

“A STUDY OF FUNGAL ISOLATES FROM CLINICALLY SUSPECTED SUPERFICIAL MYCOSES PATIENTS IN TERTIARY CARE HOSPITAL”.

Microbiology

Dr. D. Shyamala Resident, Department of Microbiology, SRTR GMC, Ambajogai.

Dr. D. M. Kulkarni Associate Professor, Department of Microbiology, SRTR GMC, Ambajogai.

Dr. S. L. Nilekar Associate Professor, Department of Microbiology, SRTR GMC, Ambajogai.

Dr. B.A. Mundhe Head of Department, Department of Microbiology, SRTR GMC, Ambajogai.

ABSTRACT

Background: Fungal diseases formed an important entity in the recent times in Tropical countries like India. Superficial mycoses are infections of skin, hair and nails caused by dermatophytes, yeasts and non-dermatophyte moulds. **Objective:** The present study was undertaken to isolate various fungal agents causing superficial mycoses among patients attending OPD in a tertiary care hospital. **Materials And Methods:** This study was conducted from October 2022 to March 2023. All clinically suspected patients of superficial mycoses visiting dermatology OPD were included in the study. Samples collected like skin scraping; hair and nail clippings were subjected to direct microscopy- KOH mount and culture on SDA and DTM media. **Result:** Among 182 samples, 89(48.9%) were KOH positive and 93(51%) were KOH negative. Culture positive was 48(26.3%) and 134(73.6%) culture negative. Male were more commonly affected (61.5%) than females (38.4%). The commonest age group affected was 21 – 30 years with 52 (28.5%) cases. The most common clinical presentation was T corporis followed by P versicolor and candidiasis. Out of 48 culture positive samples, the predominant fungal isolates were Dermatophytes (73%) followed by Candida species (18.7 %) and non dermatophytic moulds (8.3%). Among 35 Dermatophytes, *Trubrum* (33.3%) was the commonest isolate. **Conclusion:** This study signifies the importance of mycological examination in diagnosis of superficial mycoses for their effective treatment.

KEYWORDS

Fungal isolates / Prevalence/ Potassium hydroxide/ Sabouraud Dextrose Agar/ Dermatophytes

INTRODUCTION

Superficial mycoses refers to fungal infections of the outermost layer of skin and its appendages like hair and nails¹. This group includes dermatophytosis, pityriasis versicolor and candidiasis². Among these, dermatophytes are responsible for the largest number of cases³. On the basis of clinical, morphologic & microscopic characteristics three anamorphic genera are recognized as Dermatophytes: Epidermophyton, Microsporum & Trichophyton⁴. They have the affinity to keratin rich tissues and produce dermal inflammatory response, intense itching, and cosmetically poor appearance⁵.

Factors contributing to the high frequency and continual occurrences of dermatophytosis in developing nations may also consist of negative residing situations, kid interaction styles, and poor health⁶. Though several reports on dermatophytosis are available from different parts of the country, there are only few reports on non dermatophytic fungi and yeast like fungi as causes of superficial mycoses along with dermatophytosis⁷. Clinical lesions caused by the fungi are highly variable and closely resemble other skin diseases making laboratory diagnosis and confirmation necessary⁸.

Recently there has been an increase in incidence of fungal infections. This increase may be result of frequent usage of antibiotics⁹. In view of this the present study was undertaken to isolate and identify various fungal agents causing superficial mycoses among patients attending OPD in a tertiary care hospital.

MATERIALS AND METHODS

This was an observational prospective study, conducted for a period of 6 months (October 2022 to March 2023). A total of 182 clinically suspected cases of superficial mycoses patients visiting Dermatology Out-patient department in a tertiary care hospital during study period with respect to inclusion and exclusion criteria were included in the study.

Patients of extreme ages (infants and above 60 years), immunocompromised patients like Diabetics and HIV patients, patients on immunosuppressive drugs like steroids, patients who were on antifungal therapy and secondarily infected sites were excluded from the study. Ethical clearance from institutional ethics committee have been obtained. Confidentiality of the study subjects have been assured and maintained throughout the study.

A detailed clinical history including age, sex, duration, site and extent of infection, type of lesion, antifungal therapy and occupation of patients were taken.

Clinical specimens like skin scrapping, infected full-length hair (by hair plucking) and clipped nail clippings were collected in small paper envelopes after thoroughly wiping the area with 70% alcohol. The material was carefully collected by scraping skin outward from edges of active lesions with scalpel blade held at an angle of 90° to the skin surface. Full length hair root was carefully plucked for hair root sample. Samples were collected in a sterile Whatman filter paper envelope and transported immediately to Microbiology department for further processing. Samples collected were made as two portions, one portion used for direct microscopy [KOH] mount, while second was for culture.

