ORIGINAL ARTICLE

Clinico Mycological Study of Superficial Mycoses

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Abstract:

Background: Generally it is well established fact that geographical distribution of the fungi may change from time to time; hence this study was planned. *Aim and Objectives:* To analyze the prevalence of superficial mycoses, its clinical presentation and species identification of the fungal isolates responsible for the disease. *Material and Methods:* A total 125 clinically diagnosed cases of superficial mycoses visiting Dermatology and Venereology outpatient department of Bharati Hospital, Sangli, for a period of one year were included in the study. Specimens like skin scrapping, nail clipping, hair were collected and subjected to KOH mount and culture. Identification of species was done by macroscopic examination of culture, tease mount and other physiological tests including Urease test, Hair perforation tests and Germ tube test. *Results:* Superficial mycosis was more common in the age group of 21-30 years (28%) and in males (60.8%). The infection was more common in students (29.6%). Tinea corporis (42.4%) was the commonest clinical type followed by tinea cruris (22.4%). 61.6% cases were positive by direct microscopy and 60.8% cases showed culture positive. Out of 125 samples, dermatophytes were grown in 63 cases (82.89%) followed by non dermatophytic moulds in 10 cases (13.16%) and *Candida albicans* in 3 cases (3.95%). The most common isolate among dermtophytosis was *T. rubrum* (46.05%) followed by *T. mentagrophyte* (25%). *Conclusion:* It was concluded that along with dermatophytes, non dermatophytic moulds are also important to cause of superficial mycoses.

Keywords: Dermatophytoses, Tinea corporis, *T. rubrum,* Non dermatophytic moulds.

Introduction:

Superficial mycosis refers to fungal infections of skin and its appendages hair and nail [1]. It is seen in a majority of the patients of all age groups attending dermatology clinic for various skin disorder. It has been estimated that superficial mycoses is seen in 20-25 % of the world's population [2]. It includes diseases like Pityriasis versicolor, Tinea nigra, Piedra, Dermatophytoses and onychomycoses [3]. These diseases are commonly seen in tropical and temperate zone because hot and humid climate is favorable for establishment of infection [4].

The causative agent varies according to the clinical types. *Tinea versicolor* is caused by yeast –*Malassezia furfur*, a normal inhabitant of skin. Dermatophytoses caused by Dermatophytes, Tinea nigra and Black piedra by Dematiaceous fungi, White piedra by Trichosporon, onychomycosis by *Candida species* and non dermatophytic moulds like Penicillium species and Aspergillus species. Dermatophytosis is often described as 'Tinea' [1, 3]. It is the first most common type of superficial mycoses therefore studied worldwide [5]. It is caused by heterogenous group of fungi known as Dermatophytes derived from animals Zoophilic,

from soil Geophilic and some which has adopted human host known as Anthropophilic [1]. Although the disease does not show much difference clinically the predominant type of dermatophyte species involved show geographical differences. Dermatophytosis is more commonly seen in males and in the age group 21-30 years as per earlier studies [6, 7].

Tinea versicolor is the second most common type of superficial mycoses commonly seen in adults. It is characterized by patchy discoloration of skin mainly on chest, neck and back. Onychomycoses is third type which resembles *Tinea unguim* [1]. Most of these infections are asymptomatic and patients seek medical advice for mild pruritis or cosmetic purpose. Laboratory diagnosis is important before initiation of antifungal therapy because some disease need prolonged antifungal therapy while some do not need any. Many antifungal agents are used to treat superficial mycosis. Sometime same species of dermatophytes may show variable susceptibility pattern. The present study was carried out to find out the prevalence of superficial mycoses, its clinical presentation and species identification of the fungal isolates responsible for the disease.

Material and Methods:

A total 125 clinically diagnosed cases of superficial mycosis visiting Dermatology and Venereology Outpatient Department of Bharati Hospital, Sangli from June 2014 to July 2015, were included in the study. A detailed clinical history including age, sex, site of infection, type of lesions, duration, occupation, antifungal treatment, similar complaints in the family members were recorded. Written consent was taken from the patient before collecting the sample and the study was conducted after approval of Institutional Ethics Committee.

