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RESEARCH ARTICLE

ISOLATION OF NON-DERMATOPHYTIC MOULDS (NDMS) AS EMERGING OPPORTUNISTIC AGENTS OF SUPERFICIAL MYCOSIS

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ABSTRACT

In the past few decades, the incidence of superficial fungal infections has been increasing in the tropical countries like India. This study was carried out to evaluate the presence of non-dermatophytic moulds (NDMs) as emerging opportunistic agents of superficial mycosis from patients presenting at a dermatology clinic. A total of 163 skin, nail and hair samples were collected during a period of May 2012 to January 2013. The samples were divided into two parts, one for direct microscopy and another for culture on Saboraud's Dextrose Agar (SDA). The isolated fungi were identified based on the macroscopic and microscopic features. The culture positivity was found to be 122 (74.8%) cases. Among the culture positive cases, dermatophytes accounted for 73 (59.8%) cases. The isolation rate of non-dermatophytic moulds was found to be 40.2% in 49 cases. The dermatophytes are usually reported as the major cause of these infections. However, the emergence of Non-dermatophytic Moulds (NDMs) as opportunistic agents of superficial mycosis should also be considered in evaluating the culture negative cases for dermatophytes as well as those cases ending up in treatment failure after empirical treatment for dermatophytic infections.

INTRODUCTION

Over the past two decades, there have been increased rise in the incidence of superficial fungal infections with high morbidity in tropical countries especially India (Bakheshwain *et al.,* 2010). Fungi causing superficial infections affect the keratinized tissue of skin, hairs and nails of man and animals. The etiological agents (dermatophytes) of such infections are species of *Trichophyton, Microsporum* and *Epidermophyton*. In addition to the accepted pathogens, there are significant numbers of non-dermatophytic moulds (NDMs) which have been implicated in cases of superficial mycosis (Sharma *et al.,* 2012). Non-dermatophytic moulds are filamentous fungi which are commonly found in nature as soil saprophytes and plant pathogens (Tosti *et al.,* 2000). However, it is not known whether these non-dermatophytes occur as a primary ailment or exist as secondary invaders in the infection process of dermatophytosis. The non-dermatophytes which are frequently isolated and identified along with the dermatophytic mould includes *Alternaria spp., Scytalidium spp., Fusarium spp.*, *Acremonium spp.*, *Scopulariopsis spp.*, *Cladosporium* s*pp*., and *Aspergillus spp*. (Farwal *et al.,* 2011). In recent years the incidences of these Non dematophytic Moulds (NDMs) have risen dramatically due to the wide spread use of broad spectrum antibiotics, immuno-suppression, chemotherapy, diabetes and certain invasive procedures. Dermatophytes are the predominant pathogens accounting for most of the cases of dermatophytosis. However, yeasts (especially *Candida albicans*) and some non-dermatophytic moulds have also been implicated as causative agents of this condition. Hence, proper laboratory investigations are essential to differentiate between fungal infections caused by these dermatophytes and the non-dematophytic molds. An accurate diagnosis depends mainly on proper laboratory investigations and successful isolation of the organism in the culture. In this study an attempt has been made to study the occurrence of non-dermatophyte moulds (NDMs) as emerging opportunistic agents of dermatophytosis.

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MATERIALS AND METHODS

Collection of demographic details

The study group comprised of patients who are suspected of having dermatophytic infections and have not received any

prior treatment for their conditions presenting at the outpatient department of a skin care and cosmetic clinic, Krishnagiri over a period of nine months from a period of May 2012 to January 2013. The patient consent form was obtained and the study was approved by the institutional ethical committee for the enrolment of human subjects. A detailed clinical history of the patients was taken in relation to name, age, sex, address, occupation, duration of illness and involvement of more than one site. The clinical examination of the patient was made in good light which included site of lesion, number of lesions, types, presence of inflammatory margin, etc. A total of 163 samples of skin, hair, and nail clippings were collected from patients by following standard collection procedures.

Specimen Collection

The specimens were collected according to the method of Chaya and Pande, 2007.

Isolation of Fungi from Nails clippings

The affected area was cleaned with 70% ethyl alcohol and after drying, using a blunt sterile scalpel and nail clipper the skin scales, crusts and pieces of nail were collected in clean white paper packets. Nail specimen was collected by taking clippings of the infected part and scrapings beneath the nail.

Isolation of Fungi from Skin Scrapings

After cleansing the affected area with 70% ethanol, the skin specimen was collected by scraping across the inflamed margin of lesion into the apparently healthy tissue. The active border of the lesion was selected for the scrapings and were collected by moving the scalpel perpendicular to the skin surface and transported to mycological laboratory.

