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Indian Journal of Microbiology Research

Journal homepage: https://www.ijmronline.org/



Original Research Article

Spectrum of dermatophytes in a tertiary care centre in Northern India

Pavneet Kaur^{1,*}, Guneet Awal², Amandeep Singh³, Ramanjit Kaur⁴, Parmeet Kaur⁴

- ¹Dept. of Microbiology, SGRD Institute of Medical Sciences and Research, Amritsar, Punjab, India
- ²Dept. of Dermatology, SGRD Institute of Medical Sciences and Research, Amritsar, Punjab, India
- ³Dept. of Radiodiagnosis & Imaging, SGRD Institute of Medical Sciences and Research, Amritsar, Punjab, India
- ⁴SGRD Institute of Medical Sciences and Research, Amritsar, Punjab, India



ARTICLE INFO

Article history: Received 01-11-2020 Accepted 27-12-2020 Available online 15-12-2021

Keywords:
Dermatophytes
Trichophyton
Microsporum and epidermophyton

ABSTRACT

Dermatophytes is major public health challenge in many parts of the world, mainly in developing countries due to poor housing facilities, high population per capita, and poor sanitary conditions. Early diagnosis and identification is must for preventing and early treatment of dermatophytosis. Also, some studies suggest that in prepubescent children there is an inadequate amount of fungi inhibiting fatty acids synthesized predisposing them to dermatophytic infections. Reduction in the synthesis of these fungistatic triglycerides in sebum premenopausal women is also seen predisposing them to infection by dermatophytes. However, low socioeconomic status along with illiteracy and overpopulation has been a main predisposing factor to dermatophytic infections in developing parts of the world. The incidence also been increased due to the rise in the number of immunocompromised patients and considerable use of broad-spectrum antibioticsEarly finding of infection is must for prevention and early management of dermatophytosis. Dermatophytes enter keratinized tissue via keratinases, which produce a dermal inflammatory response causing burning, itching and redness

Aim & Objectives: To determine prevalence of species of dermatophytes.

Materials and Methods: This Study was conducted on 334 samples i.e. skin scrapings, nail clippings, and hair for fungal culture in the Mycology laboratory over a period of one year extending from December 2019 to December 2020. Specimens were cultured on modified Sabouraud's dextrose agar media containing antibiotics and incubated at 25°C and 37°C for a period of 4 weeks. Isolation and identification of various species of dermatophytes were done. A cross-sectional study was conducted on patients who came to our hospital in the department of dermatology or were referred to the department of microbiology over a period of one year extending from November 2019 to November 2020.

Microbiological tests of suspected patients included potassium hydroxide (KOH) mount and fungal culture examination. Cases with culture-positive results were correlated with clinical diagnosis. In the study total of 334 samples (skin scrapings, nail clippings, and hair) were received for fungal culture in the microbiology laboratory during the study period. Samples obtained were cultured on modified Sabouraud's dextrose agar (SDA) media containing antibiotics and incubated at 25°C and 37°C for a period of 4 weeks. Species identification was performed based on colony's morphology, finding of the teased mount by using lactophenol cotton blue stain (LCB) and slide culture, and also with urea hydrolysis test as seen.

Results: The Study was performed on 334 samples received from the department of dermatology for fungal culture. Fungal elements were seen in 31% of cases and were isolated in 30% of cases. In these culture-positive cases, dermatophytes were reported in 90% cases, Candida species in 4%, and another fungus was reported in 6% cases. Trichophyton species is most commonly isolated (27.6%). Microsporum and Epidermophyton species were isolated in 5.1% cases. T. mentagrophytes was the most common fungal isolate among all the culture-positive cases.

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1. Introduction

Dermatophytes are fungi that require keratin in dermis for their growth. These fungi can infect the hair, skin, and nails. The etiologic agents of the dermatophytoses are grouped in three genera i.e Epidermophyton, Microsporum, and Trichophyton. On the basis of their primary habitat, they can also be divided into anthropophilic and zoophilic. Species of all these groups can infect humans in all age groups particularly in tropics, where moisture helps in growth of the fungi. 3

In developing countries predisposing factor are poor housing facilities, high population per capita, and poor sanitary conditions. Also some studies suggest that in prepubescent children there is inadequate amount of fungi inhibiting fatty acids synthesized predisposing them to dermatophytic infections. A Reduction in the synthesis of these fungistatic triglycerides in sebum premenopausal women is also seen predisposing them to infection by dermatophytes.

