributing factor in this part of country.

Materials and Methods

This is a prospective study over a period of two and a half years from July 2009 to December 2011 Ethical clearances from institutional Ethical Committee was obtained. study conducted at S. R. T. R. Govt Medical College & Hospital, Ambajogai Beed, Maharashatra.The study population comprised of 311clinically diagnosed cases of dermatophytoses attending Dermatology outpatients department at S. R. T. R. Govt Medical College & Hospital, Ambajogai Beed. during a period of two and a half years. Our hospital which is a tertiary care hospital. Detailed history of onset of disease, duration of symptoms, trauma, occupation, drugs, associated co morbid conditions, family and personal history was taken. Enquiries were also made as to exposure to animals, cases or any other suspected sources.

Collection and processing of the sample

Samples were collected from affected lesions. Whenever the patients presented with lesions at clinically different sites samples were collected from all those sites and each of these were processed and examined individually.

Collection of samples from skin:

The affected area was swabbed with 70% ethyl alcohol and the active edge of lesion scrape sterilized blunt scalpel. The scrapings were collected from active margin of lesion without injuring the skin surfaces and

collected in black paper packet to prevent contamination.

Skin Sripping: It was taken by applying a waterproof vinyl adhesive tape to affected skin lesion and then this was carried to laboratory by adhering to glass slide in black paper packets.

From the nails

The affected nails were swabbed with 70% ethyl alcohol after which the nails were scraped deeply enough to obtain recently affected nail tissue. Nail clippings were also collected in addition to nail scrapings from the lesions whenever it is feasible.

From the scalp (hair)

The same procedure as mentioned for skin scrapings was followed, in addition few affected hairs were also epilated and collected with the help of scissor, collect the basal portion of hair (hair stub) as the fungus was usually found in this area. The nail clippings and hair samples were cut into small fragments of 1mm in size. Out of the material collected, part of it was used for direct KOH examination and remaining part was used to inoculate onto Sabouraud dextrose agar plain and with chloramphenicol& cycloheximide and Dermatophyte test medium (DTM) with supplement to isolate the causative dermatophytes. These three culture media used in our mycology laboratory were obtained as dehydrated media (manufacturer HiMedia Laboratories, Mumbai) and prepared in-house following stringent quality control measures. DTM is a selective medium recommended for the isolation and cultivation of pathogenic dermatophytic fungi. It is a modification of a commercial formulation made by Taplin et al in 1969 (Madhavi *et al.,* 2011; Taplin *et al.,* 1969)

KOH examination

Skin and hair specimens were subjected to 10% KOH solution. The preparation was kept at room temperature for 30 mins. Nail clippings and scrapings were kept overnight dipped in 40% KOH solution. Subsequently examination was done under high power objective (40x) of the microscope for branching and septate hyphae.

Culture

Skin, hair and nail samples were inoculated after reducing the size of the samples to approximately to 1-2 mm as it was mentioned earlier. Inoculations were done at four sites at well spaced interval onto Sabouraud s dextrose agar slants with chloramphenicol (0 05mg/ml) and cyclohexamide (0 5mg/ml) (Madhavi *et al.,* 2011; Emmons *et al.,* 1977). Chloramphenicol was added to inhibit the growth of bacteria and cyclohexamide was used to inhibit the growth of saprophytic fungi. Inoculations of specimens were also done on DTM slopes for isolating dermatophytes where mixed pathogens were suspected. The tubes were incubated in BOD incubator at 250c and also at room temperature to achieve good growth of some dermatophytes which prefer a little higher temperature. The tubes were examined at regular intervals for evidence of fungal growth and the progress of growth was also noted. Culture tubes not showing any growth were discarded after four weeks of incubation. Any visible growth on SDCCA was examined for colony morphology, texture, ureas production pigmentation on surface (obverse), pigmentation on the reverse. Microscopic examination of colony was done by doing a lactophenol cotton blue mount to examine the hyphal structure, different vegetative structures formed by hyphae, microconidia, macroconidia and chlamydoconidia.

