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ORIGINAL RESEARCH ARTICLE

A Clinical Survey of Tinea Suspected Patients for Dermatophytes and Related Fungal Agents

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ABSTRACT

In 17 visits on different dates, a total 77 tinea suspected patients were examined for the collection of samples in dry and humid weather conditions from March 2012 to September 2012. A clinical result showed that patients reporting at skin OPD were positive for six different tinea namely tinea barbae, tinea capitis, tinea corporis, tinea manum, tinea pedis and tinea unguium. Most dominant cases were that of tinea corporis (63.63%) followed by tinea pedis (15.58%). Skin, hair and nail samples of diseased patients were aseptically collected and transferred onto DTM (Hi Media) and incubated at 28^0C for 2-4 weeks. A total of 90 molds were isolated in which 78 isolates were of opportunistic non-dermatophytes and 12 isolates were of dermatophytes (17.91%). The predominant etiologic agents of dermatophyte in sampled cases were *Trichophyton mentagrophyte* (7.7%) followed by *Trichophyton rubrum* (3.3%), *Microsporum gypseum* and *Trichophyton tonsurans* (1.1%). Seventy eight isolates of opportunistic non-dermatophytes belonged to 13 genera, representing 24 species. The most frequent genera were *Aspergillus* followed by *Alternaria*. Tinea infection was higher in female (49.35%) than in male (44.15%) and children (6.49%). The results of this study also demonstrated a high prevalence of tinea corporis among the patients belonging from Lower Income Group (LIG) due to their unhygienic living conditions.

Key words: Dermatophytes, Tinea, Aspergillus.

INTRODUCTION

Dermatophytic infections have been considered to be a major public health problem in many parts of the world. They are transmitted by either direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in combs, hair brushes, clothing, furniture, public seats, caps, bed linens, towels, and hotel rugs and cause infections of the skin, hair and nails due to their ability to obtain nutrients from keratinized material. They colonize the keratin tissues and inflammation is caused by host response to metabolic by-products. They are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Acid proteinases, elastase, keratinases, and other proteinases reportedly act as virulence factors. Some of these infections are known as ringworm or tinea. The most common fungal disease infection is Tinea found common among the skin patients and caused by dermatophytes which are pathogenic in nature. Three fungal genera that commonly causes skin disease in animals and humans, represents anamorphic (asexual or imperfect fungi) forms of *Microsporum*, *Epidermophyton* and *Trichophyton*.

Several opportunistic fungi survive in soil on keratin substrate and can also cause skin disease [1, 2]. The opportunistic fungi associated with skin patients also cause skin diseases and are matter of concern to medical mycologist due to their pathogenic nature. Such molds are often termed “air borne fungi” or “laboratory contaminants”. They produce lesions that are similar to dermatophytes [3]. They synthesise a variety of keratinolytic enzymes which are capable of utilizing keratin in-vitro [4]. These fungi and their spores can infect sick and immunodefficient persons [5]. The patients, who receive excessive long term treatment with broad spectrum antibiotics, become susceptible to infection by opportunistic fungi.

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In the present study clinical cases of suspected tinea patients were examined during dry and humid weather periods known for outbreak of fungal infections and isolation of causative agents from their infected samples was conducted. This study also included information relating to economic group, living conditions and sex of the sampled patients.

MATERIALS AND METHODS

Collection of samples

A total of 77 suspected tinea patients were clinically diagnosed for sampling and skin, hair and nail samples with apparent infection were aseptically collected from these patients. The affected area was thoroughly sponged with 70% alcohol to remove surface contaminants and medication if any. Infected skin and nail from the border next to natural skin was scrapped gently with the help of sterilized blade and samples were collected on sterilized paper.

Infected hair was collected with the help of a pair of epilation forceps. The stumps of infected hair are usually small and epilation in such case is not at all painful.

Inoculation and Incubation

Samples were collected in sterilized paper envelope and brought to lab immediately. These samples were inoculated onto prepared plates of Dermatophytic Test Medium and Sabouraud Dextrose Agar (M063, Hi Media) amended with 0.5µg/ml of actidione (Hi Media) and were incubated at 28°C for two to four weeks. When growth was observed, prepared a permanent slide with PVA and studied under the microscope (Olympus) X10, X40 and in oil immersion. Microscopic examination of fungi under Olympus BX-40 was done and organisms were identified based on colony features and micro-morphological characteristics with the help of recent text.

Case history

The present study also included information relating to economic group, sex and living condition of the suspected patients examined during the course of sample collection. According to their economic group these were divided in to three categories: Lower Income Group (LIG), Middle Income Group (LIG) and Higher Income Group (LIG).

