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Isolation and identification of dermatophytes in a tertiary care hospital, Solapur

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Abstract
Background: Dermatophytosis is superficial fungal infection caused by dermatophytes, a group of fungi that are capable of growing by invading the keratin of skin, hair and nails. Dermatophytes include Trichophyton, Microsporum and Epidermophyton species.
Aim & Objectives: To study the distribution and frequency of Dermatophytes species according to the site of infection.
Material and Methods: A total of 130 skin scraping, hair and nail samples from clinically suspected patients were collected. Direct examination for fungal elements was done by using 20% KOH mount for skin and hair samples and 40% KOH for nail clippings. The samples were cultured on Sabouraud's dextrose agar with gentamicin and cycloheximide and Dermatophytes test medium.
Results: Majority of cases were between age groups of 31–40 years followed by 21-30 years. In this study female predominance (62.3%) was found. Tinea corporis and Tinea cruris were more common in the age groups of 21–40 years. Out of 130 cases, samples from 112 (86.15%) cases were culture positive and 100 showed KOH mount positivity (76.9%). Trichophyton mentagrophytes was the commonest isolate. Other dermatophytic species isolated were Trichophyton rubrum, Trichophyton tonsurans, Microsporum canis and Epidermophyton flocossum.
Conclusion: Diagnosis of these infections requires proper clinical examination and laboratory diagnostic aids. Early diagnosis and prevention of predisposing factors play a major role in control of Dermatophytes infection.

Keywords: Dermatophytes, Tinea infections, Trichophyton, Sabouraud's dextrose agar

Introduction

During the last 40 years, studies of mycotic infections in humans and animals have increased significantly. The prevalence of superficial mycotic infections has risen to such a level that skin mycoses now affect more than 20–25% of the world's population, making them one of the most frequent forms of infections [1]. Skin infection due to Dermatophytes has become a significant health problem affecting children, adolescent and adult especially in tropical and subtropical countries including India, where moisture plays an important role in promoting the growth of these fungi [2, 3].

Dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissues of human and other animals to produce an infection, 'dermatophytosis', commonly referred to as 'ringworm [4]. They affect keratin rich tissues like skin, hair and nails producing dermal inflammatory response and intense itching in addition to a cosmetically poor appearance [5]. Dermatophytosis in India has received increasing attention in recent years from different parts of the country. The Indian subcontinent has a remarkable varied topography and as a monsoon land, most parts of the country experience sustained periods of a combination of recurrent heat and high humidity. Such conditions favour the occurrence of mycotic infections. The fungi causing dermatophytoses vary from place to place and seasonal variations also have been observed. The immigration of labour, troop movements, emigrations and other traveling also play an important role in spreading this fungal infection [6]. The classical presentation of tinea infection is a lesion with central clearing surrounded by an advancing, red, scaly, elevated border (Fig. 1). This presentation though very typical of ringworm infection is very often confused with the other skin disorders, making laboratory diagnosis and confirmation necessary [7]. The present study was undertaken with a clinical and mycological approach wherein correlation between the age,

sex and occupation was studied and Dermatophytes species isolation and identification was done using the standard techniques [8].

Material and methods

Study and duration: This prospective study was carried out in the Department of Microbiology, during the period from August 2017 to February 2018 (7 months).

Inclusion criteria: Skin, hair and nail samples were taken from clinically suspected cases of dermatophytosis. A detailed history of selected cases was taken.

Exclusion criteria: Patients already taking treatment for fungal infection were excluded from the study.

A total of 130 skin, hair and nail samples from patients suspected to have dermatophytoses were collected. A detailed history regarding age, sex, occupation, social status and duration of complaint was taken. Samples were collected after cleaning the affected surface with 70% alcohol. From skin lesions, scales were collected from erythematous growing margins of the lesion with a sterile blunt scalpel. The hairs were plucked with sterile scalpel and forceps. Samples were collected in sterilized Whatman filter paper envelope and transported to the microbiology laboratory. Fungal spores resist drying and remain viable for several weeks when stored in paper.

The culture media used were Sabouraud's dextrose agar (plain), Sabouraud's dextrose agar with 0.05% chloramphenicol and 0.5% cycloheximide and Dermatophytes test medium. All media were received from HI media, Bombay. These media were incubated in duplicate at room temperature and at 370 C for a period of 4 weeks and observed after an interval of 2-3 days. Any fungal growth was identified based on colony morphology, pigmentation, growth rate, microscopy (LPCB), slide culture, urease test. Potato dextrose agar was used for enhancing pigment production and sporulation of the fungus. Dermatophytes test medium was incubated at room temperature and observed for up to two weeks for growth and a colour change from yellow orange to red. Physiological Tests like Urease test was done to differentiate between different species of Trichophyton (between T. Mentagrophytes and T. rubrum) by using Christensen's urea agar slant (HiMedia, Bombay).

