PREVALENCE OF DERMATOPHYTES AND OTHER FUNGAL AGENTS ISOLATED FROM CLINICAL SAMPLES

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Abstract

The common cause of skin infections are dermatophytes and opportunistic fungi. Aim of this study was to isolate and identify the fungal agents from clinical samples from patients with different mycoses. Clinical samples from 165 patients were subjected to potassium hydroxide (KOH) examination and culture isolation; causative agents were identified macroscopically and microscopically. All the 165 specimens were KOH positive and 110/165 (66.7%) samples were culture positive. Of these, highest isolation rate was obtained in opportunistic mycoses such as candidiasis (29/29, 100%). Dermatophytes were isolated in 53/80 (66.3%) specimens and *Trichophyton rubrum* was the commonest isolate in skin samples (17/24) among the patients suffering from dermatophytosis. *Phaeoannellomyces wernecki* was isolated in a patient suffering from tinea nigra. The study signifies the importance of mycological examination in the diagnosis of various mycoses for their effective management.

Key words: Clinical samples, dermatophytes, opportunistic fungi, prevalence

Although fungi are world wide, only few of them are considered pathogenic. The pathogenic fungi may give rise to infections in animals and human beings. Most of the agents cause infection of the superficial layers of the integument and only very few give rise to systemic involvement. Recently there has been an increase in the incidence of fungal infections. This increase may be a result of frequent usage of antibiotics, immunosuppressive drugs and various conditions like organ transplantations, lymphomas, leukemia and human immunodeficiency virus (HIV) infections.¹

Skin infection due to dermatophytes has become a significant health problem affecting children, adolescents and adults. Mycetoma caused by filamentous fungi (Eumycotic mycetoma) and filamentous bacteria (Actinomycotic mycetoma) need to be differentiated by culture studies. A correct diagnosis is important to initiate appropriate treatment and also essential for epidemiological purposes. In the background of immunosuppression, detection of these agents becomes mandatory for they effective management of mycoses to prevent further invasions. The present study was undertaken to isolate various fungal agents causing mycoses among the patients attending mycology section of the Department of Dermatology, Madras Medical College attached to Government General Hospital, which has got an average new outpatient turnover of 7000-8000 per year.

Materials and Methods

This study was undertaken for a period of two years from January 2001 to December 2002. All the clinically suspected 165 cases were subjected to mycological work up. The specimens included skin scales, hair, hair roots and pus in cases of superficial mycoses. Biopsy tissue and grain were the specimens in deep mycoses.²

Microscopic examination

Direct microscopic examination was undertaken in 10% potassium hydroxide (KOH) wet mount for the specimens of skin scales, pus crust, biopsy tissue and grains, while 40% KOH was employed for hair and nail specimens.³ Grains of mycetoma were also subjected to Gram stain and modified Ziehl Neelsen stain (1% H₂SO₄).

Culture study

The KOH positive cases were subjected to culture study, scraping site was cleaned aseptically with 70% ethanol and the scales were collected in a sterile slide with the help of sterile scalpel. The culture was performed in two different sets of antibiotic incorporated Sabouraud dextrose agar (SDA) media, one with chloramphenicol 50 mg/L and the other with cycloheximide 500 mg/L and in addition to chloramphenicol.² The culture tubes were incubated at 30°C and the culture growth was observed and the tubes were discarded only after six weeks in the absence of growth.

The mycological identification was based on macroscopic and microscopic examination of the culture isolates. The macroscopic examination of dermatophytes was characterized by duration of growth, surface morphology and pigment

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production on the reverse. Commeal agar (CMA) was used to
differentiate Trichophyton rubrum from T. mentagrophytes
based on pigment production on the media. In addition, hair
perforation studies were carried out to distinguish between
these two species. The microscopic examination of fungal
growth was observed with lactophenol cotton blue stain.
Nature of mycelium and conidia formation (macro and micro
conidia) helped to differentiate various genera and species.

