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Original Research Article

Isolation and Identification of Fungi from Patients Suffering from Superficial Skin Infection in a Tertiary Care Hospital

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Abstract

Introduction: Superficial fungal infections are often seen in day-to-day clinical practice, and their prevalence continues to rise worldwide. The present study attempts to find out the distribution of superficial fungal infection with respect to socio-economic and demographic variables among a symptomatic group.

Objective: The present study was undertaken with a view to isolate the fungi from patients suffering from superficial skin infection attending Dermatology OPD at NAMO Medical Education & Research Institute Silvassa, DNH and to assess the clinical profile, prevalence of dermatophytes infection in study population.

Methods: A clinical and mycological study of superficial mycosis was conducted on 100 cases (65 male & 35 female). Direct microscopy by KOH mount and culture was undertaken to isolate the fungal pathogen in each case. Further, LPCB mount was performed for microscopic morphology.

Result: A total of 100 patients were enrolled in the study. Dermatophytosis was the commonest superficial fungal infection in 47 cases), followed by Candidiasis in 8 (14.5%) cases. Commonest clinical presentation was T. corporis in 32 (39%), followed by T. cruris in 27 (33%) cases. The commonest dermatophyte cultured was *T. mentagrophyte* in 22 (22%) followed by *T. rubrum* in 15 (14.9%) cases. Non Dermatophytic Molds (NDM) were isolated in 3/55 (5.4%) cases. The commonest NDM isolated was Exophiala.

Conclusion: We conclude that along with dermatophytes, non dermatophytic fungus are also emerging as important cause of superficial mycosis. Direct microscopy and culture both are important tools for diagnosis of fungal infections.

Keywords: Dermatophytosis, Dermatophytes, T. mentagrophytes, T. rubrum

Introduction

Superficial fungal infections have been identified worldwide as being one of the most important human fungal infectious diseases. The prevalence rate of superficial fungal infection differs from place to place according to environmental condition and also due to poor hygienic condition of population [1]. In India most of the places are very hot and humid climate because of that our country has more prevalence rate of superficial fungal infections [2,3]. Superficial mycoses are limited to the stratum corneum and essentially elicit no inflammation. Cutaneous infections involve the integument and its appendages, including hair and nails. Infection may involve the stratum corneum or deeper layers of the epidermis. Inflammation of the skin is elicited by the organism or its products. Despite therapeutic advances in the last decades, the prevalence of cutaneous mycoses is still increasing [4]. Most of the people are living in very low socioeconomic status, crowded living environment, and poor medical care which adds to the increased prevalence of cutaneous fungal infections [3]. Superficial fungal infections can be caused by dermatophytes, yeasts and non-dermatophyte moulds. Among these dermatophytes are the most common agent which produce cutaneous inflammatory response and most intense itching in the poor hygienic condition [5]. It also occurs in immunocompromised persons. The yeast group of candida species is also a commonest etiological agent of superficial fungal infections [6].

Superficial candidiasis includes skin, oropharyngeal, vaginal and conjunctival tissue infections. Dermatophytic cutaneous infection occuring in children, adolescent and adults has become a most important health problem [7]. This infection does not cause any death but it may lead to morbidity and plays a more important role in health related problems. The diagnosis of a superficial fungal infections are strongly based upon the clinical findings and testing to confirm the diagnosis because a variety of cutaneous disorders may present with similar features. Direct microscopic examination, isolation, cultural features and physiological characteristics are useful to identify the genus and species of dermatophytes but these conventional methods require time and effort [5]. These methods are slow and morphological characteristics depend on lot of variables such as decreased growth rate, presence of low threshold of organisms in clinical specimens. In order to choose the most effective medication, the clinicians needs a basic understanding of the drugs available as well as their mechanisms of actions, dosages, interactions, indications and adverse effects.