RESULTS

A total of 77 samples were taken from clinically diagnosed tinea patients in which 49 cases were of tinea corporis, 12 of tinea pedis, 06 cases were of tinea manum, 04 of tinea capitis, 03 cases each were of tinea barbae and tinea unguium (**Table 1 & Fig 1**). The maximum numbers of fungal strains were isolated from the samples of tinea corporis (65.67%) followed by tinea pedis (16.41%) while minimum isolation was observed in tinea barbae and tinea unguium (2.98%). Sixty samples were positive for opportunistic fungi and twelve for dermatophytes. A total of 90 molds were isolated in which 78 isolates were of opportunistic non-dermatophytes and 12 of dermatophytes.

Table 1: Differential fungal isolates in relation to sampling of tinea patients

Type of mycoses	Cases sampled	Culture positive (%)	Number of fungal isolates	
			Opportunistic non-dermatophytes	Dermatophytes
Tineacapitis	04	4.47	04	00
Tineacorporis	49	65.67	47	09
Tineabarbae	03	2.98	04	00
Tineamanum	06	7.46	06	00
Tineapedis	12	16.41	12	03
Tineaungium	03	2.98	05	00

The maximum number of tinea in suspected patient belonged to LIG (43) followed by MIG (30) whereas minimum number from HIG (04). As for locality of suspected patient 48% were from rural and 52% from urban settlements. 49% patients occupied mud houses and 52% lived in permanent houses (**Table 2**).

Table 2: Social classes of the patients examined

S. No	Social Class	Per capita family income (INR/month)	No. of patients from different class	Settlement		Living condition	
				Rural	Urban	Permanent house	Mud house
1	Lower Income Group (LIG)	3,000 - 6,000	43	37	06	05	38
2	Middle Income Group (MIG)	6,000 - 40,000	30	-	30	30	-
3	Higher Income Group (HIG)	Above 40,000	04	-	04	04	-

Four species of dermatophytes belonging to 2 genera were isolated from corporis and pedis region of the tinea patient. These were *Trichophyton rubrum* (MTCC 11418), *Trichophyton mentegrophyte* (MTCC 11417), *Trichophyton tonsurans*, *Microsporum gypseum*

(MTCC 11584). Among the seventy eight isolates of opportunistic fungi, 47 isolates were obtained from skin, 12 from foot, 06 from hand, 04 from scalp, 05 from nail and 04 from beard belonging to 13 genera and 24 species. *Aspergillus* species were most common among all samples (37). These were the only isolate in the samples of tinea barbae, tinea capitis and tinea unguium. *Alternaria* species was reported in nine cases from among tinea corporis, tinea manum and tinea pedis. *Cladosporium* species were reported in eight cases of tinea corporis, tinea pedis and *Curvularia* species were isolated from seven cases of tinea manum and tinea pedis. *Acremonium* was reported in four cases of tinea pedis while *Chaetomium* (3*), Mycelia sterilia* (2) and *Penicillium* (2) were isolated from samples of tinea corporis. In the samples of tinea corporis single species of *Bipolaris*, *Fusarium*, *Paecilomyces* were isolated while in Tinea pedis, one species each of *Nigrospora* and *Malbranchea* was reported. Tinea infection was higher in female (49.35%) than in male (44.15%) and children (6.49%). Amongst children (0-10), tinea capitis was common and tinea corporis was most common between the age group of 21-30 years. Females of rural area were highly suspected for tinea than male (**Table 3**).

Table 3: Age and sex related cases of tinea patients

S. No	Age group of patients	Sex			Type of infection*
		Male	Female	Children	
1	0-10 yr	-	-	03	Tinea capitis
2	11-20 yr	02	03	0	Tinea corporis
3	21-30 yr	19	24	-	Tinea corporis, tinea pedis, tinea barbae*
4	31-40 yr	06	07	-	Tinea corporis, tinea pedis, tinea manum*
5	41-50 yr	04	03	-	Tinea corporis, tinea pedis*
6	51-60 yr	02	01	-	Tinea corporis
7	61-70 yr	01	-	-	Tinea corporis

*In descending order

Amongst non-dermatophytic fungal isolates species spectrum revealed maximum number of *Aspergillus* (6) followed by *Alternaria* (4), *Trichophyton* (3), *Acremonium* (2), *Cladosporium* (2), *Curvularia* (2) and one each of *Bipolaris, Chaetomium, Fusarium, Malbranchea, Microsporum, Nigrospora sphaerica, Paecilomyces, Penicillum* and *Sterile mycelia* (**Table 4**). Most dominant infective isolates of dermatophytes were the species of *Trichophyton* followed by *Microsporum gypseum.* The maximum frequency of non-dermatophytes was in *Aspergillus niger* (23.3%) while minimum in *Alternaria dianthicola, Aspergillus janus, Bipolaris australiensis*, *Fusarium* sp. *Nigrospora sphaerica, Malbranchea* sp. and *Paecilomyces lilacinus* (1.1%).