However, low socioeconomic status along with illiteracy and overpopulation has been main predisposing factor to dermatophytic infections in developing parts of the world. Incidence of dermatophytes has been appreciably increased due to rise in number of immunocompromised patients and also because of considerable use of broad spectrum antibiotics. ^{5,6}

Early finding of infection is must for prevention and early management of dermatophytosis. Dermatophytes enter keratinized tissue via keratinases, which produce dermal inflammatory response causing burning, itching and redness. Traditionally, infections by dermatophytes have been named corresponding to the anatomic locations e.g., tinea capitis for ringworm of the scalp. Bernatophyte infection can be mild to very severe depending on reaction of host's body to fungus, the virulency of the infecting species, the anatomical site of the infection, and also local environmental factors.

The occurrence of dermatophytosis in population is based on many other factors such as population type, vulnerability of individual, life style and cultural practices present in society. In many studies there is significant difference in isolation of species depending on the geographical pattern. ¹⁰

2. Materials and Methods

A cross-sectional study conducted on patients who came to our hospital in the department of dermatology or were referred to the department of microbiology over a period of one year extending from November 2019 to November 2020.

E-mail address: drpavneetkaursood@gmail.com (P. Kaur).

Microbiological tests of suspected patients included potassium hydroxide (KOH) mount and fungal culture examination. Cases with culture-positive results were correlated with clinical diagnosis. In the study total of 334 samples (skin scrapings, nail clippings, and hair) were received for fungal culture in the microbiology laboratory during the study period. Samples obtained were cultured on modified Sabouraud's dextrose agar (SDA) media containing antibiotics and incubated at 25°C and 37°C for a period of 4 weeks. Species identification was performed on the basis of colony's morphology, finding of the teased mount by using lactophenol cotton blue stain (LCB) and slide culture, and also with urea hydrolysis test as seen in Figures 1 and 2.



Fig. 1: Growth of trichophyton rubrum on sabouraud's dextrose agar

2.1. Inclusion criteria

All samples of patients clinically suspected to have dermatophytosis of either sex and of all age groups referred to the department of microbiology were included in this study.

2.2. Exclusion criteria

Infants and patients already on antifungal therapy were excluded from the study.

The study was done after ethical clearance from the institute. After informed written consent all the patients enrolled in this study.

^{*} Corresponding author.

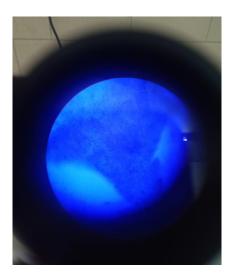


Fig. 2: Trichophyton rubrum on lactophenol cotton blue stain

2.3. Culture and microscopic examination

The sample was mounted in an aqueous solution of 10% (w/v) potassium hydroxide (KOH) on a microscopic slide. After of mounting, the preparation was examined for the presence of fungal elements. Then the clinical sample was cultured irrespective of the negative or positive direct microscopic examination results onto plates of Sabouraud's dextrose agar containing chloramphenicol with and without cycloheximidewhich were prepared according to the instruction. All inoculated plates were then incubated at inverted position for 4-6 weeks at 25-30°C aerobically. Culture plates were examined twice a week for any fungal growth. Colonies suspected of dermatophytes were subcultured into potato dextrose agar (Oxoid, Basingstoke, England) for the production of spores. Cultures of dermatophytes were identified by examining macroscopic and microscopic characteristics of their colony. Texture, rate of growth, topography, and pigmentation of the front and the reverse side of the culture were employed for the macroscopic identification. Microscopic identification of mold isolates was performed by placing pieces of a colony from SDA and/or PDA to clean microscopic slide and staining with lactophenol cotton blue. After placing a cover slip, each preparation was observed microscopically. Urea agar (Oxoid, Basingstoke, England) was used in the differentiation of Trichophyton tonsurans, Trichophyton violaceum, and Trichophyton rubrum.