Budding yeast cells of Candida spp. were identified
microscopically. Candida species were classified as albicans
and non-albicans group by the production of the
chlamydospores on corn meal agar and germ tube formation.
Olive oil (2%) was overlaid on the media for the isolation of
Malassezia spp. in clinically diagnosed cases of pityriasis
versicolor. Plain SDA medium was used in cases of pityriasias
versicolor. Plain SDA medium was used in cases of mucorales
and eumycotic species. Lowenstein-Jensen medium was
used for the primary isolation of the agents in
actinomycetoma. Eumycotic agents were confirmed based
on microscopic observation of morphology and
conidilogenesis.

Results

Eighty out of 165 cases (48.5%) were dermatophytoses, 39 (23.6%)
were pityriasis versicolor, 29 (17.1%) were condidiasis and 12 (7.1%)
were cases of mycetoma. Tinea nigra was the clinical diagnosis in one and non-dermatophyte
onychomycoses was suspected in 4 (2.4%) patients. Specimens from all these cases were KOH positive (Table 1). Those patients with actinomycotic mycetoma showed gram-
positive filaments of actinomycetes. On the whole 110 (66.7%)
cultures were obtained from the KOH positive specimens. Isolation of opportunistic pathogens was 29/29 (100%) in
candidiasis (Table 2).

Among dermatophytes 24 isolates were obtained from
skin scales, 25 from scalp and scalp hair and four from nail clips. The dermatophytes isolated were T. rubrum, T.
violeaceum, each in 21 specimens, T. mentagrophytes in six, T.
simii in three and Epidermophyton floccosum in two
specimens (Table 3). T. Rubrum was isolated from skin scales in
17 (81%) and two (8.3%) each from scalp/scalp hair and nail. Candida spp. were isolated from 29 cases, of which 26 (89.7%)
were from HIV patients presenting with oral candidiasis and
rest were women with vulvovaginal and intertrigenous
candidiasis, Candida spp. (non albicans species) were 17
(58.6%) and Calbicans were 12 (41.4%).

Malassezia species were isolated from 22 patients with
pityriasis versicolor with 14 samples from chromic type and 8
from achromatic type. Phaeoannellomyces werneckii
was isolated from a patient with tinea nigra. Non dermatophyte
such as Scopulariopsis brevicaulis and Helminthosporium
spp. could be isolated from 2 out of 4 patients suspected with
non-dermatophyte onychomycoses.

Of the 12 cases of mycetoma, nine had actinomycotic
disease while three had eumycotic disease. Isolates could be
obtained only from the cases of eumycotic and all the three
isolates were identified as Madurella mycetomatis.

Discussion

Of the total number of 165 KOH positive specimens, only
110 (67.1%) isolates could be obtained. The isolation rate of
opportunistic mycoses was more i.e., 29/29 (100%) in
candidiasis. The higher load of the organism in the
immunocompromized background could be the reason for such
higher isolation rate. Among 80 patients with dermatophytosis
isolates could be obtained from 53 (66.3%) patients. The

| Table 1: Types of dermatomycoses included in the study and their laboratory results (n=165) |
|---------------------------------|----------|--------|-----------|
| Mycoses                        | Total no. of cases | KOH positive | Culture positive | % of total isolates |
| Dermatophytosis                | 80        | 80      | 53         | 66.3          |
| Pityriasis versicolor          | 39        | 39      | 22         | 56.4          |
| Candidiasis                    | 29        | 29      | 29         | 100.0         |
| Mycetoma                       | 12        | 12      | 12         | 25.0          |
| Tinea nigra                    | 1         | 1       | 1          | 100.0         |
| Non-dermatophytic onychomycoses| 4         | 4       | 2          | 50.0          |

| Table 2: The culture isolates among the cases of mycoses in relation to the site of involvement (n= 100) |
|---------------------------------|----------|--------|----------|---------|----------|--------|---------|----------|
| Mycoses                        | Total no. of cases | Skin | Scalp | Nail | Tongue | Vulva | Foot | Total no. of culture |
| Dermatophytosis                | 80        | 24    | 25    | 4    | -      | -     | -      | 53       |
| Pityriasis                     | 39        | 22    | -     | -    | -      | -     | -      | 22       |
| Versicolor                     |            |       |       | -    | -      | -     | -      |          |
| Candidiasis                    | 29        | -     | -     | 26   | 3      | -     | -      | 29       |
| Mycetoma                       | 12        | -     | -     | -    | -      | -     | 3      | 3        |
| Tinea nigra                    | 1         | 1     | -     | -    | -      | -     | -      | 1        |
| Non-dermatophytic onychomycoses| 4         | -     | 2     | -    | -      | -     | -      | 2        |