Table 4: Per cent frequency of occurrence and relative rank of dermatophytes and non-dermatophytes in sampled patients

S. No	Fungal isolates	Frequency (%)	Relative rank*
1	*Acremonium acutatum*	2.2	7
2	*Acremonium hyalinum*	2.2	7
3	*Alternaria alternata*	4.4	5
4	*Alternaria brassicicola*	2.2	7
5	*Alternaria longipes*	2.2	7
6	*Alternaria dianthicola*	1.1	8
7	*Aspergillus flavus*	7.7	2
8	*Aspergillus fumigates*	2.2	7
9	*Aspergillus niger*	23.3	1
10	*Aspergillus repense*	2.2	7
11	*Aspergillus sydowii*	4.4	5
12	*Aspergillus janus*	1.1	8
13	*Bipolaris australiensis*	1.1	8
14	*Chaetomium globosum*	3.3	6
15	*Cladosporium cladosporioides*	6.6	3
16	*Cladosporium herbarum*	2.2	7
17	*Curvularia brachyspora*	2.2	7
18	*Curvularia lunata*	5.5	4
19	*Nigrospora sphaerica*	1.1	8
20	*Fusarium* sp.	2.2	7
21	*Malbranchea* sp.	1.1	8
22	*Microsporum* gypseum	1.1	8
23	*Pacelomyces lilacinus*	1.1	8
24	*Penicillum* sp.	2.2	7
25	*Trichophyton mentagrophyte*	7.7	2
26	*Trichophyton rubrum*	3.3	6

27	*Trichophyton tonsurans*	1.1	8
28	Sterile mycelia	2.2	7

*Based on per cent frequency of occurence

DISCUSSION

In the present study, clinical samples from 77 cases of tinea diagnosed patients were taken for isolation of fungal strains amongst which sixty seven samples (87.01%) were positive for dermatophytes and opportunistic non-dermatophytes. 18% cases showed positive result for dermatophytes while 82% for opportunistic non-dermatophytes. Falahti *et al.* [6] collected 1254 samples from clinically diagnosed tinea patients, out of which 169 (13.5%) samples were positive for dermatophytes. Selma *et al.* [7] did a survey among the students living in rural areas of Turkey and reported dermatophytic infection. Their study revealed occurrence of maximum cases of tinea corporis followed by tinea pedis. Sen and Rasul [8] studied 100 clinically suspected cases of tinea in Assam during a period of one year and found that tinea corporis was the most common. Tinea corporis has been most common type of skin infection in our study which is in conformity with previous observations [9-13].

With regard to isolation of dermatophytes, the most frequent dermatophyte was *Trichophyton mentagrophytes* (7.7%) followed by *Trichophyton rubrum* (3.3%), *Trichophyton tonsurans* (1.1%) and *Microsporum gypseum* (1.1%). Kannan *et al.* [14] reported that *T. rubrum* was the chief isolate from skin scales followed by *T. mentagrophytes.* These are the commonest agent isolated form glabrous skin of the body and the feet. Similar findings have been observed in the present study.

The literature revealed that the opportunistic fungi can also cause lesions similar to dermatopohytes. Species of *Acremonium* causes a keratitis in humans [15]. The genus *Aspergillus* contains a large number of pathogenic species and is the commonest contaminant [16]. The species *Aspergillus flavipes* cause cutaneous aspergillosis [17]. *Aspergillus janus* causes keratitis [18] while cutaneous infection has been reported by *Aspergillus niger*. A few species particularly *Aspergillus flavus, Aspergillus fumigatus* also cause skin lesions similar to dermatophytes. *Alternaria* species have been reported to cause cutaneous infections in immuno deficient patients. A superficial infection reported in an AIDS patient by *Alternaria infectoria* [19] whereas a subcutaneous infection has been recorded by *Alternaria dianthicola* [20]. *Bipolaris austaliensis* has been reported as a causative agent for subcutaneous and cutaneous infections [21, 22]. *Chaetomium globosum* caused cutaneous lesions and onychomycosis [23, 24]. The species of *Cladosporium* was causal organism of cutaneous infection and *Cladosporium cladosporioides* and *Cladosporium herbarum* were isolated from cutaneous infections [25-27]. The species of *Curvularia* were common saprophytes and occasionly caused skin infections in humans [28]. In some cases Keratitis was caused by *Curvularia brachyspora* [29]. *Fusarium* was reported in the patients of keratitis [30, 31]. A species of *Nigrospora spherica* cause skin lesions in a leukemic patient in India [32]. *Paecilomyces lilacinus* caused cutaneous infection [33] and species of *Penicillium* is reported to cause keratitis [34].

The non-dermatophyte fungal isolates cultured from infected samples viz. skin nail and scalp should not be, therefore ignored because these remain closely associated with skin infection and also cause almost identical lesions that are produced by true dermatophytes [35-38]. The study revealed that the patients from lower economic classes were more prone to fungal infection which appears to be connected with their unhygienic living conditions and ignorance about the disease. Such patients in our study were not able to afford to live in hygienic conditions largely due to their poverty. In addition their crowded living conditions provided good opportunity for the transmission of dermatophyte species [39-41].

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