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A Study of Fungal Isolates from Superficial Mycoses Cases Attending IIMS & R, Lucknow

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ABSTRACT– This study was an attempt to estimate the prevalence of fungal isolates in superficial mycoses cases attending IPD and OPD of IIMS&R, Lucknow, Uttar Pradesh. A prospective study over a period of six (6) months was conducted from January 2015 to June 2015. The suspected cases of superficial mycoses were subjected to mycological examination with direct microscopy using 10%-40% KOH depending on the types of samples (skin, nail, hair) processed and culture on Sabouraud's dextrose agar with chloramphenicol and cycloheximide (SDCCA) and also on Potato dextrose agar (PDA) medium. Causative agents were identified as macroscopically and microscopically from the growth obtained on SDCCA and PDA. Direct microscopy revealed fungal elements in 78 (66.1%) cases whereas 54 (45.7%) were positive on culture. Out of 54 (45.7%) culture positive samples 6 (15%) were negative on microscopy (KOH mount). Tineacorporis 38 (32.2%) was the most common clinical types and male is to female ratio in relation to clinical types was 2.2:1. The commonest age group affected was 21–30 years with 41 (34.7%) cases. Males were predominantly affected by 41 (75.9%) and male to female ratio being 3.1:1. 60% of the patients came from rural backgrounds. College students formed a major chunk of the cases 29 (24.6%) followed by housewives 18 (15.3%) and unskilled workers 16 (13.6%). *Trichophyton mentagrophytes* 20 (37%) was the predominat isolate followed by *T. tonsurans* 15 (27.7%), *T. rubrum* 3 (5.5%), *M. audouinii* 3 (5.5%) and *T. schoenleinii* 2 (3.7%) with no Epidermophyton species. A non-pigmented variant of *T. rubrum* was identified in this study. Both SDCCA and PDA were found equally effective in isolating fungal isolates from clinical samples in our study. We were reported the change in frequency of dermatophytes isolated from superficial mycoses cases in our region.

Key-Ward: Superficial mycoses, Non-pigmented variants, Dermatophytes

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INTRODUCTION

Superficial mycoses refer to the disease of the skin and its appendages caused by the fungi. This group includes dermatophytosis, pityriasis versicolor, tinea nigra, white piedra, black piedra and candidiasis [1]. These fungi have the capability to produce keratinase, which allow them to metabolize and live on human keratin like skin, hair and nail [2]. Dermatophyte infections are one of the earliest known infections of mankind and are very common throughout the world [3]. Although dermatophytosis does not produce mortality, it does cause morbidity and poses a major public health problem, especially in tropical countries like India due to the hot and humid climate [3]. Infection of skin or nail can also be of non dermatophytic fungi and yeast-like fungi. Over the last decades, an increasing number of non dermatophytes filamentous fungi have been recognized as agents of skin and nail infections in humans, producing lesions clinically similar to those caused by dermatophytes [4]. The causative fungi colonize only the cornified layer of the epidermis or supra-follicular portions of hair and do not penetrate into deeper anatomical sites.

MATERIALS AND METHODS

A prospective study over a period of six (6) months from January 2015 to June 2015 was conducted at Integral Institute of Medical Sciences & Research, Lucknow, Uttar Pradesh. The study population comprised of 118 clinically suspected cases of superficial mycoses attending Dermatology outpatients department at Integral Institute of Medical Sciences And Research, Lucknow. All the clinically suspected cases of superficial mycoses referred to the Department of Microbiology for isolation and identification of etiological agent were included in the study.Demographic details of every case and detailed history of onset of disease, duration of symptoms, trauma, occupation, drugs, associated co-morbid conditions, family and personal

history was taken. Enquiries were also made as to exposure to animals, cases or any other suspected sources.

Specimen collection and Procedure- The affected areas were swabbed with 70% alcohol. Skin scrapings or nail clippings or plucked hair was collected in clean white paper packets.

Skin scraping- Skin scrapings were collected by scraping across the inflammatory margin of the lesion including the healthy skin using sterile scalpel or clean slide. If vesicles are present, the top was removed with fine scissors and stored for further examination.

Nail scraping- Nail specimen was collected by taking the infected nail clippings and was scraped deeply enough to obtain the recently invaded nail tissue. In cases of paronychia i.e. where a yeast infection is suspected, exudate was expressed from the paronychial folds by probing with a flat excavator and collecting on a swab previously moistened with sterile saline.