Results and Discussion

The present study consists of 311 cases which were clinically diagnosed as cases of tinea infection. All age groups and both sex were included in the present study. Highest incidence of dermatophytosis was observed in the age group of 21-30 years (33.45%) and 31-40 years (28.30%) in the present study. The incidence was more in males (62.10%) than in females (37.90%) and male to female ratio was 1.64:1. it was observed that the higher incidence of dermatophytes infection was in the age group 21-30 years followed by 31-40 years which were more common in males as in this age group outdoor activity and exposure is more in field. The higher incidence in young males could be due to greater physical activity and increased sweating. Tinea corporis (42.12%) was the commonest clinical type encountered mainly in the age group of 21-30 years. Tinea cruris (25.08%) was the second clinical type isolated mainly in the age group of 31-40 years. Tinea unguium (12.86%) was most common in females, in the age group of 21-30 years Out of 311 cases, Tinea pedis was seen in 10.93% cases mainly in the age group of 21-30 years, In our study population most were farmers and field workers, as they were engaged mostly with farming which was increased during rainy season, therefore highest farming activity were done by this study population that to also mainly by bare foot, so this could have probably resulted in increased incidence of Tinea pedis. Low occurrence of Tinea capitis below 10 years of age in our study population may be due to awareness regarding hygiene of scalp in childhood by parents and by teacher through various school healths' check-up.

The low occurrence in female child could be due to regular application of vegetable oil over the scalp which has inhibitory and fungi static properties. Though low incidence of Tinea capitis in adults was observed by various authors we could not isolate a single case of Tinea capitis from adults. Infection was most common in low socioeconomic group (87.78%) followed by middle socio-economic income group (10.62%) and least in high socio-economic income group (1.61%), In the present study, dermatophytosis was most commonly seen in manual workers (39.87%) including farmers, labours, butchers, and carpenters, followed by students (32.80%), housewives (14.15%) and professionals (13.18%) These were likely to be due to increased physical activity and increased opportunity of exposure in manual workers during farming, increased wet work in housewives and the probable factors put forward for this association includes increased sweating in outdoor activities, constant contact with plants and soil. We also observed maximum cases of dermatophytosis in June-Sept (41.16%)

In the present study, out of 311 clinically diagnosed cases of dermatophytosis (76.85%), were positive for fungi, either by KOH and/or culture (40.19%), were positive by both KOH and culture (36.36%), were positive by KOH and negative by culture, however (12.86%) were negative by KOH but turned to be culture positive (10.30%), were negative by both KOH and culture, which was comparable with all above studies done by various authors. The variation in culture and KOH could be due to non-viability of fungal elements in some cases. Topical antifungal treatment was also important factor for the above variations.

Out of 165 isolated dermatophytes the most common dermatophyte isolate was *T. rubrum* (56.37%) followed by *T. mentagrophytes* (19.39%). *E. floccosum* was isolated from (11.52%) cases and amongst

*Microsporum spp, M. gypseum (*9.70%) and *M. canis (*2.42%).

Few species of dermatophytes like *Microsporum ferrugineum, T. soudanense* are endemic in certain parts of the world. *Microsporum ferrugineum* an anthrophilic dermatophytes is endemic in Africa and Oriental Asia, sporadic cases have been reported from other countries. In the present study out of 311 sample processed, Sabouraud Dextrose Agar showed growth of dermatophytes in (53.05%) while Dermatophyte Test Medium isolated (54.34%) dermatophytes including one case of non dermatophytic mould. From our study we observed that DTM was more useful as a general screening medium as opposed to an identification medium and the isolation of dermatophytes was rapid compared to SDA.*T. rubrum* and *T. mentagrophytes* were identified by their colony morphology, microscopical findings, urease test, and pigmentation on potato dextrose agar and hair perforation test. Urease was positive within 3 days in (93.75%) of *T. mentagrophytes* while of *T. rubrum* (2.15%) within 7 days. *T. rubrum* (95.70%) produce wine red pigment on Potato Dextrose Agar where as only (6.25%) of *T. mentagrophytes* produce less intense pigments. *T. mentagrophytes* (90.63%) showed conical perforation in hair perforation test within 15 days .whereas not a single strain of *T. rubrum* did so within 30 days. In the present study trauma (36.01%) was found as a predisposing factor for development of dermatophytosis. comparable finding were reported from foIn present study HIV infection (13.83%) was found to be a contributory and predisposing factor for development of dermatophytosis, comparable finding In the present study (12.86%) patients showed positive history of diabetes during clinical examination.

