Original Research Paper

Volume-8 | Issue-6 | June-2018 | PRINT ISSN No 2249-555X

Microbiology

CLINICOMYCOLOGICAL CO-RELATION AND PREVALENCE OF DERMATOPHYTOSES IN A TERTIARY CARE HOSPITAL

Dr. D. Sudha Madhuri — Associate Professor, Department Of Microbiology, OMC/ Niloufer Hospital 11-4-721, Red Hills, Lakdikapul, Hyderabad, Telangana 500004

Dr. Chandana Konda — Senior Resident, Department Of Microbiology, OMC/ Niloufer Hospital 11-4-721, Red Hills, Lakdikapul, Hyderabad, Telangana 500004

ABSTRACT

INTRODUCTION: Dermatophytes are keratinophilic fungi affecting 20–25% of the world's population, with higher prevalence in tropical countries like India.

AIMS AND OBJECTIVES: To determine prevalence of dermatophytoses and clinical correlation with laboratory diagnosis of dermatophytes.

MATERIAL &METHODS: A total of 75 skin, hair and nail samples from clinically suspected cases of dermatophytoses, in all age groups and both sexes were collected and subjected microscopy and culture.

RESULTS: Out of 75 samples tested, 35 were positive for dermatophytes. Prevalence of dermatophytoses is 46.66%.21-30 age group affected more with M: F=1.6:1. Most common clinical type wasTinea corporis (34.66%) followed by Tinea cruris (26.66%) and Tinea unguium (20%). T.rubrum (28.57%) was the major isolated pathogen followed by T.tonsurans (22.85%) and T.mentagrophytes (20%).

CONCLUSION: T. rubrum's ability to survive on the skin surface and to evade host defenses accounts for the high prevalence of infections with this fungus.

KEYWORDS : Dermatophytes, Dermatophyte Test Medium. T.rubrum

INTRODUCTION

Dermatophytes are keratinophilic fungi which typically infect skin, hair and nails and are unable to penetrate deeper than the stratum granulosum.[1,2] 20–25% of the world's population are affected with dermatophytoses.[3] Prevalence of dermatophytosis is more in India with favorable tropical climate like hot, humid weather and other associated factors like poverty, poor hygiene and overcrowding.[4] Dermatophytes comprise three anamorphic (asexual or imperfect) genera, *Microsporum, Trichophyton*, and *Epidermophyton*. Trichophyton species affect skin, hair, and nails.Microsporum species affect skin and hair and Epidermophyton species affect skin and nails.[5]Clinically dermatophytosis presents with ring like lesions commonly known as "Tinea "or "Ringworm". According to the site of involvement, these have been categorized into Tinea corporis (ring worm of the body), Tinea cruris (ringworm of the groin), Tinea capitis (ringworm of scalp and hair), Tinea pedis (ringworm of the feet), Tinea barbae (ringworm of the beard), Tinea manuum (ringworm of hand), and Tinea unguium (ringworm of nail). [6] Transmission of dermatophytes occurs by direct contact with infected humans or animals or indirectly by contact with contaminated fomites.[7] Though, not life-threatening, dermatophytoses can cause great discomfort. Correct diagnosis of the dermatophytosis is essential to initiate treatment and also for epidemiological purposes.

Aims and objectives:

1. To determine the prevalence of dermatophytoses
2. Clinical correlation with laboratory diagnosis of dermatophytes.

Materials and Methods

75 clinically suspected cases of dermatophytoses, attending the outpatient department of Dermatology, Venereology and Leprosy at Gandhi Hospital, Musheerabad, were enrolled into the study. Demographic data like age, sex, occupation, duration of presenting complaints, site of involvement and family history were collected. It is a prospective cross sectional study conducted from March 2014 to April 2015. Patient consent and permission from Institutional ethics committee was obtained.

Inclusion criteria

All patients with clinical suspicion of dermatophytoses in all age groups and both sexes.

Exclusion criteria

Patients with dermatophytoses on antifungal drugs and those with secondary bacterial infection.

Specimen collection: Skin, hair and nail samples were collected from clinically suspected cases of dermatophytosis under aseptic conditions into the folds of sterile filter paper and transported to the microbiology laboratory.

Skin - The skin lesions were scraped from center to edge of the infected area with a sterile blunt edge of scalpel.

Hair - In Tinea capitis and Tinea barbae, the hair with basal root portion was plucked using sterile tweezers and small portions of hair roots were epilated. Dull broken hairs were plucked from the margin of the lesion and cut into small pieces.

Nail - In Tinea unguium infection, close clipping of the infected nail and nail bed were collected by a vigorous scrapping of the nail bed and the underside of the nail plate after cleaning the affected area with 80% ethanol.

Laboratory diagnosis: All samples were subjected to microscopy and culture.Out of 75 samples in study population, 54 were skin scrapings, 18 were nail clippings and 3 were hair pluckings.