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isolation rate in this study seemed to be higher when compared to various other studies where it has ranged from 45.3-52.2%. More isolates could be obtained from scalp/scalp hair compared to skin scales and the isolates were least from nail specimens. Among the cases of mycetoma, the isolation rate was much lower (3/12) comprising only of eumycotic agents.

*T. rubrum* was the chief isolate form skin scales (17/24) in the present study similar to many other reports. This is the commonest agent isolated form glabrous skin of the body, groin folds and the feet. T. mentagrophytes was the second common isolate from the body site 4/24 (16.7%) as has been observed in other studies. E. floccosum was isolated from two specimens obtained from the scalp and this was the third common isolate from the glabrous skin.

*T. violaceum* seemed to be the chief isolate from the scalp/scalp hair (20/25). This agent is still the commonest isolate from cases of tinea capitis in India. This agent was also isolated from an specimen of skin scales (1/24). T. simii, the zoophilic species, could be isolated from scalp/scalp hair and this formed the second common isolate from the scalp 3/25 (12%). The prevalence of T. simii among dermatophytes in general has been observed to be 1%. The prevalence of T. simii in tinea capitis was found to be 1.4% in Tamilnadu, India and as high as 10% has been reported from Sri Lanka. T. rubrum was the least common isolate (2/25) from the scalp and this considered to be the common agent causing glabrous type of tinea capitis which is usually encountered in adults. The isolates from the nail specimens were *T. rubrum* (2/4) and T. mentagrophytes (2/4) and these agents are the common species infecting the nail.

*Malassezia* spp. were isolated from 22/39 (56.4%) samples and the isolates were more in chronic type 14/22 (63.6%) than achromic type 8/22 (36.4%). This was probably because of more number of chronic pityriasis versicolor included in the study group. The isolation rate of this agent in the present study seemed to be much higher compared to a study wherein a low culture positivity (27.8%) has been reported. Among the *Candida* spp. non albicans group was predominant when compared to *C. albicans* group because of more number of HIV patients included in the study.

*S. brevicaulis* and *Helminthosporium* spp. could be isolated from non dermatophyte onychomycoses and these are rare isolates that infect the nail. A rare and unusual human pathogen of *P. werneckii* was isolated from a case of tinea nigra. There have been sporadic reports of this condition from South India.

The eumycotic and actinomycotic mycetoma cannot be differentiated on clinical grounds. Histopathological diagnosis is also inconclusive with regard to the agents causing the disease and mycological examination plays an important role in the diagnosis. *M. mycetomatis* was isolated from all three eumycotic disease and it is considered as the commonest agent involved in eumycotic mycetoma. Isolation is difficult in actinomycotic disease which might involve culture in enriched media and biochemical procedures.

The present study shows that mycological examination of causative agent is necessary to differentiate and treat dermatophyte and non-dermatophyte onychomycosis. Isolation rate of all the fungi has been observed to be much higher in this study except with mycetoma and it can be concluded that isolation rate can be enhanced with aseptic and proper culture techniques.

References


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| Table 3: Isolation of dermatophytes in relation to site involved (n=53) |
|---|---|---|
| Dermatophytes | Skin | Scalp/Scalp Hair | Nail |
| *Trichophyton rubrum* | 17 | 2 | 2 |
| *Trichophyton violaceum* | 1 | 20 | - |
| *Trichophyton mentagrophytes* | 4 | - | - |
| *Trichophyton simii* | - | 3 | - |
| *Epidermophyton floccosum* | 2 | - | - |