Inclusion Criteria:

All patients visiting the Dermatology OPD showing lesions typical of superficial mycosis as diagnosed by the clinician were included in the study. Patients of all age group and both the sexes were included.

Exclusion Criteria:

Patients, who were already on antifungal therapy within 3 months prior to the commencement of the study, were excluded.

Sample Collection:

Clinical specimens like skin scrapings, nail clippings and infected hair along with follicle were collected in a sterile, small white paper envelop after cleaning the affected area with 70% ethanol [8].

Microscopic Examination:

Direct microscopic examination was undertaken in 10% potassium hydroxide (KOH) wet mount for the specimen of skin scrapping and hair plucking while 40% KOH was used for nail clipping for the presence of fungal hyphae, arthrospores and for type of hair invasion [1].

Isolation and Identification:

Clinical specimens including skin, nail and hair were inoculated on Sabouraud's dextrose agar with and without chloramphenicol (50mg/L) and cycloheximide (500mg/L). The cultures were incubated at 25^0C and 37^0C for 4 weeks. If any growth was obtained, identification was made based on colony characteristics, pigment

production; microscopic appearance in Lactophenol cotton blue (LPCB) teased mount, slide culture, urease test and hair perforation test. Yeasts were identified by standard methods [1, 8].

Results:

A total 125 clinically diagnosed cases of superficial mycosis were included in our study. Superficial mycosis was more common in the age group of 21-30 years (28%) followed by 31-40 years (20.8%). Male (60.8%) predominated the female patients (39.2%). Male to female ratio was 1.55:1. The infection was more common in students (29.6%) followed by farmer (28.8%), house wives (21.6%) and other (20%) which include business and professional workers. The most common clinical type observed was tinea corporis (42.4%) followed by tinea cruris (22.4%), onychomycosis (10.4%), and pityriasis versicolor (8.8). Tinea corporis and tinea cruris were predominantly seen in males (Fig. 1, 2) and onychomycosis was predominant in female patients. Pityriasis versicolor was the predominant superficial mycosis in adults. Mixed clinical types were also seen. (Table 1)

Out of 125 cases, 57 cases (45.6%) were positive by both microscopy and culture. Twenty cases (16%) were positive by direct microscopy only (Fig. 3) and 19 cases (15.2%) were culture positive and negative on direct microscopy. (Table 2)

The relation of etiological agents to clinical types is shown in (Table 3). The most common fungal isolate was the dermatophytes 63 (82.89%) followed by non dermatophytic moulds 10 (13.16%) and *Candida albicans* 3 (3.95%). Among dermatophytes, the most common isolate was *T. rubrum* (46.05%) (Fig. 4, 5) mainly isolated from tinea corporis, tinea cruris and tinea faciei. The second most common isolate was *T. mentagrophytes* (25%) which was most commonly isolated from tinea corporis and tinea cruris. *Candida albicans* was isolated from onychomycosis. Most of the Non Dermatophytic Moulds (NDM) was isolated from nail infections and in these patients, there was history of trauma.

