

Clinical and mycological analysis on the occurrence of superficial mycosis with special emphasis on dermatophytes

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Abstract: *Background:* Superficial mycosis is prevalent in tropical and subtropical countries including India where heat and humidity play an important role in promoting growth of these fungi. *Objectives:* To identify superficial mycotic infections and correlate clinical types with dermatophytes. *Material and methods:* A total of 112 samples were collected from patients clinically diagnosed to have superficial mycotic infections of hair, nail and skin, after recording demographic details. Direct microscopy using 10%KOH was performed for the presence of branched hyaline hyphae and the samples were cultured on Sabourauds dextrose agar for species identification. *Results:* Among the various clinical types, Onychomycosis was the common clinical type (41.1%) in age group of 20-40years and it was seen predominantly in men. Out of total 112 samples, about 38 subjects were culture positive with dermatophytes strains. Among the isolates, *Trichophytonmentagrophyte* was the mostcommon (16.96%), followed by *T.rubrum* (8.03%). Superficial mycotic infection and dermatophyte infection was seen to be more for males as compared to females ($P=0.00049$). *Conclusion:* Onychomycosis was the common clinical type. By comparing the strains of the clinical types we can classify the disease according to its severity and occurrence, which will further help in designing effective treatment strategies. Since fungal culture takes time, direct microscopy using KOH for fungal elements helps in starting empirical treatment. Appropriate management for fungal infection should be done by adapting healthy habits like not sharing the daily utilities and cleaning body on regular basis.

Keywords: Superficial Mycosis, Dermatophytes, Onychomycosis, T. Mentagrophytes, Tinea.

Introduction

Superficial fungal infection affects millions of individuals, worldwide [1-2]. Though the infection does not have significant health outcomes, cosmetic appearance is affected. Superficial mycotic infections include dermatophytosis, pityriasisversicolor, and onychomycosis. The most common infection among the three is dermatophytosis; the causative-dermatophytes include 3 genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*. The infection is commonly designated as tinea or ringworm. The clinical manifestation of these infections is affected by several factors, viz. the site of infection, host's immune response and the mycotic agent [3].

Superficial mycosis is more common in India where heat and humidity play an important role in accelerating the growth of these fungi [3]. This

is mostly seen in males as compared to females, which may be due to their use of closed footwear, exposure to public baths and sporting activities. It can also be seen that youths are more affected than the other groups due to carelessness and untidy habits like sharing of personal utilities and lack of cleanliness. The nature of these mycotic infections may vary with passage of time, living conditions and adoption of preventive measures in the society [4].

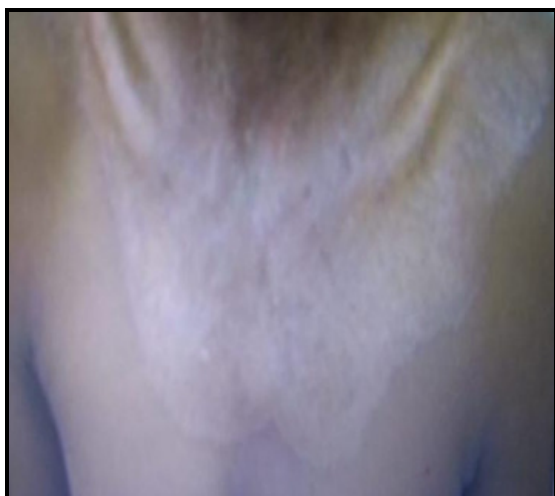
Susceptibility to chronic and persistent superficial mycotic infection, particularly dermatophytosis has been associated with a number of underlying conditions such as diabetes mellitus, immune-compromised states, and defective cell mediated immunity responses like collagen vascular diseases, that alter the clinical appearance and the course of infection [5-7].

Many serological tests have been carried out for diagnosis of fungal infections; alternative diagnostic tests are required to rule out false positive results produced by one test. KOH test is one such diagnostic tool used to differentiate dermatophytes and *Candida albicans* symptoms from other skin disorders like psoriasis and eczema [8]. This study was undertaken to identify superficial mycotic infections and to identify dermatophyte.

Material and Methods

This one-year (March 2018-February 2019), clinical study was performed in the Department of Microbiology of a tertiary care hospital in Karnataka after approval from Institutional Ethics Committee and written consent of patients. Patients clinically diagnosed with superficial mycosis, for over a period of a year, by observing lesions or piedra (Figure 1.a), were included in the study. Patients unwilling to take part in the investigation, those with infections for more than a year, and those already treated for the same were excluded from the study.

