



Mycological study of dermatophytosis in a part of South India

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Received 20 June 2016; Received in revised form 18 July 2016; Accepted 25 July 2016

ABSTRACT

Aims: *Epidermophyton*, *Microsporum* and *Trichophyton* are the genera of dermatophytes causing superficial mycoses. These infections are on rise due to increase in immunocompromised patients and favorable environmental conditions in countries like India. The present study was undertaken to identify dermatophytes causing superficial fungal infection by microscopy and culture techniques which helps in accurate diagnosis and appropriate treatment of cases.

Methodology and results: Samples were collected from affected sites after cleaning the affected surface with 70% alcohol. All samples were microscopically examined for presence of hyphal structures by digesting in 10% to 40% KOH solution. All samples were inoculated into Sabouraud dextrose agar with chloramphenicol and Sabouraud dextrose agar with cycloheximide and chloramphenicol and incubated at room temperature for four weeks. Tease mount technique and slide culture technique were used for identification of dermatophytes. One hundred and ten samples from clinically suspected dermatophytoses which includes 77(70%) from male and 33(30%) from female patients were processed for identification of dermatophytes. Samples were subjected to microscopy and culture. In 61 samples (54.54%) fungal hyphae were seen by direct microscopic examination (KOH). Fifty six samples (50%) yielded dermatophyte growth in culture. *Trichophyton rubrum* was the predominant species isolated followed by *T. violaceum* and *T. mentagrophytes*.

Conclusion, significance and impact of study: Accurate and rapid diagnosis of superficial fungal infection is essential for proper management of cases. Direct microscopy is very good method for routine diagnosis, however culture remains gold standard.

Keywords: Dermatophytes, direct microscopy, culture, Sabouraud dextrose agar

INTRODUCTION

Dermatophyte infections are earliest known fungal infections affecting mankind and are very common throughout the world (Richa *et al.*, 2012). Dermatophytes are aerobic fungi that infect the stratum corneum of the skin, the hair shaft, and the nail by producing enzyme proteases that digest keratin and allows colonization and invasion (Surendran *et al.*, 2014). Traditionally infections caused by dermatophytes (ringworm) have been named according to the anatomical locations involved, by appending the Latin term designating the body site after the word Tinea, for example, infection of scalp is called tinea capitis (Hayand and Moore, 2004). *Epidermophyton*, *Microsporum* and *Trichophyton* are the genera of dermatophytes causing superficial mycoses. Dermatophytes are also classified according to their habitat, being either anthropophilic associated with humans, zoophilic associated with animals or geophilic associated with soil. Anthropophilic species are responsible for the majority of human infections and tend to be chronic with little inflammation. Infection caused by

zoophiles and geophiles are associated with acute inflammation. Dermatophyte colonization is characteristically limited to the dead keratinized tissue of the stratum corneum and results in either a mild or intense inflammatory reaction. Although the cornified layers of the skin lack a specific immune system to recognize this infection and rid itself of it, nevertheless, both humoral and cell-mediated reactions and specific and nonspecific host defense mechanisms respond and eventually eliminate the fungus, preventing invasion into the deeper viable tissue. These organisms are assuming greater significance due to the excessive use of immunosuppressive drugs for controlling serious infectious as well as non-infectious conditions (Bhatia and Sharma, 2014).

The tinea infections are prevalent globally but they are common in tropics and may reach epidemic proportions in geographical areas with higher humidity, over-population and poor hygienic living conditions. In India due to hot and humid condition, dermatophytosis is very common superficial fungal infection (Niranjan *et al.*, 2012). The distribution, frequency and the causative agents involved

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vary from place to place depending upon the climatic, socioeconomic conditions and the population density (Das *et al.*, 2009). Measures for preventing dermatophytosis include practicing good personal hygiene, keeping the skin dry and cool at all times and avoiding sharing towels, clothing, or hair accessories with infected individuals. The treatment of dermatophytoses would be most appropriate when the selection of antimicrobial agent is based on the identity of the causative agent. For example, griseofulvin is effective only for dermatophytic infections, with no activity against *Candida* spp. And non-dermatophytic molds. Terbinafine shows fungicidal activity against dermatophytes with a cure rate of 80 to 95% but shows only fungistatic activity against *Candida albicans*. For nondermatophytic molds infections, the role of terbinafine is not well defined and topical amorolfine lacquer may be effective for select patients (Denning *et al.*, 1995). The present study was undertaken to identify dermatophytes causing superficial fungal infection by direct microscopy and culture techniques, which helps in appropriate treatment of cases and also throws light different species of dermatophytes prevalent in this part of India.

