



OCCURRENCE OF DERMATOPHYTES AND NON-DERMATOPHYTES FUNGAL INFECTION AMONG PATIENT VISITING TERTIARY CARE HOSPITAL AT JAIPUR.

Microbiology

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ABSTRACT

Background: Dermatophytosis is currently a disease of worldwide importance and a public health problem in many parts of the world particularly in developing countries. The infections caused by dermatophytes are known as ringworm or tinea infections and Non-dermatophytic fungi also produce lesions clinically similar to those caused by dermatophytic infections. Present study was conducted with objective to know the prevalence of dermatophytes and Non dermatophytes infections and species prevalent in Jaipur.

Material and Methods: A total of 250 clinically suspected cases of dermatophytosis attending the Dermatology Outpatient department of tertiary care centre, Jaipur were enrolled for present study. Specimen collected was subjected to potassium hydroxide (KOH) wet preparation. After direct microscopic examination, the specimen was inoculated into 2 sets of test tubes containing Sabouraud's dextrose agar with 0.004% chloramphenicol and 0.05% cycloheximide. Fungal isolates were identified based on colony morphology, pigmentation, growth rate, microscopy and slide culture then data entered in excel and analyses in SPSS software.

Results: Out of 250 patients most of the cases 57.2% were in the age group of 25 to 44 years of age. Out of 250 samples of suspected cases 160 (64%) were culture positive and no fungal growth was found in 90 samples (36%). Out of 160 culture positive on SDA medium 90(56.25%) were Non-Dermatophyte, followed by 40 (25%) were dermatophytes while 30(18.75%) were yeast. Out of total 160 cases, maximum (16.2%) cases were belong to *Aspergillus niger* followed by *Tricophyton mentagrophyte* (14.3%).

Conclusion: The confirmation of both dermatophytic and nondermatophytic fungal isolates from the sampled population suggests a relationship that requires further investigation and whose complete understanding has a strong implication for the control of dermatomycosis.

KEYWORDS

Dermatophyte, Non Dermatophyte, *Aspergillus niger*, *Tricophyton mentagrophyte*, fungal infection

INTRODUCTION:

Dermatophytosis is currently a disease of worldwide importance and a public health problem in many parts of the world particularly in developing countries¹. Fungal infection can be divided into three broad groups such as superficial mycosis, subcutaneous mycosis and systemic mycosis. Among superficial mycosis, dermatophytosis is the most common contagious infection. It is a fungal infection of the outermost layer of skin and its appendages such as hair and nails with scalp ringworm being the most common in children of school age and adult males, respectively². They are a group of fungi that are able to damage and utilize keratin found in the skin, hair and nails. They are classified into three genera: Microsporum, Trichophyton, and Epidermophyton. It affects 20% to 25% of the world's population².

Traditionally, infections caused by dermatophyte (ring-worm) have been named by appending the latin name of the affected body part after the word "tinea"³. These include Tinea capitis (scalp), Tinea corporis (non-hairy skin of the body), Tinea unguium (nail infection), Tinea cruris (groin), Tinea pedis (athlete's foot), and Tinea barbae (bearded areas of the face and neck)⁴.

Non-dermatophytic fungi produce lesions clinically similar to those caused by dermatophytic fungal infections. The non-dermatophytic fungal infections show a lower rate of prevalence⁵.

Dermatophytosis are more common in tropical countries due to humidity, elevated temperatures and sweating. Infection by dermatophytes is restricted to non living cornified layers of the skin.

As the epidemiology of dermatophytes and non dermatophytes is changing over time it is important to review periodically the incidence of dermatophytes and non dermatophytes and their distribution. Therefore present study was conducted to know the prevalence of dermatophytes and non dermatophytes infections and species prevalent in and around tertiary care hospital, Jaipur.

MATERIAL AND METHODS:

The current study was conducted from 1 April, 2017 to 31 March 2018 in the Department of Microbiology of tertiary care centre, Jaipur, after approval by institutional ethical committee of SMS medical college,

Jaipur. A total of 250 clinically suspected cases during study period, of dermatophytosis were enrolled for present study. After taking detailed case history, informed written consent, respective samples like skin scrapping, hair and nails were collected in sterile petridish or black paper with proper sterilization and aseptic conditions. Specimen collected was subjected to potassium hydroxide (KOH) wet preparation (10% KOH for skin and hair; 30% KOH for nail) for the presence of fungal elements.