Methodology

The study population included 100 patients with clinically suspected superficial fungal infections, who attended the outpatient department of dermatology at NAMO Medical Education & Research Institute Silvassa. Patients, who were clinically suspected with superficial fungal infections irrespective of age, sex and were not undergoing treatment for the same were included for the study. Detailed history of the patients regarding age, sex, site of lesion, occupation, and associated illness was taken and patients were examined clinically for the type & site of the lesion and classified accordingly. Before collection of the sample, patient was explained about the procedure & informed consent was taken. The sample collection site was cleaned with cotton soaked in normal saline. The Clinical specimens (like skin scrapping, infected hair taken by plucking, clipped nails) were collected in a small piece of sterile aluminium foil. Immediately after collection, 10 % KOH mount examination was done and samples were inoculated on saboraud's dextrose agar (SDA) with & without antibiotics. Nail clippings were dipped in 40% KOH solution overnight for study on the next morning . All the sample were inoculated on SDA with sterile olive oil overlay. Two bottles of SDA were incubated at different temperature, one at 25°C & another at 37°C for a period of 1 month before giving negative result. If any growth was found on culture; then the isolate was identified by colonial morphology, pigment production and direct examination of smear from the colony by tease mount,cellophane tape mount using lactophenol cotton blue preparation & slide culture technique.

Results

The study population which included 100 patients with clinically suspected superficial fungal infections was subjected to mycological examination. Out of 100 patients 35 (35%) were females and 65 (65%) were males. Out of which maximum cases with infection were between 31- 40 years of age (29%), in this age group 20 were males and 9 were females followed by 21- 30 years of age group (27%) and the least were from below 10 years (3%). Out of 100 patients from whom the specimens were collected, 42 (42%) cases did not suffer from the same infection before, 31 (31%) cases had previous history of disease, 22 (22%) patients had contact history with infected person in their house and 5 (5%) patients had contact with infected animal. Out of 100 patients from whom the specimens were collected, 32 (32%) cases were from factory workers, 23 (23%) cases were from company employees, 14 (14%) cases were from students, 22(22%) cases were from housewives. Out of total 100 specimens collected 82 (82%) were skin scrapings, 7 (7%) were hair samples and 11 (11%) were nail clippings. The maximum number of specimens were skin scraping, among these 52 specimens were collected from male and 30 from female. In hair samples 5 were male and 2 were female. In nail specimens 8 were male and 3 were females.

Table 1: Clinical types of superficial fungal infections

Specimen	Clinical type of infection	Number of case	Percentage
Skin (82)	Tinea corporis	32	39%
	Tinea cruis	27	33%
	Tinea manum	8	10%
	Tinea versicolor	7	8%
	Tinea pedis	8	10%
Hair (7)	Tinea capitis	2	28%
	Tinea barbae	3	43%
	Piedra	2	28%
Nail (11)	Tinea unguinum	11	100%

Out of 100 specimens the KOH wet mount was positive for fungal elements in 65 (65%) samples and culture positivity was 55 (55%), among these culture positive isolates 47 (47%) were dermatophytes and 8 (8%) were

non dermatophytes, 35 (35%) did not show evidence of the fungi on direct microscopy and 45 (45%) not grown in culture (***Table 2*****).**

Table 2: Comparison of culture positivity and KOH mount positivity

Details	KOH Positive	KOH Negative	Culture Positive	Culture Negative
Total Samples (100)	65 (65%)	35 (35%)	55 (55%)	45 (45%)
Culture positivity in KOH positive samples (65)	-	-	55 (77%)	15 (23%)
Culture positivity in KOH negative samples (35)	-	-	5 (14%)	30 (86%)
Total dermatophytes (47)	40 (86%)	7 (14%)	-	-
Non dermatophytes (8)	8 (100%)	-	-	-

Among the culture positive 44 dermatophytes and 11 were non dermatophytic fungus which includes 4 isolates of *Candida albicans* and 4 isolates of *Candida non albicans.* Other than *Candida species*. There was no growth in 45 (45%) of the total specimens (*Table 3*). In the total 44 dermatophytes, 39 isolates belonged to the *Trichophyton species* of which 22 isolates were *Trichophyton mentagrophytes* followed by 15 isolates of *Trichophyton rubrum, 2* isolates of *Trichophyton tonsurans*, 5 isolates belonged to *Microsporum species* of which all isolates were *Microsporum canis* (*Table3).*

