ORIGINAL ARTICLE

Clinical and laboratory correlation of dermatophytic infection in patients in tertiary care hospital

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Abstract

Background: Dermatophytosis is caused by a homogenous group of keratinophilic fungi called dermatophytes that have the capacity to invade keratinized tissue i.e. skin, hair and nails of humans and animals. Fungal infections do not receive much attention and are diagnosed very late as they are not notifiable, unlike viral, bacterial, and parasitic diseases. *Aim and Objectives:* To identify and characterize different species of dermatophytes from clinically diagnosed cases of ringworm infections. *Material and Methods:* Characterization was based upon the morphological features in direct Potassium Hydroxide (KOH) mount, growth on Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM) for primary isolation of dermatophytes from clinical samples. *Results:* In the present study, total of 176 samples were collected. Causative agents were identified macroscopically and microscopically from the growth obtained from SDA. Out of the 176 patients from whom samples were collected, males were found to be more common, with the male to female ratio being 2:1. Most common clinical type was *Tinea cruris* (39.20%) followed by *Tinea corporis* (30.11%). Most common fungal isolate was *Trichophyton rubrum* followed by *Trichophyton mentagrophytes*. Thus, it was observed that although mycological culture gives a definitive diagnosis, it is time consuming. *Conclusion:* Combined use of KOH mount and culture growth should be used for proper and definitive diagnosis of dermatophytosis.

Keywords: Dermatophytes, Sabouraud Dextro Agar, Potassium Hydroxide, Dermatophyte Test Medium

Introduction:

Superficial cutaneous mycotic infections are commonly encountered fungal diseases prevalent in most parts of the world. Dermatophyte infection is one of the most common skin infections in the rural population. This is because most of the people in rural areas are farmers with proximity to domestic animals, thereby exposing themselves to soil, compost and decaying organic material. Further, rural population often swim and bathe in village ponds where animals are also bathed. All these factors may place the rural population at a higher risk of acquiring dermatophyte infections. Dermatophytosis is the most common and clinically significant superficial fungal infection because of their widespread involvement among people all over the world [1]. Clinically, the different types of dermatophytosis are classified according to body site involvement. The most important dermatophytes that cause infection in humans are classified into three genera: *Trichophyton, Microsporum* and *Epidermophyton*. *Trichophyton* causes infection of hair, skin and

nail. Microsporum causes infection of hair and skin. Epidermophyton causes infection of skin and nails. These fungi colonize the keratin tissues and inflammation is caused by the host's immune response to their metabolic by-products. The classical presentation of tinea infections is a lesion with central clearing surrounded by an advancing, red, scaly elevated border [2-4]. Dermatophytes break down and utilize keratin as a source of nitrogen. Infection is generally cutaneous and restricted to the non-living cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts.

Ringworm infections spread most easily in settings such as schools, summer camps, prisons, and families, where there may be overcrowding with common use of facilities such as bath rooms and showers. Sharing of clothing, combs, and hair brushes along with poor personal hygienic conditions contribute to the spread of dermatophytoses [5].

The distribution, frequency and the causative agents involved vary from place to place depending upon the climatic, socioeconomic conditions and the population density [6-7].

Dermatophytosis is common in tropical countries like India and may reach epidemic proportions in areas with high rate of humidity, over population and poor hygienic conditions.

This study was undertaken on the patients attending dermatology Out-patient Department (OPD) of a teaching hospital located in a rural area with the aim to evaluate and correlate the type of dermatophytic infection with associated symptoms. These patients were clinically diagnosed as suffering from dermatophyte infections and subjected to laboratory diagnosis.

Material and Methods

This was an observational cross-sectional study in which patients attending the dermatology OPD of a teaching hospital located in a rural area with complaints of ringworm lesions on various parts of body were included. A total of 176 patients were examined within the time period of one year duration with clinically diagnosed dermatophytic infection, after obtaining informed consent. Detailed clinical history including age, sex, duration and type of lesions, family history and contact with animals and/or soil was recorded. Patients who used antifungal drugs (oral or topical) within 2 months prior to presentation in OPD and those had serious underlying systemic conditions and/or bacterial infections were excluded from the study.

Standard protocol for identification of mycotic infection was followed. The materials for the study were skin scrapings. The first step of the sample collection was to clean the infected area with 70% ethanol cotton swab to remove dirt and contaminants. Subsequently, under aseptic conditions, skin scrapings from the margin of the lesions were collected in a black thick paper which was then folded, labelled, and sent to microbiology laboratory. All the samples collected were subjected to direct microscopy and culture [8-9].