Fig-1(a): Tinea corporis of the upper part of the body over the chest showing hypopigmented patches



Specimen collection: After collecting demographic details such as age and gender from the patients, the skin scrapings and the hair samples, with respect to affected area was collected for examination. In case of nail infection, the affected part of the nail was cleaned with 70% ethyl alcohol for 1-2 mins. With the help of sterile scalpel blade, scrapings were collected from discoloured, dystrophic or brittle parts of the nail in sterile glossy paper packets.

Scalp scrapings were also collected from affected patients for further examination.

Sample analysis: Samples were examined by direct microscopy after preparation of the respective KOH mounts. Incubation time with KOH was dependent on the thickness of the sample, along with examination at 5-min intervals. Each slide was thoroughly examined under 40X magnification for the presence of fungal elements (filamentous, branched hyaline hyphae, with or without arthrospore crossing the margins of the squamous epithelial cells) [Figure 1.b].

Fig-1(b): Direct microscopy of the sample using 10% KOH wet mount preparation showing branched hyaline hyphae which indicates fungal elements

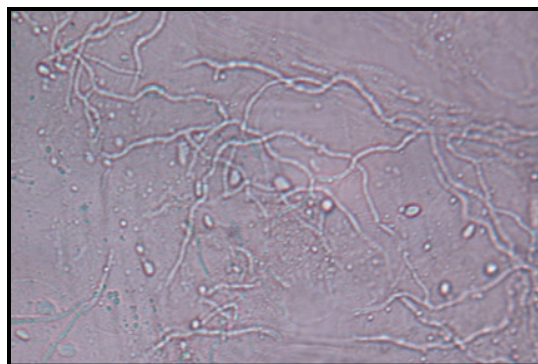
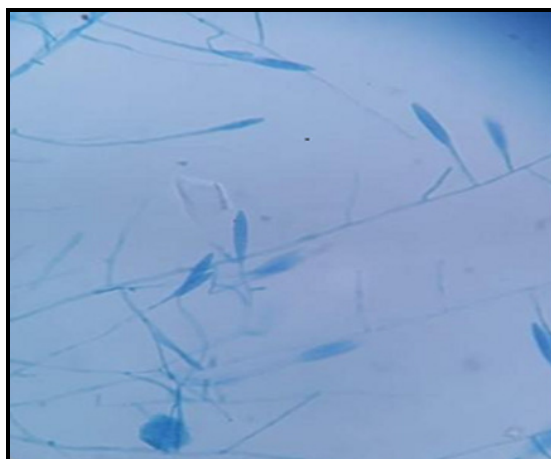


Fig 1(c): SDA slant of *Microsporium gypseum* showing cinnamon colored colonies



Fig 1(d): Lactophenol cotton blue mount of *Microsporumgypseum* under microscope showing rough walled spindle shaped macroconidia



The fungal cultures isolated from the clinical specimens were further processed by inoculating them onto Sabouraud’s dextrose agar (SDA) supplemented with cycloheximide (0.50 g) and chloramphenicol (0.04 g) and incubated at room temperature for a period of four weeks. It was considered negative for fungal growth if no growth was found after four weeks. Growth obtained on SDA was identified based on colony morphology and microscopic appearance using lactophenol cotton blue(LPCB)staining (Figure 1.c and d).

Statistical analysis: The data was recorded in Microsoft Excel and analysed using R version 3.6.0. Demographical details along with distribution of clinical samples and clinical types of infections, specifically dermatophytes, and the comparisons between these factors was represented in the form of frequency table. Chi-square test was used to determine the dependence between clinical presentation and factors such as age, gender, and dermatophytes extracted from the clinical samples, and to determine dependency between age, gender, and superficial mycotic infections. In addition, the strength of the association between superficial mycotic infections and factors such as age and gender were evaluated by calculating odds ratio.

Results

A total of 112 patients were included in the investigation after clearing inclusion criteria. Male predominance (70.54%) was observed among the patients and the patients were majorly

from age group of 21-44 years (39.29). Majority of the clinical samples were of skin scrapings (64%), whereas onychomycosis (41.1%) was the predominant clinical type of superficial infection observed. Of the dermatophyte species identified, prevalence of *Trichophytonmentagrophytes* (44.74%) was more followed by *T. rubrum* (28.95%) [Table-1].