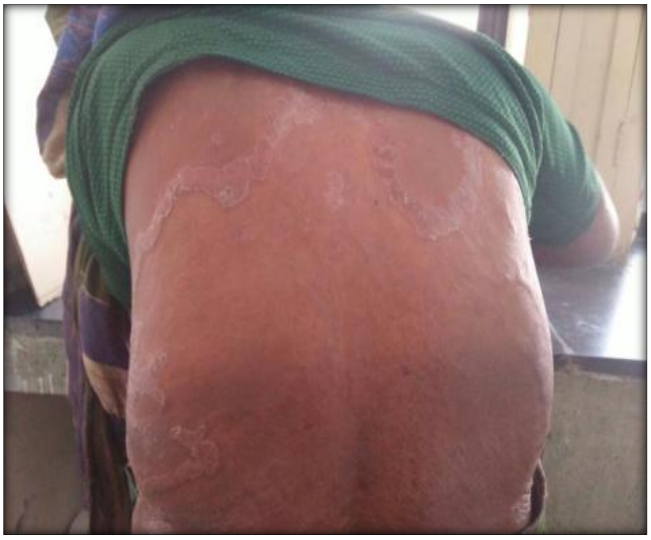


Fig 1: Patient with Tinea corporis

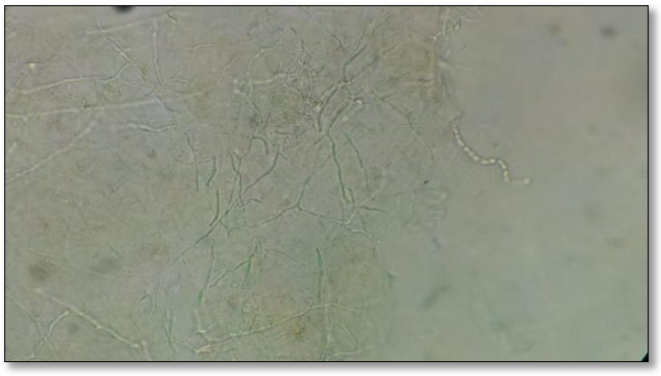
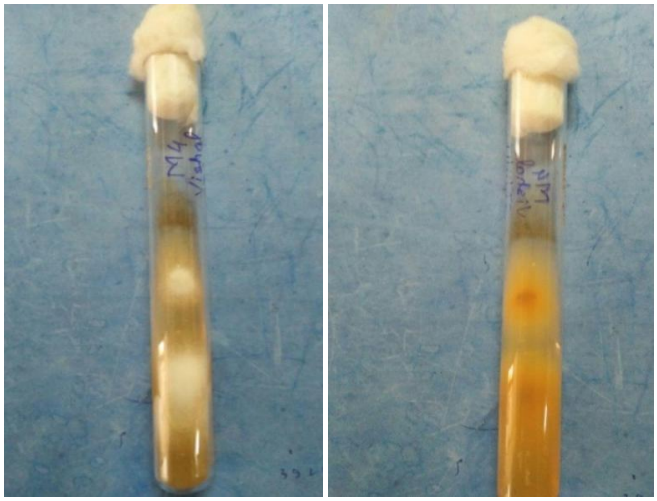


Fig 2: KOH Preparation of Skin Scrapping Showing Fungal Element (400x Magnification)



A-Obverse B- Reverse

Fig 3: SDA Slants showing Growth of T. mentagrophytes

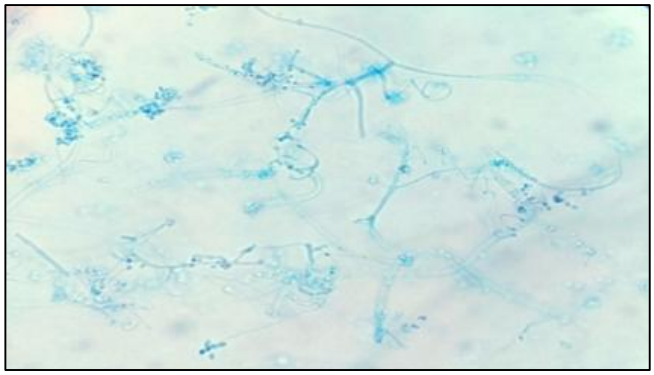


Fig 4: LPCB of T. mentagrophytes showing Spherical Microconidia in Clusters and Spiral Hyphae (400x Magnification)