Hair plucking- Hair specimen was collected by plucking the infected hair including the base of the hair shaft. The species most frequently associated with scalp ringworm cause the affected hairs to flouresce under a wood's lamp and this is a useful means of selecting material.

Direct microscopic examination

KOH mount: 10% KOH solution was used for skin and hair samples. 40% KOH for nail specimens and incubated overnight at 37^0C for clearing.Clearing can be hastened by gently heating. As soon as the specimen has cleared, examined under microscope using the 10x and 40x objectives, for the presence of filamentous, septate, branching hyphae with or without arthrospores. In case of hair, type and arrangement of spores were noted (ectothrix/endothrix). *Tinea versicolor* infections were diagnosed by the presence of round yeast cells with short, stout and curved hyphae (spaghetti and meat ball appearance).

Gram stain: Gram staining was done when growth of yeast like fungi is suspected.

Fungal culture- All the samples were collected and inoculated on two sets of test tubes containing Sabouraud's dextrose agar with chloramphenicol and cycloheximide and Potato dextrose agar. For *Tineaversicolor* infections SDA with sterile olive oil overlay was used. The fungal cultures have been identified by colony morphology, rate of growth and pigment production. Lactophenol cotton blue mount was done from the small bit of colony taken on clean glass slide and teased out using two teasing needles, to detect the presence of macroconidia, microconidia, chlamydospore and special hyphal structures. Confirmatory identification of the species was done by slide culture technique and Biochemical tests i.e. urease test, hair perforation test and rice grain test. Speciation of yeast like fungi was done by gram's stain, germ tube test, sugar fermentation and assimilation tests [10-14].

RESULTS

A total of 118 patients were enrolled in the study, comprising 82 males (69%) and 36 females (31%). None of them had any systemic diseases. *Tinea corporis* 38 (32.2%) was the most common clinical type seen (Table 1) and the male to female ratio in relation to clinical types was found to be 2.2:1 which was significant (p=0.00) (Table 2). And the predominant age group affected was 21-30 years 41 (34.7%) and say that males were affected more than female. 81(68.6%) of patients were literate, 74 (62.7%) cases belonged to low socio-economic status and 60% of cases were from rural area. College students formed a major chunk of the cases 29 (24.6%) followed by housewives 18 (15.3%) and unskilled workers 16 (13.6%).

Table 1: Clinical types in total samples of superficial mycoses

Clinical Types	Total n (%)
Tinea corporis	38 (32.2)
Tinea pedis	17 (14.4)
Tinea manuum	17 (14.4)
Tinea cruris	15 (12.7)
Tinea unguium	8 (6.8)
Tinea faceii	8 (6.8)
Tinea capitis	5 (4.2)
Pityriasis versicolor	5 (4.2)
Tinea barbae	4 (3.4)
Bulbous tineapedis	1 (0.8)
Total	118 (100.0)

Table 2: Sex distribution of clinical types of superficial mycoses Cases

Clinical types	Total n (%)	Male n (%)	Female n (%)	M:F ratio	P -value
Tinea corporis	38 (32.2)	26 (31.7)	12 (33.3)	2.1:1	< .05
Tinea pedis	17 (14.4)	9 (11.0)	8 (22.2)	1.1:1	0.808*
Tinea manuum	17 (14.4)	11 (13.4)	6 (16.7)	1.8:1	0.225*
Tinea cruris	15 (12.7)	13 (15.9)	2 (5.6)	6.5:1	N.A.
Tinea unguium	8 (6.7)	6 (7.3)	2 (5.6)	3:1	N.A.
Tinea faceii	8 (6.7)	5 (6.1)	3 (8.3)	1.6:1	N.A.
Tinea capitis	5 (4.2)	4 (4.9)	1 (2.8)	4:1	N.A.
Pityriasis versicolor	5 (4.2)	4 (4.9)	1 (2.8)	4:1	N.A.
Tinea barbae	4 (3.3)	4 (4.9)	0 (0.0)	4:0	N.A.
Bulbous tineapedis	1 (0.8)	0 (0.0)	1 (2.8)	NA	N.A.
Total n (%)	118 (100)	82 (69.5)	36 (30.5)	2.2:1	< .05

N.A- not applicable due to low sample size, (p≤0.05=significant), (*=insignificant)

Out of total 118 clinical samples processed, found 78 (66.1%) were positive by direct microscopy (KOH mount) and 54 (45.7%) were culture positive. Whereas, 30 (38.4%) samples were found to be KOH positive but culture negative, while 6 (15%) were KOH negative samples were culture positive. 34 (85%) samples were negative by both KOH and culture (table 3). Culture negative samples were excluded while calculating the prevalence of fungal isolates.