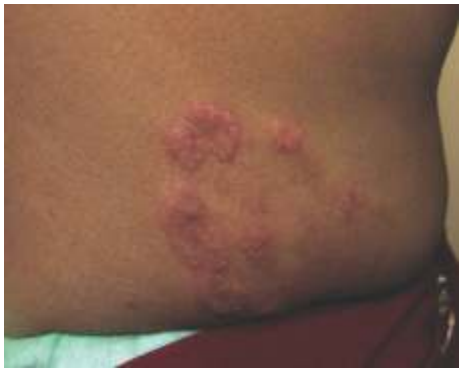

Fig. 1: *Tinea corporis*

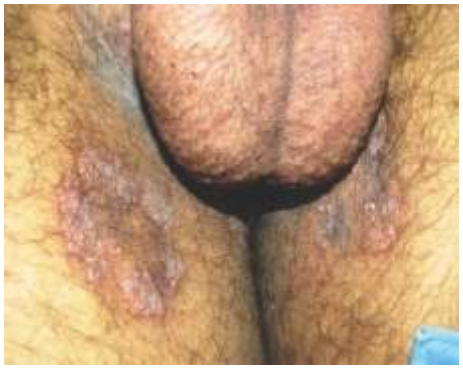

Fig. 2: *Tinea cruris*

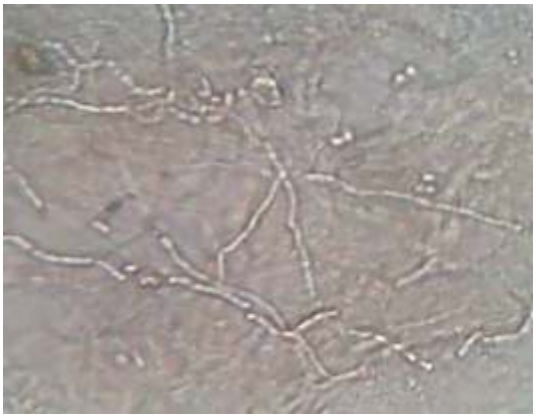

Fig. 3: Long, Branched, Septate, Refractile Fungal Hyphae seen in 10% KOH Mount (40x)

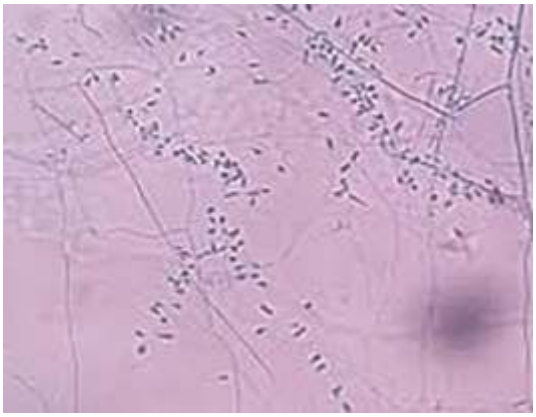

Fig. 5: LPCB Mount showing Microconidia Arranged in 'Birds-On-A-Fence' Appearance (40X)

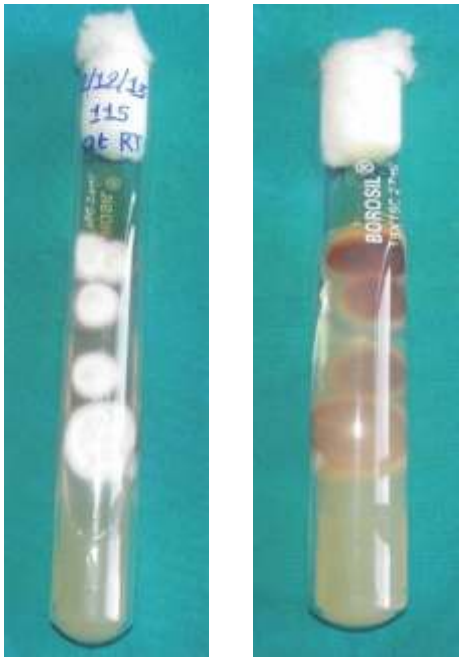

Fig. 4: *Trichophyton rubrum* Culture on SDA (Obverse and Reverse)

Table 1: Age and Sex Wise Distribution of Clinical Type of Superficial Mycoses in the Study Group