KOH wet mount: A clean glass slide is taken and a drop of KOH is placed in the centre. A small amount of sample is added to the drop and covered with cover slip.10% KOH was used for skin and hair samples (for10-15 minutes) and 40% KOH for nail (overnight).On microscopy (40x), branched hyaline septate hyphae seen and in few samples arthrospores were observed.

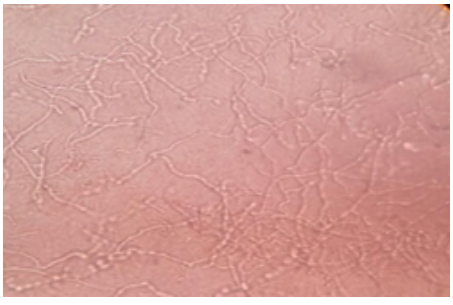

Figure 1: KOH mount -hyaline septate hyphae

Primary isolation of dermatophytes: All clinical specimens were inoculated in duplicate on Sabouraud's dextrose agar tubes (with cycloheximide (0.05g/L) and chloramphenicol (0.005g/L)) and Dermatophyte Test Media (DTM) and incubated at Room Temperature (RT,20-25°c) and 37° C for a period of 6-8 weeks. Observed every day initially for one week followed by weekly observation

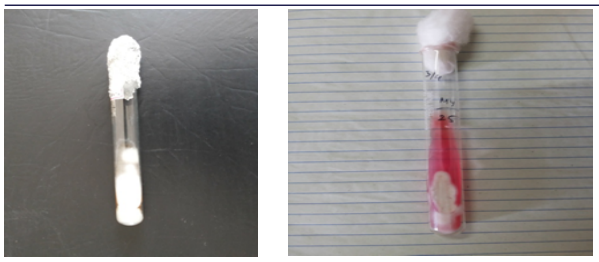

Figure 2: Growth on SDA and on DTM

Identification and speciation of dermatophytes:
Any fungal growth was identified up to the species level based on colony morphology) pigmentation (obverse and reverse), growth rate, microscopy (LPCB).All species of dermatophytes showed color change in Dermatophyte Test Media (DTM) from yellow to red.

Lacto phenol Cotton Blue (LPCB)
Dermatophyte culture growth was picked up using a sterile L-loop and placed on the drop of LPCB, teased gently and covered with coverslip. Typical morphological details like septate hyphae, macroconidia, microconidia & their arrangement were noted under 40x magnification.

Culture characteristics and microscopic morphological details observed in each species isolated.
Trichophyton species were characterised by rare, smooth macro conidia and abundant microconidia.

Trichophyton rubrum: Trichophyton rubrum was the most frequently isolated species

Colony surface is fluffy, white to rose pink and reverse is dark pink to brown.

Microscopy shows septate hyphae with numerous club shaped or tear drop micro conidia borne singly along the hyphae representing "bird on a fence" appearance. Pencil shaped macro conidia seen in few.

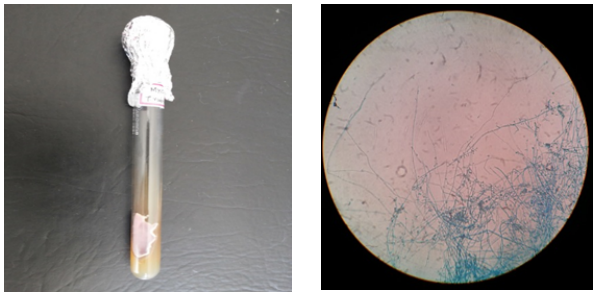

Figure 3: Trichophyton rubrum colony morphology and LPCB Mount

Important features observed in other Trichophyton species:
T.tonsurans- Tear drop micro conidia with balloon forms, intercalary and terminal chlamydospores

T .mentagrophytes - spiral hyphae

T.verrucosum - chlamydospores in chains and antler like branches

T.violaceum - Hyphae tangled, branched, irregular with chlamydospores

Microsporum species were characterized by numerous thick walled and rough macroconidia and rarely microcinidia.

M.gypseum was isolated showing septate hyphae with numerous macro conidia with no more than 6 compartments and rare microconidia

Epidermophyton species has numerous, smooth walled macroconidia and microconidia are absent.

E.floccosum was isolated where septate hyphae with club shaped macro conidia, containing two to six cells, singly or in clusters were seen. Micro conidia were absent.

Dermatophyte species were further confirmed based on urease test

Urease test: This test is to differentiate between T.mentagrophytes and T.rubrum Christensen's urea agar slant was inoculated with the test fungus observed for the colour change in the medium. with the growth of T.mentagrophytes

RESULTS AND DISCUSSION:

Clinical correlation with Culture results
Out of 75 clinically suspected Samples tested, 50 were positive by culture and 25 were negative for culture. Out of 50 positive cultures 35 were dermatophytes and 15 were non dermatophytes.Prevalence of dermatophytoses is 46.66%.

Our study correlates with the study by Sumit kumar et al, 2014[8], (42.4%) and Krishna Santhosh et al, 2015[9], (46.97%). Majeed et al [10](49.33%), Ebrahimkutty SP et al 2018 [4](43.5%) while other studies showed rates ranging from 39% to 58%.