Direct microscopic examination [KOH Mount]

All specimens were subjected to direct microscopic examination with 10-20% KOH. For nail 40% KOH was used. Sample was gently warmed by passing the slide over Bunsen burner flame and cooled. Prepared slides were observed under low power (10X) and high power (40X) objectives. Gram staining was done for suspected Candidal infections.

Malassezia is a normal skin commensal and sometimes may be responsible for infection. So, skin scrapings from clinically diagnosed cases of Pityriasis versicolor were subjected to KOH mount only and were not cultured.¹⁰

Culture examination -

Sabouraud dextrose agar, Sabouraud dextrose agar with chloramphenicol (SDA) and Dermatophyte test medium (DTM) were used for isolation of growth from clinical materials obtained. The agar slant and agar plate were inoculated by placing skin scraping, hair, or nail clipping with the help of sterile needle and forceps.

Care has been taken to ensure that the sample has been firmly attached to the agar surface and incubated at 37°C, another set was incubated at room temperature. Cultures were examined daily for first week and twice a week for subsequent period and observed for 4 to 6 weeks. Culture media were incubated for minimum one month before declaring them as negative.

Identification: Lactophenol cotton blue mount [LPCB] have been prepared for examining detailed morphological features of fungi grown on culture media. Cellophane tape mount and slide cultures were undertaken for microscopic morphological identification. Gram staining and germ tube test were done for identification of yeasts.

RESULT:

A total of 182 samples from suspected superficial mycoses patients were included in the study among which 89(48.9%) were KOH positive and 93(51%) were KOH negative. Culture positive was 48(26.3%) and culture negative 134(73.6%). A total of 45 (24.7%) samples were both culture and KOH positive, while 41(22.5%) were KOH positive but culture negative. Samples which were KOH negative but culture positive were 3 (1.64%) [TABLE I].

The commonest age group affected with superficial mycoses was 21 – 30 years (28.5%) followed by 31 – 40 years (22%) and 11-20years (19.7%). Male 112 (61.5%) were the more commonly affected than female 70(38.4%) with Male to female ratio of 1.6:1 [Table II]

Out of 182 cases, the commonest clinical presentation was Tinea corporis in 65(35.7%), followed by Pityriasis versicolor in 55(30.2%) and Candidiasis in 24(13%) [Table III]. Out of 48 culture positive samples, the predominant fungal isolates were Dermatophytes with 35(73%) followed by *Candida species* 9 (18.7 %) and non dermatophytic moulds 4(8.3%).

Among 35 Dermatophytes, *Trichophyton rubrum* 16(33.3%) was the commonest isolate followed by *Trichophyton mentagrophytes* 11 (23%), *Trichophyton tonsurans* 4(8.3%), *Microsporum gypseum* 3(6.2%), *Microsporum canis* 1(2%). Total non dermatophytic molds isolated were 4(8.3%) among which *Aspergillus species* 1(2%), *Fusarium species* 1(2%) and *Penicillium species* 2(4%).

[TABLE I] RESULT OF KOH MOUNT AND FUNGAL CULTURE POSITIVITY			
KOH MOUNT	FUNGAL CULTURE	TOTAL	TOTAL PERCENTAGE
KOH POSITIVE	CULTURE POSITIVE	45	24.7%
KOH NEGATIVE	CULTURE POSITIVE	3	1.64%
KOH POSITIVE	CULTURE NEGATIVE	41	22.5%
KOH NEGATIVE	CULTURE NEGATIVE	90	49.45%

[TABLE-II] AGE AND GENDER WISE DISTRIBUTION OF SUPERFICIAL MYCOSES			
AGE	NUMBER OF MALE AFFECTED WITH PERCENTAGE	NUMBER OF FEMALE AFFECTED WITH PERCENTAGE	TOTAL NUMBER OF CASES AFFECTED WITH PERCENTAGE
1-10 years	2(1%)	--	2(1%)
11-20 years	25(13.7%)	11(6%)	36 (19.7%)
21-30 years	35(19.2%)	17 (9.3%)	52 (28.5%)
31-40 years	16(8.8%)	24(13.2%)	40(22%)
41-50 years	20(11%)	13(7.14%)	33(18.5%)
51-60 years	8(4.3%)	3(1.64%)	11(6%)
61-70 years	6(3.3%)	2(1.1%)	8(4%)
TOTAL	112(61.5%)	70(38.5%)	182(100%)

