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Original Research Paper

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MYCOLOGICAL PROFILE OF DERMATOPHYTES

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ABSTRACT

Aims: Dermatophytoses are commonest type of fungal infections seen in human beings, invading keratinised tissue skin, hair and nails by keratinophilic fungi. Dermatophytoses can be treated effectively by antifungals. **Settings and Design:** Retrospective study **Methods and Material:** A total of 200 patients which were referred from the department of dermatology to the department of microbiology were collected from July 2020 to June 2021. After cleaning with povidone iodine and 70% alcohol, the skin was scrapped, hair was plucked and nails were clipped. Skin, Hair and Nail were treated with 10%, 20% and 40% KOH respectively to identify septate hyphae and arthrospores. The specimen was cultured on Sabouraud's dextrose agar (SDA) with and without cycloheximide. Microscopic description of the growth was done by Lactophenol cotton blue (LPCB) staining. Antifungal susceptibility testing was done by E strip method using Rosewell Park memorial institute (RPMI) media for Ketoconazole, Itraconazole, Fluconazole, Voriconazole, Posaconazole, Griseofulvin and Terbinafine. **Results:** Out of 200 samples, the commonest age group affected was between (21-30 yrs) 39.5%. Female to male ratio was 2.5:1.6. Tinea corporis was 63% and tinea cruris 27%. The commonest species isolated was Trichophyton rubrum 47.5%. Antifungal susceptibility test – Most susceptible to Itraconazole 81.4% and resistant to Griseofulvin 67.9%. **Conclusions:** Dermatophytosis is prevalent in high temperature zones. Most common isolates were T. rubrum and Trichophyton mentagrophytes in tinea cruris and tinea corporis respectively, they were most susceptible to Itraconazole and resistant to Griseofulvin. Resistance pattern of antifungal drugs helps in deciding empirical therapy for patient's better outcome.

KEYWORDS : KOH, LPCB, RPMI, SDA.

INTRODUCTION

Dermatophytosis is a superficial fungal infection. They are fungi that are keratinophilic, which produce keratinases, that cause infections of the skin, nail and hair.[1] Dermatophytes based on their habitat, are described as geophilic (soil), anthropophilic (human), or zoophilic (animal).[2]

Diagnosis is based on the combination of clinical observation and laboratory investigations. KOH mount is done for samples of skin scrapings, nail clippings, hair plucking and culture is done on Sabouraud's dextrose agar (SDA) with chloramphenicol and actidione and the fungus is identified by Lactophenol cotton blue (LPCB) from the growth. Antifungal susceptibility testing done by Epsilometer method, broth dilution and agar dilution.[2]

MATERIALS AND METHODS

Sample Population

Patients with clinical features suggestive of dermatophytes attending the OPD of dermatology department were referred to Department of Microbiology for sample collection, KOH and culture and sensitivity from July 2020 to June 2021 were taken for this study.

Sample size

Sample of 200 patients. All samples were subjected to KOH mount and culture on SDA with chloramphenicol and cycloheximide.

Methodology

Skin scrapings, hair plucking, nail clippings and nail scrapings were collected onto a sterile slide one part was used for direct microscopy and the other for culture. Rapid screening of the specimen was done by KOH microscopy using compound microscope.

Sample Collection

The appropriate area was cleaned with 70% povidone iodine and then with alcohol and allowed to dry. Skin was scraped from inflamed region to healthy region using 22.no scalpel blade, Hair was plucked with epilating forceps, Nail was cut and skin beneath the nail was also scraped, maximum amount of clinical material was collected.

Staining Procedures

KOH mount: Wet mount preparation of 10 %, 20 % and 40% for skin, hair, nail respectively and incubated for 20-30 minutes in case of skin and hair specimen and 2 hours in case of nail to detect the presence of acute angle branching with septate fungal elements/ arthrospores under low power 10X and high power 40X.[3,4]

Culture

Fungal Culture: Tubes of SDA containing chloramphenicol and cycloheximide were used for the inoculation of the specimen. The tubes were incubated aerobically at 25°C and 37 °C for 3 weeks. The tubes were examined daily to rule out contamination during the first week and twice weekly during the next two weeks. The isolates were identified by standard laboratory procedure by LPCB and slide culture when ever required.