Table 3: Comparative results of KOH & culture

KOH Results n (%)	Culture	
	Positive n (%)	Negativen (%)
Positive	78 (66.1)	48 (61.5)	30 (38.4)
Negative	40 (33.9)	6 (15.0)	34 (85.0)
Total	118 (100)	54 (45.7)	64 (54.2)

Out of total 54 culture positive cases 41 (76%) were male patients and 13 (24%) were female patients, male to female ratio was 3.1:1, which was significant (p=0.00) (Table 4).

Among 54 (45.7%) culture positive isolates *T. mentagrophytes* 20 (37%) was the commonest isolate followed by *T. tonsurans*15 (27.7%), *T. rubrum* 3 (5.5%), *M. audouinii* 3 (5.5%), *T. schoenleinii* 2 (3.7%), *Aspergillus terreus* 2 (3.7%), *Alternaria species* 2 (3.7%), *Fusarium species* 2 (3.7%), *Candida albicans* 2 (3.7%), *Candida non-albicans* 1 (1.8%), *Scytalidium species* 1 (1.85%) and *Mucor species* 1 (1.8%) (Fig. 1).

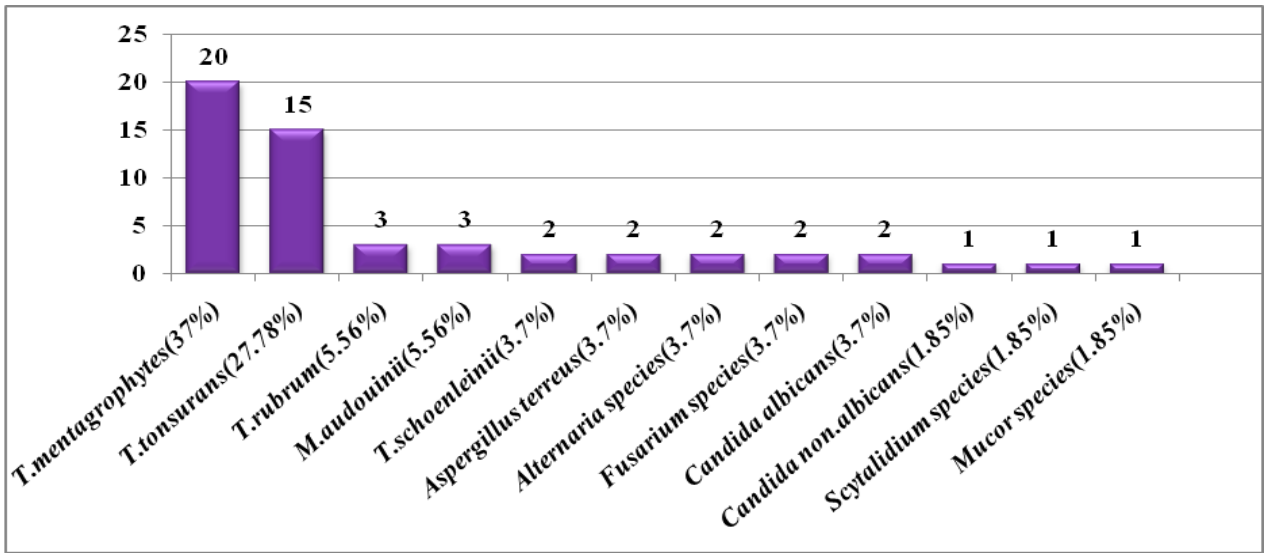

Fig. 1: Bar diagram showing frequency of fungal isolates from culture positive samples

Table 4: Distribution of fungal isolates in relation to sex

Fungal isolates	n (%)	Male n (%)	Female n (%)	P value
T. mentagrophytes	20(37)	16 (39)	4 (30.8)	< .05
T. tonsurans	15 (27.8)	13 (31.7)	2 (15.4)	N.A
T. rubrum	3 (5.6)	3 (7.3)	0 (0.0)	N.A
T. schoenleinii	2 (3.7)	1 (2.4)	1 (7.7)	N.A
M. audouinii	3 (5.6)	1 (2.4)	2 (15.4)	N.A
Aspergillus terreus	2 (3.7)	2 (4.9)	0 (0.0)	N.A
Alternaria species	2 (3.7)	1 (2.4)	1 (7.7)	N.A
Fusarium species	2 (3.7)	2 (4.9)	0 (0.0)	N.A
Candida albicans	2 (3.7)	1 (2.4)	1 (7.7)	N.A
Candida non-albicans	1 (1.9)	0 (0.0	1 (7.7)	N.A
Scytalidium species	1 (1.9)	0 (0.0)	1 (7.7)	N.A
Mucor species	1 (1.9)	1(2.4)	0 (0.0)	N.A
Total n (%)	54 (100)	41 (100)	13 (100)	< .05