The dermatophytes are among the commonest infectious agents of man and no person or geographic areas are free of them. Incidence of dermatophytosis varies according to geographical area and climatic condition of the place.

The climatic condition of our area is favourable for dermatophytes as it is 600 meters above the sea level and characterized by a mean temperature of 35-42^{0}C in summer, 25-32^{0}C in winter, rainfall about 650-700 mm per year and that too only in the period of June to September, the weather is otherwise dry.

With above knowledge the present study was undertaken to know the clinical pattern of dermatophytosis and its correlation with mycological investigation along with various predisposing factors.

Table.1 Age wise distribution of dermatophytoses in the study group

Completed age group in years	Number of cases	Percentage
1-10 years	12	3.86%
10-20 years	63	20.26%
21-30 years	104	33.45%
31-40 years	88	28.30%
41-50 years	35	11.25%
51-60 years	9	2.89%
>60 years	-	-
Total	311	100%

Table.2 Distribution of clinical types in relation to study subjects

CLINICAL TYPE	NO. OF CASES	PERCENTAGE
T. corporis	131	42.12%
T. cruris	78	25.08%
T. unguium	40	12.86%
T. capitis	07	2.25%
T. pedis	34	10.93%
T. faciei	04	1.29%
T. corporis & T. cruris	17	5.45%

Table.3 Age wise distribution of different clinical types of dermatophytes

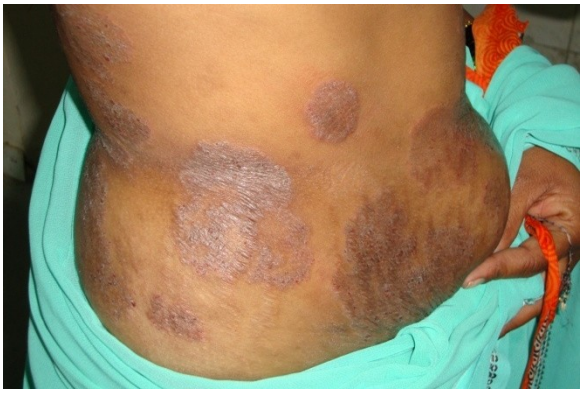
CLINICAL TYPE	COMPLETED AGE GROUP IN YEARS						
	1-10	11-20	21-30	31-40	41-50	51-60	>61
T. corporis	05	23	49	33	17	04	-
T. cruris	01	12	20	34	08	03	-
T. unguium	-	13	20	04	03	-	-
T. capitis	O5	02	-	-	-	-	-
T. pedis	01	9	12	07	03	02	-
T. faciei	00	01	01	02	00	-	-
T. corporis & T. cruris	-	03	02	08	04	-	-
TOTAL	12	63	104	88	35	09	-

Table.4 KOH preparation findings

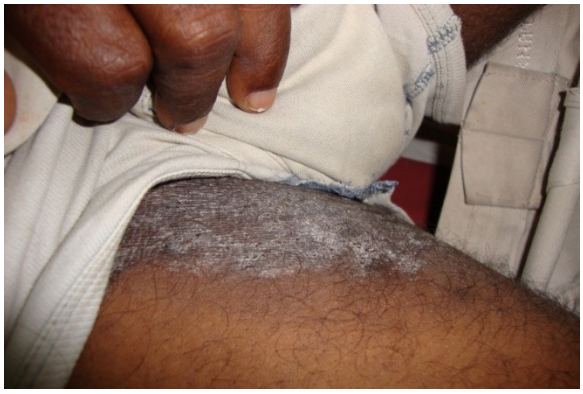
RESULT OF KOH MOUNT	NO. OF CASES	PERCENTAGE
Positive	239	76.84%
Negative	72	23.16%
Total	311	100%