Among the skin specimens *Trichophyton mentagrophytes* was the predominant isolate (19), followed by *T. rubrum (11), Candida species (4), T. tonsurans (2), M. canis (5) and.* No growth was shown in 38 specimens (*Table 3)*. From the hair specimens, maximum number of isolates were *Trichophyton mentagrophytes* (3). No growth was shown in 4 specimens. Among the nail specimens *Candida species* was the predominant isolate (4) along with *T. rubrum* (4). No growth was seen in 3 specimens (*Table 3).* Among the clinical condition, *T. mentagrophytes* which was the predominant isolate 22 (22%), followed by *T. rubrum* 15 (15%), *T. tonsurans* 4 (2%)*, M. canis* 5 (5%), *Candida species* 4 (8%) and growth was not present in 45 (45%) patients (Table 3). In *T. cruris* infection *T. mentagrophytes* was the predominant isolate 5 (18.5%), along with *T. rubrum* 5 (18.5%), *M. canis* 4 (14.8%), *Candida species* 2 (7.4%) and no growth was seen in 11 (40%) patients (*Table 3*). Non dermatophyte infection of *Tinea versicolar* and *Tinea piedra* showed no growth in all the specimen(*Table 3*). *T. mentagrophytes* was the predominant and only isolate 1 (50%) in *Tinea capitis* infection. No growth was seen in left 1 sample. In *Tinea barbae* infection, *T. mentagrophytes* was the predominant isolate 2 (66.6%) and no growth were seen in other specimen. Non dermatophyte infection of P*iedra* showed no growth in all the specimens. *Candida species* 4 (36.3%) was the predominant isolate grown in *Tinea unguinum* infection along with *T. rubrum* (4) and no growth in 3 specimens (*Table 3)*.

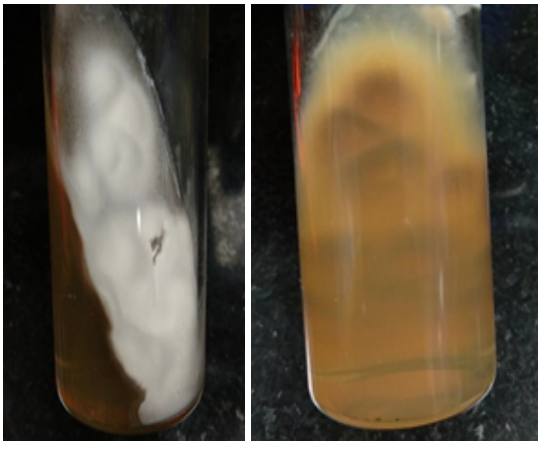

Figure 1: **Colony morphology of *Trichophyton mentagrophytes* (***Picture showing flat colony, white to cream colour and raised central tufts leads to formed central folding on SDA, with brownish red pigmented reverse side.)**

Figure 2: **Chrom agar showing different colour colonies of *Candida species*.**

Table 3 : Total isolates

Specimen Total (100)	Clinical types of lesions	Number of cases	Trichophyton rubrum [15]	Trichophyton mentagrophyte [22]	Trichophyton tonsurans (2)	Microsporum canis [5]	Exophiala species [3]	Candida species [8]	No growth [45]
Skin [82]	Tinea Corporis	32 (39%)	4 (12.5%)	8 (25%)	2 (6.2%)	-	2 (6.2%)	2 (6.2%)	14 (43.7%)
	Tinea cruris	27 (33%)	5 (18.5%)	5 (18.5%)	-	4 (14.8%)	-	2 (7.4%)	11 (40.7%)
	Tinea mannum	8 (10%)	-	4 (50%)	-	-	-	-	4 (50%)
	Tinea Versicolor	7 (8%)	-	-	-	-	-	-	7 (100%)
	Tinea pedis	8 (10%)	2 (25%)	2 (25%)	-	1 (12.5%)	1 (12.5%)	-	2 (25%)
Hair [7]	Tinea capitis	2 (28%)	-	1 (50%)	-	-	-	-	1 (50%)
	Tinea barbae	3 (43%)	-	2 (66.6%)	-	-	-		1 (33.3%)
	Piedra	2 (28%)	-	-	-	-	-	-	2 (100%)
Nail [11]	Tinea Unguium	11 (100%)	4 (36.3%)	-	-	-	-	4 (36.3%)	3 (27.2%)

Discussion

In the present study out of 100 patients 35(35%) were females and 65 (65%) were males. Male predominant occurs due to their occupation, frequent interaction with overcrowded people, poor personal hygiene and most of them were working as exhaustive physical worker like factory workers.