Table 1: Age and gender wise distribution of culture positive cases in study population.

Out of 42 samples from male patients 22 (52.38%) were positive and out of 33 females, 13 (39.39%) were positive for dermatophytes with M: F=1.6:1 and more common in 21-30 age group just as clinically suspected cases of dermatophytoses.

Age group in years	Males 42	Females 33	Total no of cases enrolled	Males culture positive	Females culture positive	Total culture positive cases
up to 20	5	4	9(12%)	4	2	6(8%)
21 – 30	18	8	29(38.66%)	8	5	13(17.33%)
31 – 40	8	12	17(22.66%)	7	4	11(14.66%)
41 – 50	4	4	8(10.66%)	2	1	3(4%)
51 – 60	4	2	6(8%)	1	1	2(2.66%)
> 60	3	3	6(8%)	0	0	Nil
TOTAL	**42**	**33**	**75(100%)**	**22**	**13**	**35(46.66%)**

Table 2: Distribution of clinical lesions based on site:

CLINICAL DIAGNOSIS	Males (n=42)	Females (n=33)	Percentage (n=75)
Tinea capitis	1(2.38)	1(3.03%)	2(2.66%)
Tinea facei	3(7.14%)	1(3.03%)	4(5.33%)
Tinea barbae	2(4.76%)	-	2(2.66%)
Tinea corporis	12(28.57%)	14(42.42%)	26(34.66%)
Tinea manuum	2(4.76%)	0	2(2.66%)
Tinea cruris	12(28.57%)	8(24.24%)	20(26.66%)
Tinea unguium	8(19.04%)	7(21.21%)	15(20.00%)
Tinea pedis	2(4.76%)	2(6.06%)	4(5.33%)
Total	42	33	75

Most common clinical type observed was Tinea corporis (34.66%) followed by Tinea cruris (26.66%) and Tinea unguium (20%).This coincides with the study by Majeed et al, 2016 Kerala, Ebrahimkutty SP et al[4].The most common clinical type was Tinea corporis 75 (50%) followed by Tinea cruris 40 (26.67%).

Table 3: List of dermatophytes spp isolated from different clinical samples

Dermatophyte	Skin n=54 (72%)	Nail n=18(24 %)	Hair n=3(4%)	Total% n=75	Percentage % (n=35)
T.rubrum	9(16.66%)	1(5.55%)	0(0%)	10(13.33%)	28.57%
T.tonsurans	8(14.81%)	0(0%)	0(0%)	8(10.66%)	22.85%
T.mentagrophytes	7(12.9%)	0(0%)	0(0%)	7(9.33%)	20%
T.verrucosum	5(9.25%)	1(5.55%)	0(0%)	6(8%)	17.14%
T.violaceum	1 (1.85%)	0(0%)	1(33.3%)	2(2.66%)	5.71%
E.floccosum	1(1.85%)	0(0%)	0(0%)	1(1.33%)	2.85%
M.gypseum	1(1.85%)	0(0%)	0(0%)	1(1.33%)	2.85%
Total	32(59.25 %)	2(11.11 %)	1(33.33 %)	35(46.66%)	70%

In our study, skin lesions were the predominant clinical presentation72% followed by nail 24% and hair 4%. Our study coincides with the study by Bhatia V.K et al [11] in which skin comprises of 72.77%, nail 23.26% and hair 3.96%.

In our study T.rubrum (28.57%) was the major pathogen, 9 were isolated from skin and 1 from nail followed by T.tonsurans (22.85%) and T.mentagrophytes (20%). This correlates with studies by (Lakshmanan et al, 2015, [12] Surendran et al, [13]2014, Jitendra Kumar Chaudhary et al [14], 2016 where T.rubrum was the most common isolated pathogen. Majid et al, 2016 reported T.tonsurans as the second most common isolate after T.rubrum in their study. Other studies reported T.mentagrophytes as commonest agent. (Sowmya Nasimuddin, 2014[15], Vikesh Kumar Bhatia et al 2014, Venkatesh V N et al, 2016 [16])

In a study period of one year out of 75 clinically suspected cases studied, prevalence of dermatophytosis is 46.66%.Tinea corporis (34.66%) was commonest clinical presentation followed by Tinea cruris (26.66%) and Tinea unguium (20%). Men were more commonly affected (56 %) than women (44 %) predominantly in age group of 21 – 30 years. Most common isolate was T.rubrum followed by T.tonsurans. T. rubrum's ability to infect and its ubiquitous presence account for the high incidence of infections. T. rubrum is especially suited to survive on the skin surface and also its ability to evade host defenses, accounts for the high prevalence of infections with this fungus.

Acknowledgement: We express our gratitude to the Department of Dermatology, Gandhi hospital for extending their support and co-operation in sample collection and laboratory staff of the Department of Microbiology for their assistance throughout the work at Gandhi Medical College and Hospital.

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