Isolation of Fungi from Hair clippings

The infected hairs were removed by plucking with the roots intact using epilating forceps along with the base of the hair shaft around the follicle. Scrapings were also collected from the scalp. All the specimens were collected in folded strips of sterile paper packed in clean, sterile zip-lock covers, labelled with the patient's number, name, and date of collection. The clinical specimen was then transported to the medical mycology laboratory for further processing. The clinical specimen was divided into two parts; one part was kept on the glass slide and a drop of KOH (10-20% for skin and hair while 40% KOH for nail samples) was added and covered with a cover slip by applying a gentle pressure. The slides were examined under low and high power objectives for the presence of fungal hyphae. The other part of the sample was inoculated on to Sabouraud Dextrose Agar (Hi-media, India) plates with chloramphenicol (50 mg/l), with and without cycloheximide (500 mg/l) and incubated at room temperature and examined regularly for the presence of fungal growth (Kannan *et al.,* 2006). The significant growth was interpreted if the direct microscopy was positive and the organism was isolated on more than one media. The growth of the fungi was examined macroscopically for colony morphology and microscopically by lactophenol cotton blue staining.

RESULTS

Out of 163 suspected cases superficial mycosis, 115 were male and 48 were female and the male-female ratio was found to be 2.39:1. The age group most affected was 15-30 years, with a mean of 22.5 years (Table 1). The culture positivity was found to be in 122 (74.8 %) cases. Among the culture positive cases, dermatophytes accounted for 73 (59.8%) cases, and non-dermatophytic moulds accounted for 49 (40.2%) cases, which included fungi mostly belonging to hyaline and dematecious hyphomycetes. The isolation rate of non dermatophytic moulds was found to be comparably high. Among the dermatophytes *Trichophyton rubrum* was the predominant followed by *Trichophyton tonsurans* and *Trichophyton mentagrophytes var interdigitale* (Table 3). Among the non dermatophytic (NDM) moulds, isolated in this study included *Aspergillus flavus*, *Exophiala dermatitis Cladosporium spp., Fusarium spp.* and *Penicillium marneffii* (Table 2).

Table 1. Age wise distribution of dermatophytosis in co-relation with gender

S.No	Age in Years	Male	Female	Total (%)
1	0-15	12	5	17
2	15-30	51	14	65
3	30-45	26	19	45
4	Above 45	26	10	36
	Total	115	48	163

Table 2. Pattern and frequency of Non-dermatophytic moulds (NDMs) isolated during this study (n=49)

S.No.	Non-Dermatopytic moulds (NDMs)	Number of isolates (%)	Percentage (%)
1	*Acremonium spp.*	2	4.08%
2	*Alternaria spp.*	5	10.20%
3	*Aspergillus flavus*	5	10.20%
4	*Aspergillus niger*	4	8.16%
5	*Aspergillus spp.*	4	8.16%
6	*Cladosporium spp.*	4	8.16%
7	*Cunninghamella spp.*	3	6.12%
8	*Exophiala dermatitis*	5	10.20%
9	*Fusarium spp.*	4	8.16%
10	*Malassezia furfur*	3	6.12%
11	*Penicillium spp.*	3	6.12%
12	*Penicillium marneffii*	4	8.16%
13	*Rhizopus spp*	3	6.12%
	Total	49	100%

Table 3. Pattern and frequency of dermatophytes isolated (n=73)

S.No	Dermatophytes	Number of isolates	Percentage (%)
1	*Trichophyton rubrum*	16	21.92
2	*Trichophyton tonsurans*	15	20.55
3	*Trichophyton mentagrophytes var interdigitale*	10	13.69
4	*Trichophyton species*	9	12.33
5	*Trichophyton mentagrophytes var mentagrophytes*	8	10.96
6	*Trichophyton verrucosum*	4	5.48
7	*Microsporum gypseum*	5	6.85
8	*Microsporum canis*	3	4.11
9	*Epidermophyton floccosum*	3	4.11
	Total	73	100

DISCUSSION

A better understanding of the fungal pathogenesis was only made after the high mortality and morbidity of the

dermatophytic infections throughout the world (Havlickova and Czaika, 2008). These fungal infections range from mild superficial to sub-cutaneous and systemic mycosis causing a great concern (Bakheshwain *et al.,* 2010). Over the past few decades there have been increased report of Non-dermatophytic moulds (NDM) as agents of skin and nail infections along with the dermatophytic mould in the isolation producing similar lesions as that of dermatophytic agents (Aggarwal *et al.,* 2002; Patel *et al.,* 2010). This present study, also reports the occurrence of such non-dermatophytes as the causal agents of dermatophytosis. In our study the isolation in pure culture of these non dermatophytic (NDM) was found in 40.2% cases which was more or less similar to the one observed by Sharma *et al.,* 2012 who reported the NDM in 36.66% cases. The Nondermatophytic moulds (NDM) reported in this study not only causes superficial infections but if untreated can develop in to serious and sometimes causes fatal mycosis (Warnok, 2006). Hence, these nondermatophyte moulds should always be kept in mind while investigating and treating the case of dermatophytosis and the common practice of discarding them as contaminant should be avoided.

Conclusion

Dermatophytes remain to be the predominant cause of dermatophytosis. But the role of these non dermatophytic mould should not be ignored and should also be given due importance. These non dermatophytic moulds (NDM) have to be taken in to consideration while deciding the therapy as if not treated properly may penetrate deeper into the tissues. Hence proper clinical management is very essential in the treatment of these non dermatophytic moulds. The clinician should plan the therapy of these cases as well as those cases ending up in treatment failure after empirical treatment for dermatophyte infections.

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