Small portion of each sample was subjected to 10% Potassium Hydroxide (KOH) mount for 30 minutes, and the remaining portion of the sample was inoculated by stab method on Sabouraude's Agar (SDA) and Dermatophyte Test Medium (DTM). The test tubes were incubated at room temperature for 3 weeks. Colonies on the slant were examined for their morphology, texture, pigmentation (obverse and reverse), etc.

From the culture, bits of mycelia growth were subjected to Lactophenol Cotton Blue (LPCB) mount and microscopically observed for characteristic spore and hyphae formation. A drop of LPCB stain was taken on a grease free glass slide and the fungal growth from the slant were teased and a cover slip was carefully placed over the preparation and observed under high power objective lens. The growth of dermatophytes in DTM was observed as change in colour of medium to red, denoting the shift in pH to alkaline.

Urease test was used as an adjunct to microscopic examination for the differentiation of dermatophyte species since most of them can produce urease enzyme which hydrolyse urea.

Pathogens were identified to species level based on microconidia, macroconidia and morphology on hyphae. Identification was confirmed by gross morphology of growth, typical microscopic characteristics, supplemented with slide culture and urease test. Data were entered in Microsoft excel and percentage were calculated.

Results

In our study, a total of 176 patients were included who were clinically diagnosed as suffering from dermatophyte infections. Age of these patients ranged from 20 to 80 years with a mean of 46.48 years. We had maximum number of patients in the age group of 41-50 years (36.93%) followed by 21-30 years (26.13%) (Table 1). Males were more commonly infected (66.47%) than female (33.52%) with the male to female ratio being 2:1. In our study, the risk factors for dermatophyte infections were not considered.

Table 1: Age and Sex wise distribution of the cases

Age of patients (Years)	Male (Number)	Female (Number)	Percentage (%)
21 – 30	29	17	46 (26.13)
31 – 40	11	6	17 (9.65)
41 – 50	41	24	65 (36.93)
51 – 60	15	9	24 (13.63)
61 – 70	15	3	18 (10.22)
71 – 80	6	-	6 (3.40)

In our study, the most common clinical presentation of dermatophytic skin lesions were *Tinea cruris* (39.20%) which was followed by *Tinea corporis* (30.11%), *Tinea incognito* (19.31%), *Tinea faciei* (6.81%), and *Tinea barbae* (4.5%). (Table 2). When KOH mount was compared with culture, it was found that more samples were culture positive than KOH positive. Of the 176 samples, 134 were culture positive of which 76 were KOH mount positive and 58 were KOH mount negative (Table 3). The reason behind samples being KOH positive but culture negative may be the non-viability of fungal elements in a culture medium and could be due to absence of the fungi in the portion of the sample used for culture. Some samples were KOH negative but culture positive and it could have been because some of the microscopic field may be missed during direct microscopic observation, especially when ample amount of sample is available for the study. The same sample when cultured in an appropriate medium, fungus grows well and flourish into the whole plate. This could also be attributed to the inactive sporulating phase of the fungi which is difficult to be seen by microscopy and depends on the skill of the observer.

The dermatophyte species which was most frequently isolated was *Trichophyton rubrum* (21.59%) followed by *Trichophyton mentagrophyte* (14.77%), *Trichophyton tonsurans* (8.5%), *Microsporum ferrugenium* (5.11%), *Microsporum audinii* (3.4%), and *Trichophyton verrucosum* (3.4%). Other non-dermatophytic growth obtained on culture were *Aspergillus spp* (14.77%), *Candida spp* (10.22%) and other contaminants (6.8%). Of the total 176 samples which were cultured, 14 (7.95%) showed no growth even after 3 weeks. (Table 4).

Table 2: Clinical presentation of dermatophytosis

Clinical presentation	Cases (Number	Percentage (%)
Tinea cruris	69	39.20
Tinea corporis	53	30.11
Tinea incognito	34	19.31
Tinea faciei	12	6.81
Tinea barbae	8	4.5

Table 3: Comparison of KOH mount and culture

Culture	Positive	Negative
KOH positive (91)	76	15
KOH negative (85)	58	27
Total (176)	134	42

Table 4: Distribution of dermatophytic species isolates

Dermatophyte spp	Number	Percentage (%)
Trichophyton rubrum	38	21.59
Trichophyton mentagrophyte	26	14.77
Trichophyton tonsurans	15	8.50
Microsporum ferrugenium	9	5.11
Microsporum audinii	6	3.40
Trichophyton verucosum	6	3.40
Trichophyton violacium	6	3.40
Aspergillus spp	26	14.77
Candida spp	18	10.22
Other contaminants	12	6.80
No growth	14	7.95

Discussion

The dermatophytes are by far the most significant cutaneous fungi because of their widespread involvement of population at large and their worldwide prevalence [8].