MATERIALS AND METHODS

Clinically suspected 110 cases of dermatophytoses attending the Out Patient Department of a tertiary care hospital were included in present study.

Collection of samples

Samples were collected after cleaning the affected surface with 70% ethanol in order to remove the dirt and environmental contaminants. From skin lesions, scales were collected from margins of the lesion with a sterile blunt scalpel, hair samples were plucked with sterile surgical forceps and infected nails using nail clippers. Samples were collected in paper envelope, labelled and transported to the microbiology laboratory.

For direct microscopy the sample collected was screened for the presence of filamentous, septate, branched hyphae by using potassium hydroxide mount (KOH), which acts as keratinolytic agent and makes fungal hyphae visible. A 10% KOH was used for hair samples, 20% KOH for skin scrapings and 40% KOH for nail samples. The sample (hair, skin and nail clipping) was placed on a clean glass slide and a drop of KOH (10%-40%) solution was added and slide passed through a burner flame to hasten keratolysis. When keratolysis softened the sample, a clean glass cover slip was kept on the sample and pressed to prevent the formation of air bubbles. The sample was kept in KOH for a variable duration ranging from 5 min to 2 h, depending upon the thickness of the sample.

For culture one set of Sabouraud dextrose agar with chloramphenicol and another with Sabouraud dextrose agar with cycloheximide and chloramphenicol were inoculated with clinical material. The inoculated agar slopes were incubated in room temperature and observed

daily for growth. If no growth was noticed by four weeks of incubation, culture was considered negative and discarded.

Dermatophytes grown from clinical samples were identified by observing rate of growth, colony characteristics and microscopic morphologies by doing tease mount technique using lactophenol cotton blue stain and slide culture technique.

RESULTS

In the present study out of 110 clinically suspected dermatophytoses, 77(70%) samples were from male patients and 33(30%) were from female patients. Maximum numbers of samples (27.27%) were from patients in the age group of 21-30 years. Age and sex wise distribution is shown in Table 1 and Figure 1.

78(70.9%) skin, 17(15.45%) hair, 15(13.63%) nail samples were processed by direct microscopy using KOH mount and culture by inoculating SDA media. Results of KOH mount and culture are shown in Table 2. Approximately half the number of samples yielded the growth of dermatophytes, whereas the remaining samples did not yield growth of dermatophytes but some samples yielded the growth of non-dermatophytes like *Aspergillus*, *Fusarium* and yeast like *Candida* especially from nail samples.

Trichophyton rubrum (31) was predominant dermatophyte isolated followed by *T. violaceum* and *T. mentagrophytes*. Different types of dermatophytes isolated from various clinical types are shown in Table 3.

Table 1: Age and sex wise distribution of cases.

Age in years	Male	Female	Total
0-10	5	2	7
11-20	13	5	18
21-30	24	6	30
31-40	15	6	21
41-50	11	8	19
>50	09	6	15
Total	77	33	110

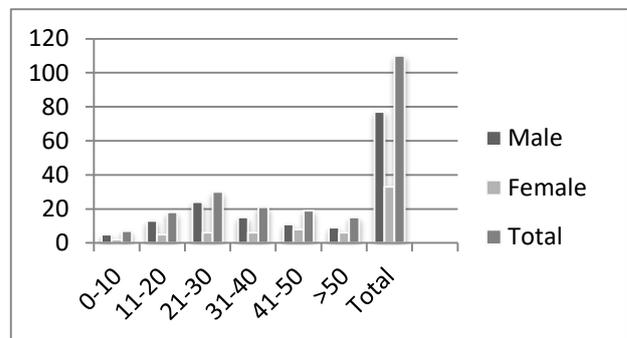


Figure 1: Bar chart showing age and sex wise distribution of cases.

Table 2: KOH and culture results of the samples.

Test method	KOH positive	KOH negative	Total
Culture positive	47	09	56(50%)
Culture negative	14	40	54(49.09%)
Total	61(54.54%)	49(45.45%)	110(100%)

Chi square, $\chi^2 = 35.13$; $P < 0.001$.

