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Mycological Pattern of Dermatophytes and Non-Dermatophytes in a Tertiary Care Hospital

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ABSTRACT

Background: Dermatophytosis is currently a disease of worldwide importance and a public health problem in many parts of the world particularly in developing countries.
Objective: This study was carried out to determine the pattern of dermatophytes in a tertiary care hospital from July 2015 to April 2019.
Methods: Nail, skin, and scalp scrapings were received from 657 patients and were used for microscopy and culture study. Fungal pathogens were identified by studying the macroscopic and microscopic characteristics of their colonies.
Results: Amongst 657 clinical samples received, 280 (42.62%) were found to be positive microbiologically. Out of 280 positive samples, 189 (28.76%) were KOH positive while 91 (13.85%) were culture positive. Amongst 91 fungal isolates obtained on culture, 53 were dermatophytes and 38 were non-dermatophyte fungi. *Trichophyton mentagrophytes* represented 60.38% of dermatophyte isolates.
Conclusions: Coupling of clinical diagnosis with laboratory diagnosis appeared to be essential for better diagnosis as the cost and long duration of fungal therapy underline the significance of accurate diagnosis of the condition before starting therapy.

Keywords: Dermatophytosis, Mycological Pattern, Dermatophytes, Non-Dermatophytes

INTRODUCTION

The incidence of dermatophytosis is increasing over the last few years and there are many cases which are recurrent and chronic. Dermatophytes are aerobic fungi that produce proteases which digest keratin and allows colonization, invasion and infection of the stratum corneum of the skin, the hair shaft, and the nail. [1] Although these skin-related problems are not generally life threatening, they are among the most common diseases and disorders of mankind. Infection is generally cutaneous and restricted to the nonliving cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts. Reactions to a dermatophyte infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors. [2]

Dermatophytes are ascomycetes with septate hyphae. There are three genera of dermatophytes, *Trichophyton*, *Microsporum*, and *Epidermophyton*. There are at least 40 species of dermatophytes that infect humans, and many of these fungi can cause disease in more than one body location. Although the species were historically divided into these genera by morphology and physical attributes, recent analysis by rRNA sequencing indicates that the dermatophytes as a whole are a cohesive

group, with no clear distinction between the three genera. The closest relatives to any one *Microsporum* species might be two *Trichophyton* species. Thus, no comparisons should be made between genera without consulting the rRNA-based phylogenetic tree. [3]

Epidemiology is important in infection control and public health issues related to the different types of dermatophytosis. [4] Dermatophytes spread by direct contact from other people (anthropophilic), animals (zoophilic), and soil (geophilic), as well as indirectly from fomites. [5] Large population size, low socioeconomic status, inadequate health facilities, and exchanging of foot-wears, clothes, and barbershop materials among people in developing nation have been recognized as potential risk factors for the proliferation of the disease. [6]

Infections caused by dermatophytes (ringworm) have been named according to the anatomic locations involved by appending the Latin term designating the body site after the word tinea,. The clinical manifestations are as follows: (i) tinea barbae (ringworm of the beard and mustache); (ii) tinea capitis (scalp, eyebrows, and eyelashes); (iii) tinea corporis (glabrous skin); (iv) tinea cruris (groin); (v) tinea favosa (favus); (vi) tinea imbricata (ringworm caused by *T. concentricum*); (vii) tinea manuum (hand); (viii) tinea pedis (feet); and (ix) tinea unguium (nails). Several anatomic sites may be infected by a single dermatophyte species, and different species may produce clinically identical lesions. [4]

Less frequently, superficial skin infections are caused by non-dermatophyte fungi (e.g., Malassezia furfur in tinea /pityriasis versicolor) and candida species. [5]

The present study was undertaken to assess the pattern of dermatophytic and non-dermatophytic infection to identify the species of fungi and to correlate the clinical diagnosis with potassium hydroxide (KOH) smear positivity and culture positivity.

MATERIAL METHODS

The study involved 657 patients that were clinically diagnosed for superficial mycosis and referred to the Department of Microbiology, Government Medical College, Amritsar for laboratory diagnosis from the period of July 2015 to April 2019. The specimen was collected by scraping the active edge of the affected skin, nail scraping, clipping and epilation of the infected and lusterless hair. Material was subjected to direct microscopic examination using 10%-40% KOH. For primary isolation Sabouraud's dextrose agar slopes were used. Fungi were then identified by studying the macroscopic and microscopic characteristics of their culture. Texture, rate of growth, topography, and pigmentation of the obverse and the reverse side of the cultures were employed to characterize fungi macroscopically. Lactophenol cotton blue mount of each fungal isolate was used to characterize fungal isolates microscopically. Slide culture was done to study the micromorphology of microconidia, macroconidia and special structures such as spirals, pectinate, racquet hyphae, and chlamydospores. Special tests were performed when necessary, viz, hair perforation test and biochemical test like urease test was done for species identification. Many mycological laboratory texts and manuals [7-9] were used as reference materials in process of identification. Yeasts were identified by means of conventional routine diagnostic methods [9]

RESULTS

Out of 657 clinical samples received, 280 (42.62%) were found to be positive for both dermatophytic and non-dermatophytic fungi microbiologically. Amongst 280 positive samples, 189 (28.76%) were KOH positive while 91 (13.85%) were culture positive. Fungi were neither detected nor showed visible fungal growth in 377 (57.38%) clinical samples.

Table 1: Correlation of direct microscopy and culture (n=657) .

Test procedure	Number	Percentage
KOH positive	189	28.76
Culture positive	91	13.85
KOH negative culture positive	29	4.41
KOH positive culture negative	127	19.33
KOH and culture positive	62	9.43
KOH and culture negative	377	57.38

Table 2 : Frequency and distribution of culture positive dermatophytes (n=53).