Table-1: Distribution of age, gender, clinical types and dermatophytes

Variable	Sub-category	Number of subjects (%)
Age (in years)	≤ 20	20 (17.86)
	21-40	44 (39.29)
	41-60	32 (28.57)
	61-80	16 (14.29)
Gender	Male	79 (70.54)
	Female	33 (29.46)
Clinical sample	Nail	47 (41.96)
	Skin	64 (47.14)
	Hair	1 (0.89)
Clinical types of mycotic infections	Onychomycosis	46(41.1)
	T.corporis	20 (17.9)
	T.corporis + T.cruis	32 (28.6)
	T.corporis + T.fasciae	1 (0.9)
	T.incognito	3 (2.7)
	T.cruis	6 (5.4)
	Pityriasisversicolor	1 (0.9)
	T mannum	1 (0.9)
	OT capitis	2 (1.8)
Dermatophyte species	<i>Microsporum Gypseum</i>	1 (2.63)
	<i>Trichophytonmentagrop hytes</i>	17 (44.74)
	<i>Trichophytonrubrum</i>	11 (28.95)
	<i>Trichophytonterrestre</i>	2 (5.26)
	<i>Trichophyton tonsurans</i>	7 (18.42)

With respect to distribution of clinical types with age, all age groups demonstrated clinical symptoms of onychomycosis. However, the factors i.e., clinical presentation and age was statistically not significant (P = 0.9215; Table 2). On the other hand, gender and clinical presentation was found to be dependent and

significant ($P < 0.0001$), with onychomycosis being predominant presentation in females (54.55%); in males, onychomycosis (35.44%) and *T.corporis* and *T.cruis* (31.65%) were common (Table 3). Also, the odds of the patients being affected by superficial mycotic infection was 3.063 (CI: 1.1360, 8.2629) times more for males than females. Based on the culture identification,

higher number of clinical types was positively identified by KOH method (59.82%; Table 4). Interestingly, there was no growth observed for 36.61% cultures on SDA which in turn were identified to be positively identified by KOH method (Table 5). However, there was no statistical significance in between SDA and 10% KOH ($P=0.306$) methods.

Table-2: Distribution of clinical types with respect to age

Clinical presentation	Age distribution				p-value‡
	Less than 20	21-40	41-60	61-80	
Onychomycosis	11 (55.00%)	16 (36.36%)	13 (40.63%)	6 (37.50%)	0.9215
Pityriasisversicolor	0	0	1 (3.13%)	0	
T corporis	5 (25.00%)	6 (13.64%)	6 (18.75%)	3 (18.75%)	
T corporis + T Cruris	3 (15.00%)	13 (29.55%)	11 (34.38%)	5 (31.25%)	
T corporis + T fasciae	0	1 (2.27%)	0	0	
T Capitis	0	2 (4.55%)	0	0	
T Cruris	1 (5.00%)	4 (9.09%)	0	1 (6.25%)	
T Incognito	0	1 (2.27%)	1 (3.13%)	1 (6.25%)	
T Mannum	0	1 (2.27%)	0	0	

‡p-value obtained by Monte-Carlo simulation

Table-3: Distribution of clinical types with respect to gender

Clinical presentation	Gender		P-value
	Female	Male	
Onychomycosis	18 (54.55%)	28 (35.44%)	0.0004998*
Pityriasisversicolor	1 (3.03%)	0.00%	
T corporis	5 (15.15%)	15 (18.99%)	
T corporis + T Cruris	7 (21.21%)	25 (31.65%)	
T corporis + T fasciae	0.00%	1 (1.27%)	
T Capitis	1 (3.03%)	1 (1.27%)	
T Cruris	1 (3.03%)	5 (6.33%)	
T Incognito	0.00%	3 (3.80%)	
T Mannum	0.00%	1 (1.27%)	

* p-value obtained by Monte-Carlo simulation.