After direct microscopic examination, irrespective of demonstration of fungal elements, the specimen was inoculated into 2 sets of test tubes containing Sabouraud's dextrose agar with 0.004% chloramphenicol and 0.05% cycloheximide. One set was incubated at 25°C and other set at 37°C for up to 4 weeks. If no growth was found after 4 weeks, it was taken as negative for growth of fungi. Fungal isolates were identified based on colony morphology, pigmentation, growth rate, microscopy (Lactophenol Cotton Blue mount), and slide culture. Special tests were done when necessary viz: hair perforation test and urease test for species identification. Germ tube test and chrome agar were done to identify the Candida species. For statistical analysis, qualitative data were expressed in proportion and percentages, and the quantitative data expressed as mean and standard deviations. Significance level for tests was determined as 95% (P< 0.05).

RESULTS:

Out of 250 patients, most of the cases 57.2% were in the age group of 25 to 44 years of age followed by 21.2% in the age group of 45 to 64 years. Out of 250 patients 149 (59.6%) were male and 101(40.4%) were female (Table-1).

Table 1: Association of Sample type with Gender distribution:

Sample	Male Number (%)	Female Number (%)	Total
Nail	117 (60.6)	76(39.4)	193
Skin	32(56.1)	25(43.9)	57
Total	149(59.6)	101(40.4)	250

P value: >0.05 (not significant) chi square value: 0.36 df: 1
The male to female ratio was 1.47:1. Out of 160 samples, 126(50.4%) samples were positive in KOH examination while 124(49.6%) were

negative for KOH examination (Table-2).

Table 2: Association between direct microscopy and Culture of study population (n=250)

Result of culture	KOH result		Total
	Positive	Negative	
Culture positive	126	34	160
Culture negative	0	90	90
Total	126	124	250

P value <0.05 (statistically significant) Chi square- 142.89 df-1

All the samples were cultured on two sets of SDA slants for isolation of fungal species. Out of 250 samples of suspected cases 160 (64%) were culture positive while no fungal growth was found in 90 samples (36%). Out of remaining 160 positive patients, 126 were correctly diagnosed on both culture and KOH (True Positive). Fungus was grown in all the samples which were KOH positive. 36 patients which were positive in culture but negative in KOH considered as (false negative) In our study, sensitivity, specificity, PPV, NPV and diagnostic accuracy of KOH against the culture were 52%, 100%, 100%, 72% and this is statistically significant association of KOH Findings with Culture was observed (P=0.001). (Table-2)

Out of 160 culture positive on SDA medium 90(56.25%) were Non-Dermatophyte, followed by 40 (25%) were dermatophytes while 30(18.75%) were yeast. Out of total 160 cases, 16.2% of cases were belongs to *Aspergillus niger* followed by *Trichophyton mentagrophyte* (14.3%) (Table-3).

Table 3: Mycological profile of the study (n=160)

Fungal species	Number	Percentage %
Dermatophytes		
<i>Trichophyton mentagrophyte</i>	23	14.3
<i>Trichophyton rubrum</i>	7	4.3
<i>Trichophyton tonsurans</i>	6	3.7
<i>Trichophyton verucosum</i>	2	1.2
<i>Microsporum gypseum</i>	2	1.2
Non Dermatophytes		
<i>Aspergillus niger</i>	26	16.2
<i>Aspergillus flavus</i>	11	6.8
<i>Aspergillus fumigatus</i>	10	6.2
<i>Alternaria spp.</i>	8	5.0
<i>Rhizopus spp</i>	8	5.0
<i>Penicillium</i>	7	4.3
<i>Curvularia spp</i>	7	4.3
<i>Cladosporium</i>	6	3.1
<i>Mucor spp</i>	4	2.5
<i>Fusarium spp</i>	2	1.2
<i>Scopulariopsis</i>	1	0.6
Yeast		
<i>Candida albicans</i>	11	6.8
<i>Candida glabrata</i>	3	1.8
<i>Candida parapsilosis</i>	6	3.7
<i>Candida tropicalis</i>	10	6.2
Total	160	100.00

Out of 160 diagnosed cases of fungal infection mostly (45.6%) belongs to farmer population doing agricultural work and remains continuously contact with cattle. Other predominant groups were student (23.1%), office workers (15.6%), Housewife (9.3%). Laborers were having only 6.2% of confirmed cases.