N.A-non applicable due to low sample size, p≤0.05=significant

DISCUSSION

Out of total 118 cases of superficial mycoses, 78 (66.1%) were positive in direct microscopic examination (KOH). 54 (45.7%) were culture positive. Similar findings were reported in other studies also, which is mentioned in (Table 5).

We found an isolation rate of 48 (61.5%) among the samples which showed positive KOH mount. Out of 54 (45.7%) culture positive samples 6 (15%) were negative on microscopy (KOH mount). Similar findings were reported in other studies which are mentioned in (Table 6).

Table 5: Comparison of KOH positivity between present and other studies

	Present study	Madhulika *et al.* [15] (West Bengal)	Vyas *et al.* [16] (North India)	Singh *and Beena*[17] (Baroda)	Bindu *et al.*[18] (Calicut)
KOH positive	66.1%	75.7%	62.5%	60.3%	64%
KOH negative	33.9%	24.3%	37.5%	39.7%	36%

Table 6: Comparison of culture positivity between present and other studies

	Present study	Madhulika*et al.*[15] (West Bengal)	Bhatia and Sharma [19] (Shimla)	Vyas *et al.* [16] (North India)	Singh and Beena [17] (Baroda)
Culture positive	45.7%	46.28%	36.6%	37.5%	44.62%

In the present study,*Trichophyton mentagrophytes* was the predominate isolate followed by *T. tonsurans, T. rubrum, M. audouinii*and *T. schoenleinii*with the frequency of 37%, 27.7%, 5.5%, 5.5% and 3.7% respectively. However, we did not observe any involvement of *Epidermophyton species* in the study.

Majority of the studies have reported *T. rubrum* as main dermatophytic isolate from superficial mycoses cases in India [17,20-24]. However, we have found *T. mentagrophytes* as predominant species followed by *T. tonsurans* and *T. rubrum.* Our findings are similar to recent studies done by others [19, 25,26], and hints towards change in frequency of dermatophytes in our region.

Table 7: Showing fungal isolates of present and other studies performed in India

Isolate	Present study	Agarwal *et al.* [25] (North-west India)	Bhatia & Sharma [19] (Himachal Pradesh)	Sahai & Mishra [26] (Central India)
T. mentagrophytes	37%	37.9%	63.5%	25%
T. tonsurans	27.7%	8.3%	–	20%
T. rubrum	5.5%	34.2%	35.1%	5%
T. schoenleinii	3.7%	–	–	7%
M. audouinii	5.5%	–	–	5%

CONCLUSION

Our study has given us insights into the clinic-mycological aspects of superficial mycoses in our region. The study reveals that skin infections are more common than the hair and nail infections in dermatophytoses cases. Common clinical types are *T. corporis, T. pedis, T. manuum* and *T. cruris*. Unhygienic conditions among low-socioeconomic group, frequent migration of labourers, workers to this region may be some of the contributing epidemiological factors.Dermatomycoses was seen in 94.5% and superficial candidiasis in 5.5% cases. *T. mentagrophytes* was implicated asthe predominating species followed by *T. tonsurans, T. rubrum, M. audouinii, T. schoenleinii, Aspergillus terreus, Alternaria species, Fusarium species, Candida albicans, Candida non-albican, Scytalidium species and Mucor species.* Isolation ofnon pigmented variants of *T. rubrum* confirms our findings of change in distribution of dermatophytes in our region. India is a growing economy and during last couple of decades interstate migration of population and increase in national and foreign tourism might be an important reason for change in frequency of dermatophytes and uncommon fungal isolates in clinical practice. We are reporting a change in frequency of dermatophytosis isolated from superficial mycoses cases in our region. However, the present study was a small study that focuses primarily on the prevalence of different dermatophytes species in Northern Lucknow and a systematic study covering larger population and over a longer period of time would give a better insights into the epidemiology of dermatophytes in Lucknow and neighboring region.

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