Although fungi have worldwide distribution, only a few of them are considered as pathogenic. The approach to fungi identification in the developing countries is through gross morphological features, whereas in the developed countries it is through the molecular approach. The fungi causing cutaneous mycosis are assuming greater significance due to excessive use of immunosuppressive drugs for controlling serious infectious as well as non-infectious conditions. The incidence of fungal infections has increased dramatically in the past 20 years partly because of the increase in the number of people whose immune systems are compromised by aging or Acquired Immunodeficiency Syndrome (AIDS), organ transplantation or cancer therapy [11]. In our study, dermatophytosis was more common in the age group of 41-50 years (36.93%). This is comparable to the study done by Mahale *et al*. [12] which found that dermatophytosis was seen more commonly in the fourth decade (23.1%) of life. Also in our study, males were more commonly affected than females which is in concordance with the findings by Bhagra *et al*. [13]. This could be due to the fact that males are more involved in outdoor physical activities leading to excessive sweating which is a favourable environment for the growth of fungus.

Our study found that culture was more sensitive than KOH mount microscopy in diagnosis of dermatophytosis. This correlates with the studies by Singh *et al*. [14] and Nasimuddin *et al*. [18] where microscopy was found to be less sensitive than culture. For culture, the media which were used for isolation were SDA and DTM.

Cultivation of fungi on different culture media provides definitive diagnosis of dermatophytosis. Selective culture media are required as the contaminating moulds hamper the recovery of dermatophytes. DTM and SDA, being selective media, can be used as a rapid screening media for the rapid detection and isolation of dermatophytes. The rate of positive culture by SDA and DTM showed no statistical difference in our study which correlates with the study by Singh *et al*. [14], where both media were found to be technically good for primary isolation.

Ringworm infection may have varied range of clinical manifestation in different areas of the body. Many studies have found tinea corporis as the most common infection with high positive rate followed by tinea cruris. But in our study, the most common infection was found to be *Tinea cruris* (39.20%) followed by *Tinea corporis* (30.11%).

The predominant species of dermatophyte which was isolated in our study was *Trichophyton rubrum* (21.59%) followed by *Trichophyton mentagrophytes* (14.77%) which is consistent with the findings reported by Jain *et al*. [3], Gupta *et al*. [16] and Lakshmanana *et al*. [17].

The transmission of dermatophytoses or tineas occurs by direct contact with infected animals and humans or by indirect contact with contaminated fomites. The clinical forms vary according to the etiologic agent (species) and the anatomical site involved. Dermatophyte infection is one of the most common skin infections in the rural population. In our study, it was also observed that 31.81% of patients suffering from dermatophytic infections were farmers by occupation, while 16.47% patients were in contact with cattle and 10.22% were in contact with dogs. Dermatophytes are among the few fungi causing communicable diseases, that is, diseases acquired from infected animals or birds or from fomites. Most of the fungal spores naturally inhabit the skin surface. Under normal conditions, they don't harm the host but in hot and humid conditions with inadequate personal hygiene, fungi grow and multiply by feeding on dead keratinized skin layers resulting in infection [10-11]. The tinea infections are prevalent globally but they are common in tropics and may reach epidemic proportions in geographical areas with higher humidity, overpopulation, and poor hygienic living conditions [18]. Hot and humid climate of India makes dermatophytosis a very common superficial fungal infection of skin [19].

Conclusion

Although dermatophytes do not cause outbreaks or pandemics, the importance of fungal infections has increased significantly, because of the increasing awareness regarding the cosmetic problems. Dermatophyte infections are common in tropical countries where hot and humid climate in association with poor hygienic conditions play an important role in the growth of fungi. Clinical manifestations of fungal infections are not specific and like other infective disease, a high degree of suspicion and awareness on the part of patient is required for early diagnosis and optimal management of infections. Thus, all the clinically diagnosed fungal infections need to be confirmed by laboratory diagnosis for proper treatment. To avoid a misdiagnosis, identification of dermatophyte infections requires both fungal culture on Sabouraud's agar media and a light microscopic mycological examination. Thus, clinical diagnosis supported by laboratory evidence of fungal infection with proper antifungal treatment will help in eradication of infection that spreads infection among close contact.

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