DISCUSSION:

Dermatophytes and Non-dermatophyte fungi implicated as a cause of dermatophytosis have been recorded all over the world, but with variation in distribution, incidence, epidemiology, clinical manifestations, and target hosts from one location to another. Differences in geographical location, health care, climatic factors, culture, and socioeconomic conditions are known to govern these discrepancies⁶.

Dermatophytosis has been a common contagious disease and remain an important public health problem among people worldwide and particularly in developing countries. This is evident by the present study in which the prevalence of dermatophytes, non dermatophyte

and yeast were 40(25%), and 90(56.25%) respectively with the overall prevalence of 64%. Our results are consistent with Ndako et al⁷ who reported prevalence of 53% dermatophyte and 38% non dermatophyte fungi which is comparably slightly higher than our finding. Another similar study conducted in Nigeria by Adefemi⁸ revealed that a prevalence of 5% dermatophyte and 15.4% non-dermatophyte fungi that is comparatively lower than our finding. Similarly, an overall prevalence of dermatophyte and non dermatophyte fungi of 66.7% and 71% have been depicted in similar studies conducted in India by Kannan P et al⁹. This is due to the differences in the lifestyle, socioeconomic conditions, risk factors associated with study subjects and environmental factors of study area. In this study we found that persons of all age groups were susceptible to dermatophytosis but it appeared to be more common in adults. Out of 250 cases most 57.2% were in the age group of 25 to 44 years of age followed by 21.2% in the age group of 45 to 64 years. The higher incidence may be due to increased physical activity, increased opportunity for exposure and increased perspiration. We found that out of 250 patients 149 (59.6%) were male and 101(40.4%) were female. The male to female ratio was 1.47:1. Our findings are in accordance with Vijay Kumar et al¹⁰ (male to female ratio 4:3), who also found males were more affected than females. However Gebreabiezgi Teklebirhan¹¹ found that more females were affected by dermatophytes than males, female male ratio being 2.2:1. The reason for increased percentage of males may be due to the fact of increased outdoor exposure and more physical work that results in increased sweating and less cosmetic consciousness compared to females¹².

In this study we found that out of 250 cases, 193 cases were of nail samples and 57 cases were of skin samples in which 60.6% of cases in nail sample and 56.1% of cases in skin samples falls under male category out of 149 male cases whereas 43.9% in skin sample and 39.4% in nail sample falls under female category out of 101 female cases. (table-1)

In this study we found that out of 250 cases, 50.4% of cases belong to positive KOH examination and 49.6% of cases belong to negative KOH examination. This may be due to the non viability of fungi due to use of antifungal agents prior to sample collection or could be due to absence of the fungi in the portion of the sample used for culture. Our findings are in accordance with Gupta et al¹³ and Bhagra et al¹⁴ in which KOH positivity was 85% and 80% respectively. In various studies, KOH positivity rate varied from 35.6% to 100% and culture positivity rate varied from 36% to 66.7%¹⁵.

Direct microscopy by KOH preparation plays an important role in diagnosis of fungal infections but culture gives definitive diagnosis. In our study, 28 specimens that were negative in KOH examination revealed fungal growth on culture medium. This may be because the fungus could have been in an inactive sporulating phase that is difficult to be seen by microscopy but able to grow in appropriate media¹⁶.

In present study we found that out of 250 cases 160 fungal growths were isolated. Among that 40(25%) were dermatophyte, 90(56.25%) were non dermatophyte and 30(18.75%) were yeast. There are various studies^{7,17} who reported dermatophytes dominant to non dermatophytes while in our study we found non dermatophytes are more in number. This may be due to variation in sample size, occupation of the patients and specimen collected. As we have collected most of the specimen from nail followed by skin and none of the sample was hair.