The study was done after ethical clearance from the institute. After informed written consent all the patients enrolled in this study.

2.4. Isolation and Identification of Dermatophyte spp

Trichophyton species were implicated in 98.6% (73/74) cases while Microsporum species was detected only in

1.35% cases. However, none of the Epidermophyton species was recovered in the present study. Among the Trichophyton spp., T. mentegrophyte was the predominant organism (64.9% cases) followed by T. rubrum (35.1% cases) (Table 2). The identification of these dermatophyte species was based on cultural characteristics, growth rate, texture, colony size and pigmentation produced on obverse and reverse sides of SDA slants. T. mentagrophyte grew rapidly (3-5 days) on SDA, the growth was powdery to fluffy, cream to white on obverse and yellow to brown on reverse. On microscopic examination, well septate spiral hypahe with numerous spherical microconida were visible (Figure 3). T. rubrum grew relatively slower (10-15 days), the growth was powdery to velvety with reddish tinge on obverse and rusty brown to deep red on the reverse. Well septate, pencil shaped hyphae with numerous spherical microconidia along with macroconida were visible on microscopic examination. M. gypseum grew rapidly (3-5 days), the growth was powdery to granular with rosy pink on obverse and yellow to brownish on reverse. As shown in Figure 3, pyriform septate hyphae were visible and microconidia were not seen as this organism rarely produces microconidia. T. mentagrophyte, the predominant species was found associated mainly with Tinea corporis 40.4% and Tinea cruris 25.5%. However, it was seen in all other tinea conditions also.



Fig. 3: Growth of trychophyton mentagrophyton on sabouraud's dextrose agar

2.5. Statistical analysis

Microsoft excel was used for interretation and analysis of the data.

3. Results

A total of 334 samples were received for fungal culture. Out of which 72%, 25% and 3% samples were from skin scrapings, nail clipping and hair samples, respectively (Table 1). From male patients a total of 49.2% samples were obtained and from females 50.7% samples were obtained (Table 2). The overall male and female ratio in both groups is approximately 1:1. Most of the patients belonged to the age group of 21–40 years (54%). Potassium hydroxide mount examination was positive for fungal components in 31% cases and culture was positive for fungal elements in about 30% cases. In these culture positive cases we have seen dermatophytes, candida and other fungus in 90% 4%, and 6% cases respectively.

From all positive cultures dermatophytes were isolated from 72.3%, 26.8%, and 0.9% of skin scrapings, nail clippings, and hair samples respectively. Trichophyton species was isolated in about 89.8% cases, Microsporum species in 3.1% cases and Epidermophyton species in .1% cases. Trichophyton mentagrophytes (67.3%) was the most common fungal isolate among all the culture positive cases.

Table 1:

Sample	Percentage
Skin	72%
Nail	25%
Hair	3%

Table 2:

Gender	Percentage
Male	49.3
Female	50.7

Table 3:

Species	Percentage
Trichophyton	89.8
Epidermophyton	7.1
Microsporum	3.1

4. Discussion

Dermatophytes constitute a worldwide problem and are more prevalent in the developing world. Hot and humid environment of various parts of India considered to be best suited for the dermatophyte infections. The overall male and female ratio in both groups is approximately 1:1. High prevalence among male patients has also been reported by other studies done in India contrary to our study, as there was approximately equal number of cases among both groups. ^{11,12} This may be due to active involvement in outdoor activities. On basis of the clinical presentation Tinea corporis (75%) was the most common clinical condition diagnosed followed by jock itch (18.75%) among all the culture positive cases in this study. In our study, T. mentagrophytes was the predominant dermatophytes seen in 67.3% of cases, isolated followed by T. rubrum and T. Interdigital. Microsporum species and Epidermophyton species were isolated in 5.1% cases each. In some other studies done in India they have also described T. mentagrophytes as a predominant dermatophyte. ^{12–15}

In the study conducted by Sudip Das et al ¹⁶ of the total 53.4% of cases of dermatophytes in the culture, contamination was seen in approximately 52% of cases and Trichophyton was the predominant organism along with T. verrucosum, followed by T. rubrum, and T. mentagrophytes. All species of Trichophyton were most sensitive to itraconazole amongst systemic antifungals and luliconazole amongst topical antifungals.