Lactophenol cotton blue preparation mount- Cultures were examined microscopically by removing a portion of growth with a spud and placed on a glass slide into a drop of lactophenol cotton blue which was gently teased with a pair of teasing needles and the cover-slip was placed on it. The LPCB mount was observed under low and high power objective of microscope, for the presence of septate hyphae, macroconidia, microconidia and other accessory structures of vegetative hyphae and the characters of each were noted.[5,6]

Antifungal Susceptibility Test

It was performed using Epsilometer method (E-test) as per clinical laboratory Standards institute (CLSI) approved guidelines suggested for dermatophytes.[2,]

RPMI 1640 media prepared with L glutamine without bicarbonate, pH supplemented with 2% glucose and was poured onto 120 mm petri dishes with depth of 4.0 mm. RPMI 1640 sterility control was performed by inoculation on a blood agar plate, to check for any contaminants.[7,8]

Antifungal agents –E –test strips from HIMEDIA were obtained and stored at -20C until tests were performed. The concentration assay ranged for Fluconazole - 0.016 to 256/mL^{-1} and Griseofulvin, Itraconazole, Ketoconazole, Posaconazole, Terbinafine, Voriconazole with range 0.002 to 32μg/mL^{-1}.[9,10]

Procedure- All isolates were tested against seven antifungal agents using the E-test according to the manufacturer's instructions. The inoculum suspensions were prepared in a 5ml sterile water tube from colonies from SDA where growth was seen and confirmed with LPCB. The suspension was adjusted to a concentration of 10^5 to 10^6 conidia/mL with 1 Mcfarland optimum inoculums for antifungal suscepitibility test verified by quantitative hemocytometer. RPMI agar surface was inoculated by dipping a sterile swab into the inoculums suspension and streaking it evenly in three directions at 60. After excess moisture was absorbed into the agar and surface was completely dry, four E-strip were applied in each 120 mm plate. The plates were incubated at 28C in biological oxygen demand incubator and results were read at 72-96 hours and looked for growth.

RESULTS

Out of the 200 patients, direct microscopy with KOH mount showed positive in 175 (87.5%) and negative in 25(12.5%) negative and on culture, 162 (81%) dermatophytes were isolated. Females 138 (69%) were infected more than males 62 (31%).

It was more common in age group of 21-30 years 64 cases (39.5%), followed by 31-40 years seen in 46 cases (23%).

Tinea corporis were clinically diagnosed in 126 cases (63%) and tinea cruris in 54 cases (27%) followed by tinea corporis with tinea cruris 18 (9%), tinea facei in 2 cases (1%).

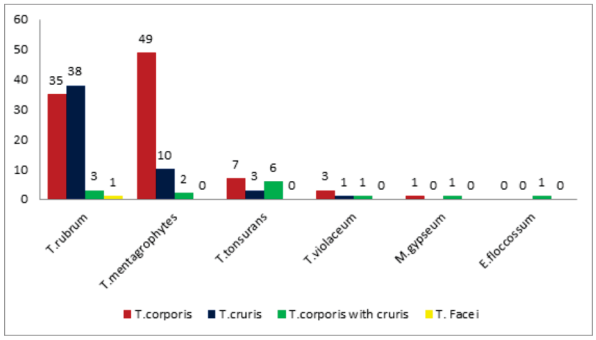
Trichophyton rubrum was in 77 cases (47.5%) ,Trichophyton mentagrophytes was in 61 (37.6%), Trichophyton tonsurans in 16 (09%), Trichophyton violaceum in 5 cases (03%), Microsporum gypseum in 2 cases (01%) and Epidermophyton floccossum in 1 case (0.6%).

In tinea corporis, out of 126 cases, 95 (75%) were isolated with predominantly T. mentagrophytes 49(51.5%), T. rubrum in 35 cases (36.8%), T.tonsurans 07 (07%), T.violaceum 03 (03%) and M. gypseum 01 (01%).

In tinea cruris, out of 54 cases 52 (96%) culture isolates were recorded, T. rubrum in 38 cases (73%), T. mentagrophytes 10 (19.2%), T.tonsurans in 03 (05%) and T.violaceum in 01 (01%) case .

Out of 18 tinea corporis with tinea cruris 15 (83%) which showed growth of dermatophytes, T.tonsurans were 06 (33%), T.rubrum 03(02%) cases, T.mentagrophytes 02 cases (01%) T.violaceum 01 case (01%), M.gypseum in 01 case (01%) and E.floccossum in 01 case(01%). Out of 02 tinea facei, only 01 showed culture positive with T.rubrum.