Our study showed that maximum of 29% had tinea infections in age group between 21 to 30 years. Different observation was found in study by Sarma *et al (14)* who observed 39% between 21 to 30 years, Patel *et a*l [8] observed 30% and Grover S, Roy et al [9*]* observed 40%. They showed high prevalence of tinea infections compare to our observation.

Tinea corporis was the commonest lesion accounting for 39% of the cases in present study followed by *Tinea capitis* 21% and *Tinea cruris* 18%. This observation correlated with Sarma and Borthakur *et al [*10] observed *T. corporis* 42% followed by T. cruris 11% cases . These studies showed *Tinea corporis* was the most predominant infection among superficial fungal infections. Among the tinea corporis infections most of the clinical condition include lesion in exposed part of the human skin. Similar findings have been shown by Venkatesan *et al* [11].

The KOH mount was positive for fungal elements in 65% of samples collected, culture positivity was 55% isolates and 35% did not show evidence of the fungi either on direct microscopy and culture. Different results were found by Sen SS *et al* [12] which showed KOH mount positive for fungal elements in 49% which did not correlate with the present study. But culture positivity was 51% which had correlated and 33% did not show evidence of the fungi either on direct microscopy or culture which also correlated with the present study [12] Another difference found by S, Singh and P.M. Beena *et al* [13] who had shown KOH mount was positive for fungal elements in 60.38% cases, culture positivity was 44.6 and 53.38% cases did not show evidence of the fungi either on direct microscopy and culture.This study showed that KOH mount was positive for fungal elements in 65% cases which correlated with Singh S and Beena PM *et al* [13] who had shown KOH mount positivity as 61%. Different results were found in a study by Kucheria M *et al* [14] reported as 80% which showed higher than our study.

The present study showed that out of 100 patients 55 (55%) were culture positive whereas Bindu *et al* (15) and Singh *et al* [13] conducted study at Calicut and Baroda respectively showed that 45.3% and 44.6% culture positive. However Patwardhan *et al* [16] had shown very less culture positivity 22.8%. our study showed high culture positivity compare to Patwardhan *et al,* Bindu *et al* and Singh *et al* probably because of good culture techniques with aseptic precautions which prevents the contaminants to over grow in the culture.

In the present study among 100 specimens cultured 47 dermatophytes were isolated which was comparable with Kannan P *et al* [22] which showed that out of 165 patients, dermatophytes were isolated in 48% of culture which correlates with our study**.** Different observation were found in study by N dako JA *et al* [17] showed that out of 100 patients, dermatophytes were isolated as 58% which was higher than our study and Ellabib MS *et al* [18] showed as 28% which was lesser than our study**.**

Our study had shown *T. mentagrophytes* (46%) as the predominant isolate followed by *T. rubrum* (31%) and *T. tonsurans* (4.2%) in *Tinea corporis* infection which is concordance with Patel P *et al* (8) showed *T. rubrum* (20%), *T. mentagrophytes* (7%). Different results were found in study by Mohammad et al [19] observed that *T. verrucosum w*as the most predominant isolate in *Tinea corporis* infection. (16)Al-Sheikh *et al* [20] conducted a study on dermatophytosis and observered that T. *mentagrophytes* were 35.4% and *T. rubrum* 27.1% isolated from tinea cruris infection which was almost similar to our study. Our present study showed non dermatophyte of *Candida species* (36%) was predominantly isolated in t*inea unguinum* infection followed by *T. rubrum* (36.3%) which was comparable with Batawi MM *et.al* [21].

Conclusion

Dermatophytic infections are one of the most common infectious diseases. Isolation rate of fungus depends on good culture techniques and good sample collection, with aseptic precautions which prevents the contaminants to over grow in the culture. *Trichophyton species* was the predominant causative agent of dermatophytic infections. Primitive diagnosis of dermatophytosis can be done by KOH mount and culture, which takes longer time to report and can differentiate at the level of genus and species level. Dermatophytosis was the commonest clinical presentation, followed by and Candidiasis. The commonest dermatophytosis was Tinea corporis (39%). The commonest dermatophyte isolated was *Trichophyton mentagrophyte* (22%). The KOH positivity rate was 65% and total culture positivity rate was 55%. Along with dermatophytes and candida, dematiaceous fungi like *Exophiala jeanselmi (3%)* are also emerging as important causes of superficial mycosis. Direct microscopy and culture both are important tools for diagnosis of the fungal infections.

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