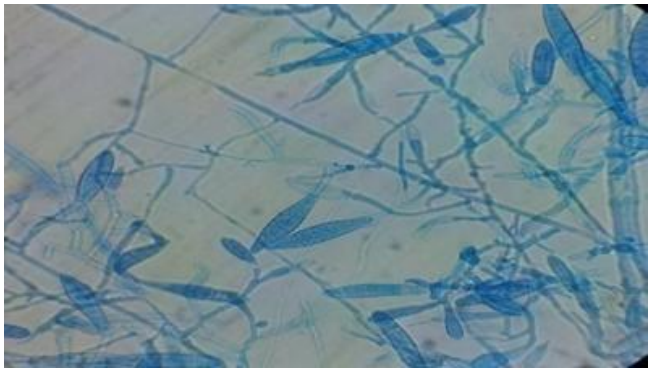


Fig 5: LPCB mount of Microsporum canis Showing macroconidia (400x Magnification)

Results

In the present study the highest number of dermatophytosis were seen in the age group of 31-40 years (40.76%) followed by 21-30 years (21.54%) (Table-1).

Among 130 cases 81 were females (62.3%) and 49 were males (37.7%).

The samples were further analyzed depending upon the clinical manifestations and it was found that out of 130, 74 cases had only Tinea corporis (56.9%), 37 had Tinea cruris (28.46%), 7 had Tinea faciei (5.38%), 2 had Tinea capitis (1.53%), 2 had Tinea unguium (1.53%) and 8 had multiple site infection (6.15%) (Table-2).

In gender wise correlation of clinical presentation, among 81 females 44 had Tinea corporis, 28 had Tinea cruris, 3 had Tinea faciei, 1 had tinea unguium and 5 had multiple site infections. So in females, Tinea corporis was the commonest lesion followed by Tinea cruris.

Among 49 males, 30 had Tinea corporis, 9 had Tinea cruris, 4 had Tinea faciei, 2 had Tinea capitis, 1 had tinea unguium and 3 had multiple site infection. Here also in males Tinea corporis was the commonest lesion followed by Tinea cruris.

Out of 130 samples, 112 were positive by culture (86.1%) and 100 showed KOH mount positivity (76.9%). Among dermatophyte species, 74 culture isolates were Trichophyton mentagrophytes (66%), 31 isolates were Trichophyton rubrum (27.7%), 3 isolates of E. flocossum (2.7%), and 2 isolates of Microsporum canis (1.8%) and T. tonsurans (1.8%) each. It was observed that Tinea corporis and Tinea cruris are predominantly caused by Trichophyton mentagrophytes.

Table 1: Age wise distribution

Age in years	No. of Patients	Percentage
0 – 10	5	3.84
11 – 20	11	8.46
21 – 30	28	21.54
31 – 40	53	40.76
41 – 50	21	16.15
51 – 60	12	9.23
Total	130	100

Table 2: Clinical Presentation

Diagnosis	Number of Patients	Percentage
Tinea corporis	74	56.92
Tinea cruris	37	28.46
Tinea faciei	7	5.38
Tinea capitis	2	1.53
Tinea unguium	2	1.53
Multiple sites	8	6.15
Total	130	100

Table 3: Gender and clinical presentations

Diagnosis	Female (%)	Male (%)
Tinea corporis	44 (54.3%)	30 (61.2%)
Tinea cruris	28 (34.6%)	9 (18.4%)
Tinea faciei	3 (3.7%)	4 (8.2%)
Tinea capitis	-	2 (4%)
Tinea unguium	1 (1.2%)	1(2%)
Multiple sites infection	5 (6.2%)	3 (6.1%)
Total	81 (100%)	49 (100%)

Table 4: Dermatophyte Species Isolated

Species	No. of isolates	Percentage
T. mentagrophytes	74	66
T. rubrum	31	27.7
T. tonsurans	2	1.8
Microsporum canis	2	1.8
E. flocossum	3	2.7
Total	112	100