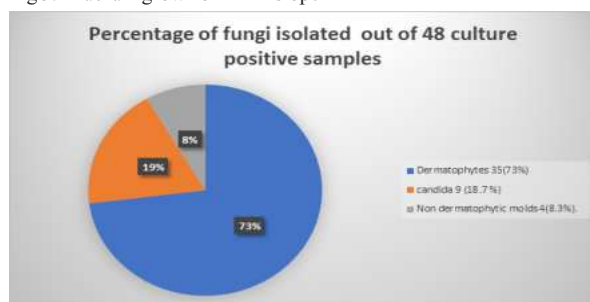
[TABLE III] DISTRIBUTION OF CLINICAL PRESENTATION AMONG SUPERFICIAL MYCOSES CASES		
MYCOSES	NUMBER OF CASES	PERCENTAGE
T. corporis	65	35.7%
T. capitis	15	8.2%
T. cruris	8	4.5%
T. faciei	5	2.7%
T. pedis	4	2.2%
Onychomycosis	6	3.5%
P. versicolor	55	30.2%
Candidiasis	24	13%
TOTAL	182	100%



Fig 1: *T. rubrum* grown on DTM slope with pigmentation on reverse.

Fig 2: *T. rubrum* grown on SDA slope,

Fig 3: *T. rubrum* grown on DTM slope



DISCUSSION

Superficial fungal infections are prevalent globally, but they are common in tropics and in geographical areas with higher humidity, overpopulation and poor hygienic living conditions¹⁵. In our study a total of 182 samples from suspected superficial mycoses patients were included where male patients were more commonly affected (61.5%) than female (38.4%) with Male to female ratio 1.6:1, similar high incidence of male being affected was noted by Tiwari et al^[11], Grover et al^[12] Reena Ray et al^[28] and Parul Patel et al^[13]. Higher incidence in male may be because of more outdoor physical activity. The commonest age group affected was 21 – 30 years (28.5%) followed by 31 – 40 years (22%) which was also similarly stated by Parul Patel^[13] and Grover et al^[12] where as Tiwari et al^[11] stated that 11–20 years (29%) as the most commonly affected age group followed by 21–30 years (20%) which was contrast with our study. In present study among the age group of 31 – 40 years, female patients were mostly affected (13.2%) than male patients (8.8%) this variation might be because of increased household works and wet occupation among this age group females.

In our study the commonest clinical presentation was T. corporis (35.7%) which was also reported by Parul Patel^[13] and Agarwal et al^[29], whereas in contrast, Reena Ray et al^[28] reported that *Tinea unguium* was the most common presentation which may differ according to the living habitat and climatic conditions of people in various regions of our country. The predominant fungal isolate was Dermatophytes (73%) which was similarly reported by Parul Patel et al [13] and also Grover et al^[12]. Higher dermatophytic isolation can be because of the occupation nature, physical activity and age group of study participants. In present study *Candida species* isolated was (18.7 %), other studies also reported *Candida* being isolated from cases of superficial mycoses. Tiwari et al^[11] Parul Patel et al^[13] and Grover et al^[12] reported increasing incidence of non dermatophytic molds isolation from superficial mycoses which is also similarly reported in present study with (8.3%) of non dermatophytic molds.

In present study direct microscopy with KOH mount shows higher positivity (48.9%) when compared with culture positivity (26.3%), which was also reported by Bhavsar et al^[7], Parul Patel et al^[13] and Tiwari et al^[11] whereas Kaur et al^[18] reported more culture positivity 46% than KOH positivity 37% which was contrast to our study. The variation in culture and KOH could be due to non viability of fungal elements in some cases depending on culture conditions and climatic conditions of the study area, this also signifies the importance of both direct microscopic examination and culture in definitive diagnosis of fungal infections.

According to Tiwari et al^[11], Madhavi S et al^[14] and Parul Patel et al^[13] *T. rubrum* was the commonest dermatophyte isolated which was similar

to our study with *T. rubrum* (33.3%) as the commonest isolated followed by *T. tonsurans* (8.3%) where as Grover et al^[12] reported that *T. tonsurans* as the commonest isolated while Agarwal et al^[29] reported that *Trichophyton mentagrophytes* as the most common isolate. This variation in dermatophytic isolation may be because of change in climatic and geographical areas of the studies.

CONCLUSION

Our study concludes that the commonest dermatophytosis was Tinea corporis and the commonest dermatophyte isolated was *Trichophyton rubrum*. Along with dermatophytes, nondermatophytic fungi are also emerging as important causes of superficial mycoses. Males were predominantly affected when compared with female patients. Main reason for increased frequency among the population is due to ignorance of the illness and presence of multiple lesions and failure to seek medical attention at the earliest. Both direct microscopy and culture are important tools of diagnosis for fungal infections where KOH examination is inexpensive and more sensitive than culture aiding in early diagnosis and management of superficial mycoses.

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