Table.5: Sex wise distribution in the study group

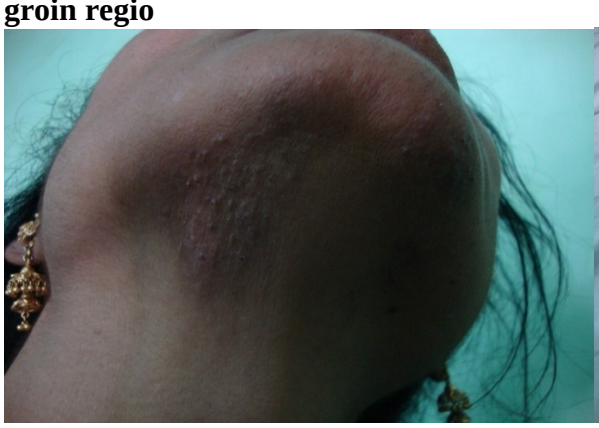
SEX	NO. OF CASES	PERCENTAGE
Male	193	62.10%
Female	118	37.90%
Total	311	100%



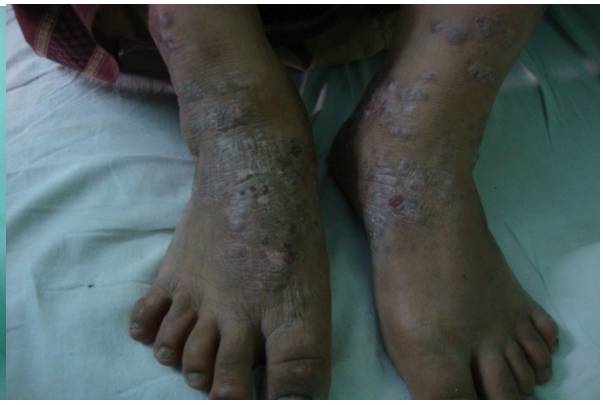
a) Tinea corporis showing circular lesions lesions over the groin regio



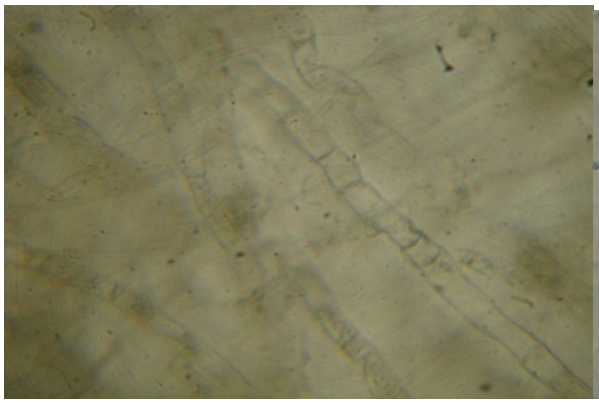
b) Tinea cruris showing on the waist



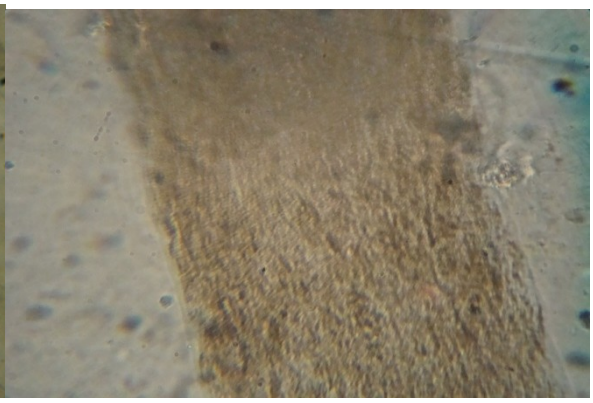
c) Tinea faciei showing circular lesion



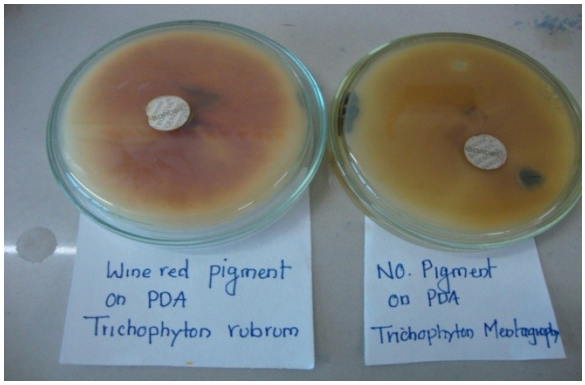
d) Tinea pedis showing lesions on the dorsum of foot