We found that among the dermatophytes *Trichophyton* and *Microsporum* species were isolated none of the isolates of *Epidermophyton* were grown in present study. Out of total 40 dermatophyte isolates, *Trichophyton mentagrophytes* (57.5%) was the most common isolate followed by *Trichophyton rubrum* (17.5%), *Trichophyton tonsurans* (15%) and *Trichophyton verucosum* (5%) were isolated. 2 isolates (5%) belonged to *Microsporum spp.* Our findings are consistent with Shilpa Dayanand Putta et al¹⁸ who also reported *T.mentagrophytes* was most common isolate though they found different isolation rate. Most remarkable observation we had in our study, *T.mentagrophytes* as the most common etiological agent among dermatophytes. This is in contrary to studies¹³⁻¹⁴ conducted in India, in which *T.rubrum* was the predominant agent commonly involved in etiology of dermatophytosis whereas Sharma et al¹⁹ found *M. audouinii* as the commonest dermatophyte species. This variation in the distribution of dermatophyte species could be explained on the

basis of different climatic conditions and geographic distribution.

In present study Nondermatophyte fungi were also isolated. Among non-dermatophyte molds isolated in the present study, *Aspergillus* species stood first. Our result supported the findings of Aikaterini et al²⁰. The most common non dermatophyte was *Aspergillus niger* 26/90 (28.8%) followed by *Aspergillus flavus* 11/90 (12.2%), *Aspergillus fumigates* 10/90 (11.11%), *Alternaria spp.* and *Rhizopus spp* both 8/90(5%), *Penicillium* and *Curvularia spp* 7/90(4.3%), *Cladosporium* 6/90(6.6%), *Mucor spp* 4(3.1%), *Fusarium spp* 2/90(1.2%) and *Scopulariopsis* 1/90(0.6%). The prevalence of non dermatophytes in our study is high as compared to other studies conducted by Grover and Roy²¹ who reported a low proportion of Nondermatophyte molds (34%) on different clinical types of Dermatormycosis.

In another study conducted by A Lakshmanan et al²² isolation of nondermatophytes was reported as 24.4% with *Candida* (60%) as commonest species followed by *Aspergillus*, *Fusarium*, *Alternaria* and *Curvularia* species. Mohammed Azam Bokhari et al²³ in his study reported 11% isolation of nondermatophyte molds in samples of Nail clippings which included *Fusarium* 4%, *Aspergillus* 2% and *Alternaria* 1% in addition to other opportunistic fungi¹¹. Extreme variation in isolation rate in few other studies may be due to differences in techniques used for sample collection, culture and other methods of identification.

Similarly, yeasts were isolated from 30 cases (18.75%) in our study with *Candida albicans* as a major isolate accounting 36.66% of the total yeast isolates followed by *Candida tropicalis* 33.33%, *Candida parapsilosis* 20% and the least were *Candida glabrata* 10%. Our results are in accordance with Gebreabiezi T et al¹³ and Satpathi et al²⁴ who also reported *Candida albicans* as a major isolates among yeast.

Though the prevalence of non dermatophyte molds and yeasts in the present study was well in reported ranges, a comparatively high prevalence rate of non dermatophyte (56.25%) was achieved. The significance of such non-dermatophyte molds in causing skin-related infections has been demonstrated in many other studies²⁵. It is not known whether non dermatophyte fungi occur as true pathogen to the body of a health individual or exist as secondary invader in already damaged tissues and cause secondary tissue destruction. However, the non-dermatophyte fungi may be considered as important pathogen with a high index of suspicion in evaluating the patients with culture negative for dermatophytes or those subjects ending up in treatment failure.

CONCLUSION:

The confirmation of both dermatophytic and nondermatophytic fungal isolates from the sampled population suggests a relationship that requires further investigation and whose complete understanding has a strong implication for the control of dermatomycosis. Our study suggested that non-dermatophyte fungi should be considered as important pathogen with a high index of suspicion in evaluating the patients with culture negative for dermatophytes or those subjects ending up in treatment failure.

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