Clinical type	Age Group (years)									Sex		Total (%)
	≤ 10 (%)	11-20 (%)	21-30 (%)	31-40 (%)	41-50 (%)	51-60 (%)	61-70 (%)	71-80 (%)	81-90 (%)	Male (%)	Female (%)	
Tinea corporis	-	11 (20.75)	15 (28.3)	12 (22.64)	9 (16.98)	3 (5.66)	1 (1.89)	2 (3.77)	-	33 (62.26)	20 (37.74)	**53 (42.4)**
Tinea cruris	-	6 (21.43)	5 (17.86)	7 (25.0)	5 (17.86)	2 (7.14)	1 (3.57)	-	2 (7.14)	19 (67.86)	9 (32.14)	**28 (22.4)**
Tinea faciei	1 (14.29)	2 (28.57)	3 (42.85)	-	1 (14.29)	-	-	-	-	5 (71.43)	2 (28.57)	**7 (5.6)**
Tinea manuum	-	-	2 (66.66)	-	-	-	1 (33.33)	-	-	3 (100)	-	**3 (2.4)**
Tinea pedis	-	-	-	-	1 (100)	-	-	-	-	-	1 (100)	**1 (0.8)**
Tinea corporis with cruris	-	1 (14.29)	-	3 (2.85)	2 (28.57)	-	1 (14.29)	-	-	3 (42.86)	4 (57.14)	**7 (5.6)**
Tinea corporis with faciei	-	-	-	-	1 (100)	-	-	-	-	-	1 (100)	**1 (0.8)**
Tinea corporis with pedis and unigium	-	-	-	-	-	1 (100)	-	-	-	1 (100)	-	**1 (0.8)**
Onychomycosis	2 (15.38)	-	4 (30.77)	2 (15.38)	3 (23.08)	2 (15.38)	-	-	-	5 (38.46)	8 (61.54)	**13 (10.4)**
Pityriasis versicolor	-	2 (18.18)	6 (54.55)	2 (18.18)	-	1 (9.09)	-	-	-	7 (63.64)	4 (36.36)	**11 (8.8)**
Total	**3 (2.4)**	**22 (17.6)**	**35 (28.0)**	**26 (20.8)**	**22 (17.6)**	**9 (7.2)**	**4 (3.2)**	**2 (1.6)**	**2 (1.6)**	**76 (60.8)**	**49 (39.2)**	**125 (100)**

Table 2: Comparative Study of Direct Microscopy and Culture

Culture	KOH Positive	KOH Negative	Total (%)
Positive	57 (45.6%)	19 (15.2%)	76 (60.8%)
Negative	20 (16%)	29 (23.2%)	49 (39.2%)
Total (%)	77 (61.6%)	48 (38.4%)	125

Table 3: Distribution of Fungal Isolates in Relation to Clinical Type

Clinical type	Dermatophytes							NDM					
	T. rubrum	*T. mentagrophytes*	*E. floccosum*	*T. tonsurans*	*T. verrucosum*	*T. interdigitale*	*T. violaceum*	*A. niger*	*A. flavus*	*Penicillium species*	*Fusarium species*	*C.. albicans*	*Total No. of Isolates*
Tinea corporis	14 (43.76)	11 (34.38)	2 -6.26	1 (3.12)	1 (3.12)	1 (3.12)		1 (3.12)	-		1 (3.12)	-	**32 (42.1)**
Tinea cruris	13 (65)	5 (25)	1 (5)	-		-		-	-	1 (5)	-	-	**20 (26.31)**
Tinea faciei	3 (60)	-	-	-	1 (20)	-	1 (20)	-	-	-	-	-	**5 (6.57)**
Tinea manuum	1 (50)	1 (50)	-	-	-	-	-	-	-	-	-	-	**2 (2.63)**
Tinea pedis	1 (100)	-	-	-	-	-	-	-	-	-	-	-	**1 (1.31)**
Tinea corporis with cruris	2 (50)	2 (50)	-	-	-	-	-	-	-	-	-	-	**4 (5.26)**
Tinea corporis with faciei	-	-	-	1 (100)	-	-	-	-	-	-	-	-	**1 (1.31)**
Tinea corporis with pedis and unigium	1 (100)	-	-	-	-	-	-	-	-	-	-	-	**1 (1.31)**
Onychomycosis	-	-	-	-	-	-	-	4 (40)	1 (10)	1 (10)	1 (10)	3 (30)	**10 (13.15)**
Pityriasis versicolor	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	**35 (46.05)**	**19 (25.0)**	**3 (3.94)**	**2 (2.63)**	**2 (2.63)**	**1 (1.3)**	**1 (1.3)**	**5 (6.57)**	**1 (1.3)**	**2 (2.63)**	**2 (2.63)**	**3 (3.93)**	**76 (100)**

Discussion:

Superficial mycosis is very common in our country because hot and humid climate is favorable for establishment of infection. Identification of fungal agent responsible for the superficial mycosis is of importance not only for the epidemiology but also for therapy since long term treatment is required in some cases. Students and farmers were most commonly affected because this study was done in rural population; they are usually unaware of the disease, neglect

the initial lesions and do not take any treatment and so present with lesions at multiple sites.