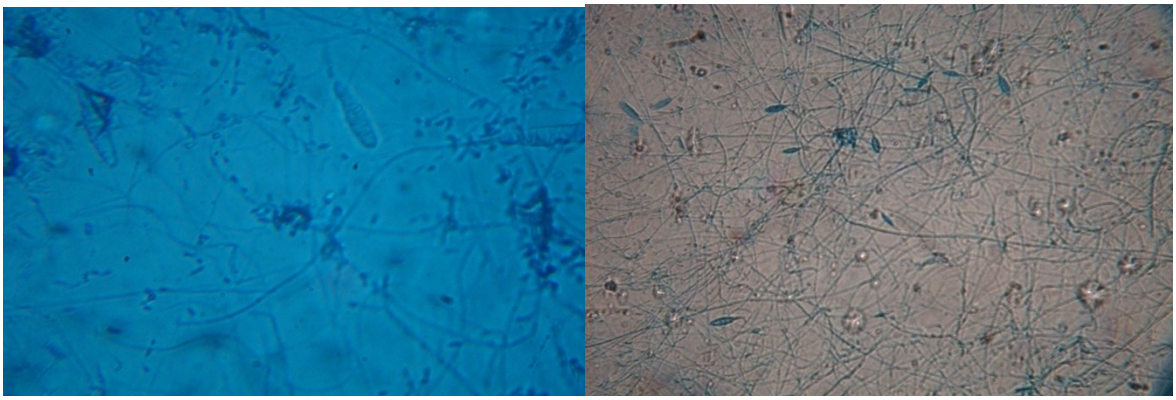
e) Bamboo hyphae of M. ferrugineum on specimen (endothrix infection)



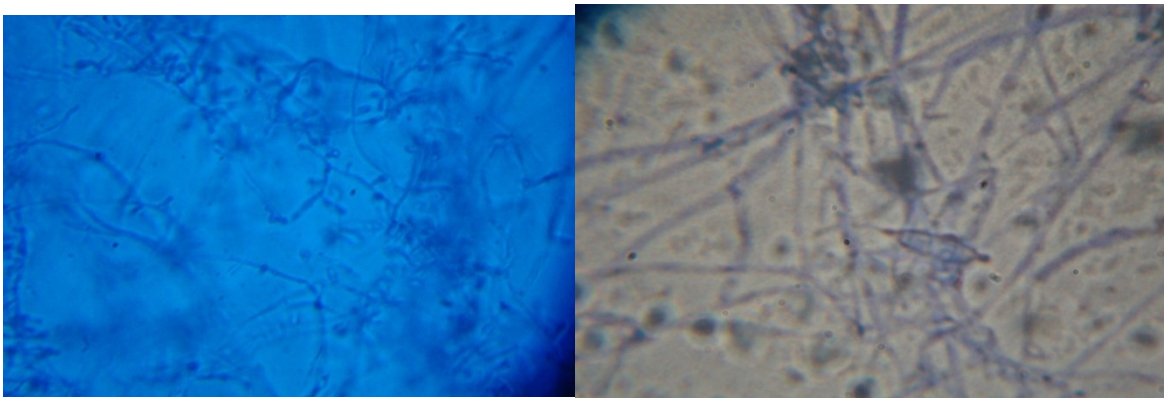
f) KOH preparation of hair slide culture



g) Trichophyton rubrum on (SDA and DTM) **h)Pigmentation PDA**



i) LPCB Trichophyton rubrum (LPCB mount) **j) Microsporum canis (LPCB mount)**



k) Trichophyton mentagrophyte (LPCB mount) **l) Microsporum gypseum (LPCB mount)**

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