Fungal Isolates	Hair	Skin	Nail	Total
Trichophyton mentagtophytes	0	28	04	32 (60.38%)
Trichophyton rubrum	0	16	02	18 (33.96%)
Trichophyton tonsurans	0	01	01	02 (3.77%)
Microsporum gypseum	0	01	00	01 (1.89%)
Total	0	46	07	53 (100%)

Table 3 : Frequency and distribution of non-dermatophytic fungi (n=38)

Fungal Isolates	Hair	Skin	Nail	Total
Aspergillus flavus	0	2	3	5
Aspergillus niger	0	0	2	2
Aspergillus terreus	0	1	1	2
Absidia	0	0	1	1
Acremonium	0	0	1	1
Alternaria	0	0	1	1
Candida species	0	4	12	16
Curvularia	0	1	1	2
Fusarium	1	0	2	3
Malassezia furfur	1	1	0	2
Mucor	0	1	0	1
Phaeoid fungus	0	2	0	2
Total	2	12	24	38

DISCUSSION

Superficial fungal infections are caused by dermatophytes, non-dermatophytic moulds and commensal yeasts. Dermatophytes, the most common causative agents, are assuming high significance in developing countries like India. The lesions may become widespread and may have significant negative social, psychological, and occupational health effects, and can compromise the quality of life significantly. These alarming aspects regarding dermatophytosis and their impact on the quality of life, warrant timely address. The recent prevalence of dermatophytosis in India ranges from 36.6–78.4%. [10] In the current study, 280 (36.83%) clinical samples received were found to be positive for both dermatophytes and non-dermatophytic fungi microbiologically. Among 280 samples only 53 samples were culture positive for dermatophytes whereas 38 samples showed culture growth of non-dermatophytic fungi. Fungi were neither detected nor showed visible fungal growth in 377 (57.38%) samples which were suspected of having superficial mycosis.

According to Expert Consensus on The Management of Dermatophytosis in India (ECTODERM India) KOH mount microscopy was recommended as a point of care testing. [10] With fungal culture, speciation of the exact organism can be done. In the clinical practice, positivity of culture or KOH smear is accepted as indicative of infection. [11] In our study, 189 (28.76%) clinical samples were KOH positive while 91 (13.85%) were only culture positive and 62 samples were both KOH and culture positive (Table1).The adequacy of the sample, and the appropriateness of the collecting tool and expertise decide the sensitivity and specificity of the KOH. Interestingly, our study found that the percentage of patients presenting with a clinical diagnosis of tinea that had cultures positive for fungus were only 13.85%. Two other studies showed similar incongruence between the clinical diagnosis of tinea and fungal culture, with less than a third of patients presenting with a clinical diagnosis of the disease having positive cultures. [11,12] The reasons of how culture may miss a diagnosis of tinea include sampling error, using defective culture medium, and mishandling of the culture medium..The perfect gold standard for infection must be a triple confirmation: positive KOH smear, positive culture, and a clinical exam consistent with tinea. [11]

Trichophyton species were implicated in 98.1% (52/53) cases while *Microsporum* species was detected only in 1.89% cases (Table 2). However, none of the *Epidermophyton* species was recovered in the present study. Among the *Trichophyton spp.*, *T. mentegrophyte* was the predominant organism (60.38%) followed by *T. rubrum* (33.96%). This was in concordance with the study conducted by Bhatia *et al.* [13] Soniya *et al*. and Sahai *et al* also reported Trichophyton mentagrophyte as the most common isolate in their studies. [14,15] In our study *T. tonsurans* was also isolated from only two clinical samples. Mahajan *et al*. and Sahai *et al* also isolated *T. tonsurans* in their studies. [14] The least common species was M.gypseum (1.89%). This finding correlates with the study done by Konda et al in Secundrabad and Ramana VP et al in Guntur. [16,17]

Non-dermatophytic fungi were isolated from 41.76% culture positive study subjects (Table3). The significance of non-dermatophyte fungi species in skin-related infections has been highlighted in other many published studies. However, the extent to which non-dermatophyte fungi actually cause dermatophytosis when a dermatophyte is present concurrently is still a subject of debate. Therefore, further investigations demonstrating how this group of fungi causes infection are needed. Among non-dermatophyte molds isolated in the present study, Aspergillus species was the most common. [18] Our result supported the findings of Aikaterini et al. and Nouripour-Sisakht et al. [19,19] Absidia, Acremonium, Alternaria, Candida, Curvularia, Fusarium , Malassezia furfur, Mucor and phaeoid fungi were other isolated non-dermatophyte fungi recorded in our study. The significance of such non-dermatophyte molds in causing skin-related infections has been demonstrated in many other studies. [21,22] Candida albicans is a major cause of tinea unguium as documented in many studies. [18,23] Similarly, in our study also Candida albicans has been isolated in 12 subjects with nail infection. Recovery of large number of non-dermatophyte fungi along with dermatophytes in our study showed that non-dermatophyte fungi are emerging as important causes of dermatophytosis, warranting the implementation of intensive epidemiological studies of dermatophytosis across the country.

Dermatophytes have a large impact on human health, as they are found on the skin and are frequently associated with disease. Coupling of clinical diagnosis with laboratory diagnosis appeared to be essential for better diagnosis as the cost and long duration of fungal therapy underline the significance of accurate diagnosis of the condition before starting therapy. PCR amplification directly from the samples could be a better tool for diagnosis of dermatophytosis. Molecular diagnostic tools are being developed for rapid and early identification of these pathogens which in combination with conventional methods would facilitate early management of dermatophytosis. [13]

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