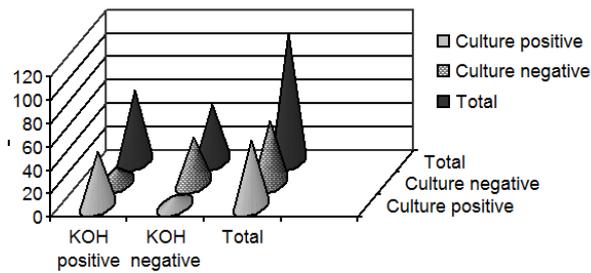


Figure 2: KOH and culture results of samples.

Table 3: Different types of dermatophytes isolated from samples.

Dermatophyte	Tinea corporis	Tinea Cruris	Tinea capitis	Tinea pedis	Tinea unguium	Total
<i>T. rubrum</i>	20	06	03	01	01	31(55.35%)
<i>T. violaceum</i>	04	-	06	-	-	10(17.85%)
<i>T. mentagrophytes</i>	04	02	-	02	01	09(16.07%)
<i>T. tansurans</i>	-	-	02	-	-	02(3.57%)
<i>Microsporum gypsiium</i>	02	-	-	-	-	02(3.57%)
<i>Epidermophyton floccosum</i>	-	02	-	-	-	02(3.57%)
Total	30	10	11	3	2	

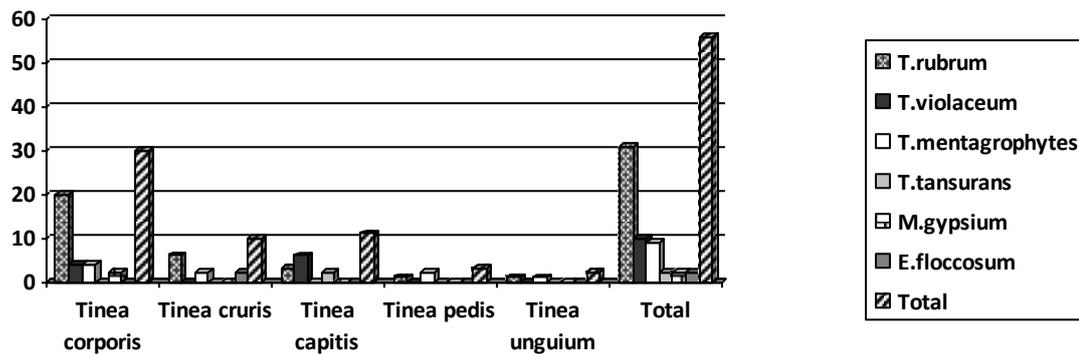


Figure 3: Types of dermatophytes isolated.

done by Sen and Rasul (2006) and Sumana and Singaracharya (2004), the highest prevalence of dermatophytosis in the same age group were also

DISCUSSION

Earth has been documented as a natural territory for fungi which cover individual kingdom with evolution (Sharma et al., 2015). One fifth of the world's population suffers from mycoses (Bhadauria et al., 2001). The Mycoses caused by fungal infections of the skin, hair and nails are widespread and the most numerous group amongst all Mycoses. Primary infection starts through small skin break. These breaks are coming out through secretion of enzymes that digest keratin. The excreted enzyme plays a vital role in the process of infection and considered as primary virulent factors (Sharma et al., 2012). Apart from the clinical symptoms superficial fungal infections can cause debilitating effects on a person's quality of life. In India studies on dermatophytosis has received increased attention of physicians and microbiologists in recent years due to the excessive use of immunosuppressive drugs for controlling serious infectious as well as non-infectious conditions and presence of favorable environmental conditions.

High incidence of dermatophytosis in present study was seen in the age group of 21-30 years. In studies

reported. Increased incidence of dermatophytosis in younger age group is probably due to high physical activity which in turn increases the risk of exposure to

fungal spores. About 70% of the samples in this study were from male patients. Similar observations were noted by other authors in their studies (Sumana and Singaracharya, 2004). Male predominance could be due to their increased outdoor activity when compared to females.