Table-4: Distribution of clinical types with respect to medium

Clinical presentation	KOH Positive	Culture Positive
Onychomycosis (46)	22 (47.83%)	12 (26.09%)
Pityriasisversicolor (1)	1 (100.00%)	0
T corporis (20)	14 (70.00%)	8 (40.00%)
T corporis + T Cruris (32)	20 (62.50%)	12 (37.50%)
T corporis + T fasciae (1)	1 (100.00%)	0
T Capitis (2)	2 (100.00%)	1 (50.00%)
T Cruris (6)	4 (66.67%)	3 (50.00%)
T Incognito (3)	2 (66.67%)	2 (66.67%)
T Mannum (1)	1 (100.00%)	0
Total	67 (59.82%)	38 (33.92)

Table-5: Comparison of results of 10% KOH medium and culture medium

10% KOH medium	Culture medium	
	Negative	Positive
Negative	33 (29.46)	12 (10.71)
Positive	41 (36.61)	26 (23.21)

With respect to assessment of clinical types of dermatophytes, 12 onychomycosis presentations

were identified to be of dermatophytes. The other major clinical presentation identified to be of dermatophytes was the mixed culture of *T.corporis* + *T.cruis* (12). However, the presentations were identified to be majorly of *T. mentagrophytes* (17) and *T. rubrum* (11). The clinical presentations were observed to be associated with the extracted dermatophytes (P=0.018; table 6).

Table-6: Distribution of clinical types with respect to isolated dermatophytes

Clinical presentation	Isolated Dermophytes						Negative for dermatophytes	p-values
	Total	M. gypseum	T. mentagrophytes	T. rubrum	T. terrestre	T. tonsurans		
Onychomycosis	12	0	5	6	1	0	34	0.01849**
Pityriasisversicolor	0	0	0	0	0	0	1	
T corporis	8	0	4	3	0	1	12	
T corporis + T Cruris	12	0	7	2	0	3	20	
T corporis + T fasciae	0	0	0	0	0	0	1	
T Capitis	1	0	0	0	0	1	1	
T Cruris	3	1	0	0	0	2	3	
T Incognito	2	0	1	0	1	0	1	
T Mannum	0	0	0	0	0	0	1	
Total	38	1	17	11	2	7	74	

*** For Chi square test, negative to dermatophytes column is not considered and 0.5 is added to each cell and p-value is obtained by Monte-Carlo simulation.*

Discussion

Superficial mycosis is the common fungal diseases affecting most of the people worldwide. The incidence of superficial mycosis in the present investigation was highest in 21- 40 years of age which is consistent with findings by researchers [9-11]. A socioeconomic analysis in previous studies indicates that most patients with superficial mycotic infection belongs to lower socioeconomic group that could be due to physical activity induced by perspiration [8-10].

Male predominance was observed in the present study, in accordance with the published literature. This can be due to sporting activities and

preference for closed footwear among other factors, resulting in higher perspiration which facilitated fungal growth. Additionally, the social stigma surrounding the females in rural areas limits the report of fungal infection. This could be due to less immunity and more exposure to various environment. Frequent shaving of scalp and sharing of caps can also be a contributory factor. Patients on immunosuppressant could experience extensive disease with mixed clinical types. Additionally, these infections thrive among populations of tropical countries due to hot and humid weather conditions resulting in higher prevalence in Indian population [12].

Cutaneous mycoses have been reported to be increasing in the Indian population as reported by Tripathy in 2016. In accordance with the report, the present study also observed higher number of samples from the affected skin regions. Upon observation of the clinical presentations, onychomycosis was most observed. The incidence of onychomycosis is reported to be increasing in the worldwide scenario and accounts for almost 50% of nail related infections. The findings of the present study were similar to other studies conducted in the Indian population *T.mannum*, *T.pedis*, and *Pityriasisversicolor* exhibited the lowest incidences similar to other studies [9-15].

The present study is concurrent with earlier observations [19] that have suggested *T.mentagrophytes* to be the common etiological agent for most of the dermatophytes clinical types. This was similar to several observations in the literature, especially the research conducted in India [20, 21]. Since fungal culture takes time, therefore 10% KOH has been used as a clearing agent for direct demonstration of fungi in skin, hair and nail scrapings [12, 15]. It permits quick

and clear examination without warming and prevents the rapid drying of fluid [14-23]. Comparison of different strains of clinical types can help in distinguishing the severity and occurrence of the diseases which can further help in designing adequate treatment strategies. Limitation of present study is the lack of record of follow-up treatment, small sample size, non-dermatophyte exclusion and comparison with other demographic factors. In future it is recommended to follow up with the patient so that the reoccurrence of infection can be prevented, and adequate treatment is provided.

Conclusion

The fungal disease can be controlled and treated with a systematic approach, but in most people the disease reoccurs due to lack of local immunity or inadequate treatment. If treatment is stopped before all fungi are shed, then the disease will re-establish itself and cause more damage to the patient. Therefore, a proper follow up is required to totally eradicate the disease.

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Conflicts of interest: There are no conflicts of interest.

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