Itraconazole was most susceptible 132 cases (81.4%) followed by Posaconazole 126 (77%),Voriconazole 120 cases (74%) Fluconazole 99(61%) Ketoconazole 87(53.7%).The most resistant were Griseofulvin 110 (67.9%) and Terbinafine 102(62.9%).



Dermatophytes Isolated (N=162)

Antifungal Susceptibility test N=162

Antifungals	Susceptibility	Intermittent	Resistant
Fluconazole	99(61)	04(2.4)	59(36.4)
Griseofulvin	50(30)	07(4.3)	110(67.9)
Itraconazole	132(81.4)	13(8)	17(10.4)
Ketoconazole	87(53.7)	09(5.5)	66(40.7)
Posaconazole	126(77)	06(3.7)	30(18.5)
Terbinafine	51(31.4)	09(5.5)	102(62.9)
Voriconazole	120(74)	08(4.9)	34(20.9)

DISCUSSION

Dermatophytosis forms a large group of patients which were referred from department of Dermatology to microbiology. The high temperature and body sweating in tropical area of India facilitates fungal growth. It was common due to its symptomatic nature (pruritis) leading for early medical advice. Indian subcontinent varied topography is highly favorable for the acquisition of fungal infections. It is necessary to identify fungi causing infections and to find out the source of infection for treatment and to prevent their occurrence.

Out of the 200 patients, Females (69%) and males (31%), with a male to female ratio of 1.6:2.5, which is comparable with the studies done by Cordeiro RA et al [11]reported that females being more commonly affected than males 1.6:2.2, female predominance may be due to tight fitting clothes (Sarees and salwar being tightly tied around the waist line in South India).

The age group between 21-30 years were commonly seen with dermatophyte infection in 64 cases (39.5%) followed by 31-40 years seen in 46 cases (23%) which was reinforced by Bhagra S et al [12]21-30 years in 28% cases and Veer P et al [13] 31-40 years in 39.4%. The youth showing preponderance due to high exposure to sunlight and more work load.

In the present study, out of 126 cases (63%) of tinea corporis, 95 (75%) were isolated with most common fungi being T. mentagrophytes in 49 (51.5%) cases which is comparable to the studies done by Kalita JM et al [14]with 55% cases. The studies done by Uthansingh K et al[15] and Bindu V et al[16]showed T.rubrum as more common isolate in 78.2% and 68% respectively.

In our study, out of 54 cases of tinea cruris 52 (96%) which were isolated, the most common isolate was T.rubrum in 38 cases (73%) which was comparable with the study done by Patel SS et al[17]seen in 57.4% cases. It differed from the study done by Mishra N et al [18]where T.mentagrophytes was commonly seen in 49% cases suffering from tinea cruris. The more exposure could be because of poor hygiene and sweating. In our study, T.mentagrophytes were isolated more in tinea corporis patients and T.rubrum in tinea cruris cases, this change may be explained due to urbanization where the etiological agents are better adapted to human keratinized tissues.

In 15 isolates of mixed infection, T.tonsurans was predominantly seen in 06 cases (33%).

In the present study, 162 dermatophytes were isolated and tested for their antifungal susceptibility to seven antifungal drugs viz Fluconazole, Griseofulvin, Itraconazole, Ketoconazole, Posaconazole, Terbinafine and Voriconazole by E- test method. 81.4% dermatophytes (T.rubrum, T.mentagrophytes, T.tonsurans, T.violaceum) were susceptible to Itraconazole respectively, followed by Posaconazole in 77% isolates, 74% of Voriconazole, Fluconazole in 61% and Ketoconazole in 53.7% cases. This was comparable with the study done by Basak P et al [19] with 97.9% dermatophytes susceptible for Itraconazole.

In our study, Griesofulvin were found to be resistant to 67.9%, followed by Terbinafine (62.9%). In the study done by Verma S et al[20]. Griseofulvin and Terbinafine showed similar resistance (50%), whereas in the study done by Sharma S et al[8] and Perea S et al[21] maximum isolates were resistant to Fluconazole in 70% and 83% respectively.

There has been a rise in antifungal resistant strains of fungi, therefore early treatment with appropriate antifungals is essential for proper treatment and prevention in spread of the disease. [22]

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