In a study done by Lakshmanan et al. 17 it was seen that among nondermatophytic fungi, Candida, Aspergillus, Alternaria, Curvularia, and Fusarium was seen thus pointing that nondermatophyticmolds are emerging cause of superficial mycoses. It is of utmost importance that clinical diagnosis of dermatophytoses should be correlated with microbiological findings as these infections have many mimics, which can produce identical lesions. For example, Tinea corporis may be confused with eczema, Tinea capitis may be confused with alopecia areata, and onychomycosis may be confused with dystrophic toe-nails due to repeated minor trauma. Before starting therapy physicians should firstly confirm dermatophytoses with help of KOH mount preparation or culture. Tinea corporis, jock itch, and athlete's foot generally answer topical antifungal agents, but oral antifungal agents should be considered for severe disease, failed topical treatment, immunocompromised patients, or severe moccasin-type Tinea pedis. Oral terbinafine should be used as a first-line drug for Tinea capitis and onychomycosis as it has tolerability, low cost and high cure rate. However, kerion should be treated with griseofulvin unless Trichophyton has been established as the pathogen. The limitations of this study are its retrospective nature, in which antifungal susceptibility was not performed. Genomic and proteomic studies weren't performed, which could have given better clarification about fungal species.

Several studies in India reported dermatophyte infection is most common in the 30-40 years of age, in the present study, it was found almost all ages were susceptible to various dermatophytic infections with preference in age 21-40. Kanwar AJ et al. ¹⁸ in their study also stated a similar range of age during which dermatophyte infection is

prevalent i.e., 16-45 years. Tinea corporis was the foremost common clinical pattern among 21-30 years and 31-40 years during this region. Common occurrence in this age group could be due to strenuous outdoor manual work with excessive perspiration in this age group compared to inactive behavior in the later years. ¹⁸ Whereas, in 11-20 years jock itch was found common and in 1-10 years of school going age, tinea was common. This finding has been accepted universally that tinea, an infection preferably found in children. Tinea unguium was more common among 41 to 50 years.

In this study, Trichophyton mentagrophytes were the predominant dermatophyte isolated. This is in accordance with study carried out by authors in which other species of dermatophytes such as Epidermophyton floccosum, Trichophyton verrucosum and Trichophyton violaceum were also isolated although in small numbers whereas the commonest dermatophytes isolated from hair and nail samples were Trichophyton mentagrophytes. Similar results were seen in other studies where the authors pronounced that Trichophyton rubrum and Trichophyton mentagrophytes were the common species infecting the nails. Microsporum nail infections are rare, this finding has been upheld in the present study. However, Microsporumaudouinii was isolated during a study by Adhikari L et al., in Sikkim. ¹⁹

Predisposing factors contributing to dermatophytosis such as lack of hygiene, poor nutrition, lower socioeconomic standard, their occupation, living conditions were not presented in the present study due to space constraint.

5. Conclusion

From our present study we can comprehension about the prevalence and distribution of dermatophytoses in northern India. Most frequently diagnosed clinical complaint was Tinea corporis followed by Tinea cruris. *T. mentagrophytes* was the most commonly isolated species followed by *T. tonsurans* and *T. rubrum*. Thus from data obtained in our study we could help in the timely diagnosis of the disease and further hampers its spread with specific control measures

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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Author biography

Pavneet Kaur, Associate Professor

Guneet Awal, Associate Professor

Amandeep Singh, Professor

Ramanjit Kaur, Resident

Parmeet Kaur, Resident

Cite this article: Kaur P, Awal G, Singh A, Kaur R, Kaur P. Spectrum of dermatophytes in a tertiary care centre in Northern India. *Indian J Microbiol Res* 2021;8(4):297-301.