In our study superficial mycosis was more common in the age group of 21-30 years (28%) and affecting males (60.8%) more commonly than females (39.2%). These results are comparable with other studies done by Sen *et al.* [6], Sharma *et al.* [7]. Whereas, Karmarkar *et al.* [9] reported that most common age group affected was 0 to 10 years (28.4%) and Madhuri *et al.* [10] reported that females (51.96%) were more commonly affected than males (48.04%). The higher incidence in young males could be due to greater physical outdoor activity with increased sweating.

Tinea corporis was the commonest clinical type encountered (42.4%) in adult males followed by tinea cruris (22.4%), tinea faciei (5.6%) which is comparable with Patel *et al.* [5], Patwardhan *et al.* [11] and Balakumar *et al.* [12]. It was more common in males because excessive physical activity in males, less aeration due to tight clothing and high rate of sweating in groin region make this site more vulnerable to dermatophyte. Tinea pedis and onychomycosis were more common in females which are comparable with other studies done by Madhuri *et al.* [10], Patwardhan *et al.* [11]. Pityriasis versicolor was encountered in (8.8%) cases, most commonly affected in the young adults 21-30 years (54.55%) because lipophilic nature is requirements of growth of *Malassezia species*, the yeasts are rarely found on the skin of children and older individuals but are usually present during years when sebum production is highest. These findings are comparable with other studies done by Kannan *et al.* [13], Bhavsar *et al.* [14], and Das *et al.* [15].

On comparison with KOH and culture findings, KOH it showed that sensitivity was (75%) and specificity was (59%). In our study the KOH preparation has shown good sensitivity in comparison with culture. These findings are comparable with other studies done by Karmakar *et al.* [9], Bindu *et al.* [16], Singh *et al.* [17] was reported KOH sensitivity. Twenty cases (16%) were positive by microscopy and negative by culture. This variation could be due to non-viability of fungal elements in some cases and inadequacy in sampling due to very small lesions. Nineteen (15.2%) were negative by microscopy but culture positive. These variations are due to the inadequate material as well as misdiagnosed due to presence of short fungal elements.

T. rubrum 35 (46.05%) was the commonest fungal isolates in the majority of clinical types followed by *T. mentagrophytes* 19 (25%), *E. flocossum* 3 (3.94%) cases. This was comparable to other studies done by Sen *et al.* [7], Patwardhan *et al.* [11] and Ranganathan *et al.* [18] *T. tonsurans* accounted 2 (2.63%) in our study. The incidence of *T. verrucosum* was 2 (2.63%) and it was isolated from tinea corporis and tinea faciei each one cases. The high incidences of *T. verrucosum* have been reported by Sahai *et al.* [19], Mathur *et al.* [20]. *T. interdigitale* was isolated only in 1 (1.3%) cases. There was no evidence of *T. interdigitale* infection found in other studies. In our study, most of the non dermatophytes 7 (70%) and *Candida albicans* 3 (30%) were isolated from onychomycosis. NDM included, *Aspergillus niger* 4 (40%), *Aspergillus flavus, Penicillium and Fusarium* species 1 (10% each). These finding is comparable with Madhuri *et al.* [10]. NDM were considered significant only if they were isolated repeatedly in pure culture and with a positive KOH finding.

Conclusion:

The superficial mycosis is caused by not only dermatophytes but also non-dermatophytic moulds. Thus, species identification is very important to initiates prompt and appropriate antifungal therapy. It is essential that good laboratory methods are available for rapid and precise identification of the dermatophytes

involved, in order to apply appropriate treatment and preventive measure.

The changing profiles of human dermatophytoses among countries have further necessitated the development of improved diagnostic methods for identification of dermatophytes. Thus molecular methods should be considered in the detection of dermatophytes for rapid diagnosis and prompt initiation of treatment.

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