In the present study, 61 samples (54.54%) were positive for fungal hyphae by direct microscopic examination (KOH), whereas 56 samples (50%) yielded growth of dermatophytes by culture. Forty samples (36.36%) were both culture and KOH negative. The present study was supported by other studies. Singh and Beena (2003) reported microscopy positive in 60.38% of samples and culture in 44.6% of samples. About 53.38% samples did not show evidence of fungus either on direct microscopy or on culture. In a study done by Lakshmi *et al.* (2015) reported KOH positivity in 58.18% of samples and culture positivity in 56.36% of samples. In the present study culture positivity was more in KOH positive samples (83.92%) compared to KOH negative samples (16.07%). This difference is statistically significant $\chi^2 = 35.13$; $P < 0.001$. Similar findings were observed by Lakshmi *et al.* (2015). In their study, 85.9% samples were culture positive in KOH positive samples and 15.2% were culture positive in KOH negative samples. This shows that direct microscopy by KOH mount is a good screening test in the laboratory diagnosis of dermatophytosis.

Various tinea conditions in the present study were diagnosed by the clinician based on the clinical presentation. Tinea corporis was the most common clinical condition observed in which various exposed parts of the body are affected followed by tinea cruris and tinea capitis. Tinea conditions are as a result of exhaustive physical work and prolonged exposure to sun leading to excessive sweating. In addition, the tight fitting and synthetic clothing particularly in males provide damp, sweaty and warm skin conditions. All these factors favour the growth of dermatophytes. Tinea pedis and tinea unguium might result from wearing of socks and shoes for a long period providing damp conditions especially in inter-digital space. These findings were also reported by (Bhatia and Sharma, 2014).

Predominant species isolated in the present study was *T. rubrum* from 31(55.35%) samples. Other authors who reported *T. rubrum* as predominant isolate in their studies were Singh and Beena (2003) about 73.27%, Surendran *et al.* (2014) about 67.5% and Lakshmi *et al.* (2015) about 58.06%. Second commonest species isolated in present study was *T. violaceum*, from 10(17.85%) samples. However, in contrast to present study other studies have reported *T. mentagrophytes* as second commonest organism (Bindu and Pavithran, 2002; Peerapur *et al.*, 2004; Surendran *et al.*, 2014). From tinea unguium cases only 2 *Trichophyton* species were isolated. However, five samples from tinea unguium cases yielded non-dermatophytes, which includes two *C. albicans*, two *Aspergillus niger* and one *Fusarium* species which were not included in the Table 3. It is a well-known fact that infections of nail is caused by dermatophytes as well as by yeasts and moulds like *Aspergillus*, *Fusarium*,

Curvularia etc. This could be one of the reasons for isolating less number of dermatophytes from nail samples in present study.

Neoscytalidium dimidiatum can cause clinical lesions resembling those induced by *T. rubrum*. *Neoscytalidium* spp. are moulds responsible for foot dermatomycoses in tropical and subtropical areas, and an increasing number of cases are being reported in temperate countries among immigrants from tropical areas. As there is no effective oral or topical treatment for skin and nail infections due to *Neoscytalidium* spp., accurate etiologic diagnosis is required to discriminate these infections from those due to dermatophyte species. Thus, improper antifungal treatments, often expensive and sometimes associated with drug toxicity, can be avoided (Kinda *et al.*, 2012).

Identification of individual dermatophyte species causing infection is also important for several reasons. For example, *M. canis* commonly indicates a cat (rarely a dog) as a persistent inoculum source, while *M. gypseum*, causing similar lesions, indicates contact with contaminated soil. Second, the actual treatment regimens may differ for different dermatophyte species: for example, *Trichophyton tonsurans* in tinea capitis tends to require shorter treatment times than *M. canis*. The latter fungus to some extent evades drug exposure by forming arthroconidia outside the hair shaft, while the former forms arthroconidia inside the hair shaft where contact with conventional anti-fungal drugs is relatively high. Third, especially in onychomycosis, culture and species identification may be needed to distinguish dermatophytes from non-dermatophytic species causing dermatophytosis-like infection that does not respond to anti-dermatophyte therapy (Graser *et al.*, 2008).

CONCLUSION

Dermatophytosis is one of the commonly encountered fungal infections in developing countries like India. Accurate and rapid diagnosis is essential for proper management of cases. Direct microscopy is very good method for diagnosis of superficial fungal infections in routine laboratory especially in resource poor setting, however culture remains gold standard which provide information about different species of dermatophytes prevalent in the region and also helps in discriminating infections resembling dermatophytosis like infections caused by *Neoscytalidium* spp.

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