Discussion

Superficial fungal infections are world-wide in distribution. In superficial mycosis, Dermatophytes are the major causes of cutaneous mycosis and remain a general public health problem. The prevalence of superficial fungal infections varies in different geographical locations. The high rate of prevalence is accounted for by various factors such as high levels of humidity throughout the year along with heavy rainfall. The high humidity and temperature provides a fertile ground for the growth of fungi causing superficial infections. The problem is further compounded by other risk factors such as low socioeconomic status and conditions like overcrowding, poverty and poor personal hygiene [3]. This is true for a tropical country like India. Studying the prevalence of Dermatophytes infections and their various clinical presentations helps in the early diagnosis and treatment of Dermatophytosis. In general, superficial mycoses are known to affect all age groups. However, they tend to predominate in younger age groups and adults comprising the working age population. In this study, it was observed that 40.76% cases affected with Dermatophytosis were in the age group 31-40 years. Similar findings were reported by C Roopa *et al* from Karnataka in 2015 [23]. A study by Prasad P.V. *et al* [28] also showed that the common age group involved in Dermatophytoses is 21-40 yrs. The higher incidence of superficial mycoses in young age may be due to increased physical activity, increased opportunity for exposure and hormonal pattern. It is obvious that the mean age of 30 years is the period where the labourers exert more physically, resulting in increased perspiration which produces a hot, humid, environment in the body, favouring the growth of Dermatophytes. Excessive perspiration also washes away fungus killing oils in the skin making it more prone to Dermatophytes infection.

Many studies observed that males are more commonly affected population like studies by Prasad PV *et al* [28], Suman *et al* [24] and SS Sen *et al* [25]. Higher incidence in males might be due to greater physical activity and increased sweating. However in the present study superficial fungal infections were more common in females (62.3%) than in males (37.7%). Male to female ratio was 0.60:1. In the study conducted by Sweta R. Prabhu *et al*, male to female ratio was 0.74:1 [21]. Also the study conducted by Bassiri- Jehroomi *et al* in Tehran from 2000 to 2005 showed male to female ratio of 0.68:1 [22]. In the present study the higher female predominance can be associated with the ethnic practices such as use of veil covering the whole body which was not subjected to daily washing and also associated with sharing of clothes among family members. Majority of these cases belonged to low socioeconomic group.

Of the 130 cases analyzed in this study, Tinea corporis was the commonest presentation [56.9%] followed by Tinea cruris [28.46%] which corresponds to study by Kanwar AJ *et al* [11], Prasad PV *et al*, Suman *et al* [24]. Similar findings

were reported by Maity *et al* 2014, Kumar *et al* 2014 and Bhatia VK and Sharma PC 2014 [29, 26, 18, 19]. In this study Tinea capitis was seen in 1.53% of patients. All Tinea capitis cases were in the age group of 0-10 yrs. This corresponds to the study by Grover C *et al* [30], Ghannoum M *et al* [31] and Elewski BE [32] in which Tinea capitis was predominantly a disease of children. Post pubertal changes in hormones resulting in acidic sebaceous gland secretions can cause decrease in the incidence with age [5]. It is said that pubertal changes in hormones results in acidic sebaceous gland secretions which is responsible for decrease in incidence of Tinea capitis in adults. The incidence rate of tinea faciei in our study correlates with the previous studies done by Kumar *et al* 2014 (3.2%), Bhatia VK and Sharma PC 2014 (3.4%) and Sharma M and Sharma R 2012 (1.1%) [26, 18, 19].

In this study, diagnosis of dermatophytosis cases was made by demonstrating dermatophytes under microscope by KOH mount and culturing the specimen on SDA with cycloheximide media and proved that direct KOH mount was found to be a good screening test for dermatophytosis because 76.9% samples were positive on KOH mount while 86.1% were positive on culture. The study by Kannan, C. Janaki *et al* [12] and Suman S *et al* [24] also showed that KOH mount positivity was seen in 80% of cases. But in contrast to this study, the culture positivity was only 45%. Shah A.K *et al* in 1976 found that the culture positivity was higher than the KOH wet mount [33].

In the present study, Trichophytons were the most common dermatophytic species isolated (95.5%) followed by Epidermophytons (2.6%) and Microsporums (1.8%).In our study Trichophyton mentagrophyte was most common isolate and similar findings were seen in studies like Bhatia VK and Sharma PC 2014 [18] and Alkhafajii KA and Alhassnawei HH 2014 [27] who also reported T.mentagrophytes as the predominant isolate. Microsporum canis was isolated from 2 cases of tinea capitis. The variation in the distribution of dermatophyte species could be explained on the basis of different climatic conditions and geographic distribution.

Conclusion

Superficial fungal infections are very common in tropical climate. Poor hygienic conditions and overcrowding play a significant role in the growth of these fungi. Early diagnosis and treatment can limit the spread of these infections. Both direct microscopy as well as culture is important and both should be done simultaneously in all cases of clinically suspected superficial mycosis. The present study highlights the importance about creating awareness of good hygienic practices to prevent the occurrence of these fungal infections. Preventive measures such as maintenance of personal hygiene, avoidance of tight and restrictive clothing and early diagnosis and treatment plays a major role in control of these infections. Patients need to be educated regarding the continuation of medications until mycological cure in order to prevent recurrence.

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