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A Study On The Prevalence Of Dermatophytosis In Patients In A Tertiary Care Centre

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Abstract

Introduction: Dermatophytosis is an infection of the hair, skin, or nails caused by a dermatophyte, which is most commonly of the Trichophyton genus and less commonly of the Microsporum or Epidermophyton general. Skin infections due to dermatophytes have become a significant health problem affecting children, adolescents, and adults. Accurate diagnosis is important to initiate appropriate treatment and is also essential for epidemiological purposes.

Aim of The Study: To Find out the prevalence of dermatophytosis in patients attending tertiary care hospitals.

Material And Methods: This Observational Cross-sectional study was done on 130 suspected dermatophytosis cases who are Attending the outpatient department of Sri Lalithambigai Medical College and Hospital Adayalampattu, Maduravoyal, Chennai in June 2022 Direct KOH mount was done for all the specimens and culture was done in Sabouraud's dextrose agar, containing chloramphenicol (0.04gms/litre) and cycloheximide (0.5g/litre). For observing the microscopic appearance, using a teasing needle, mounts from the culture were made in Lactophenol cotton blue [LCB]. Slide culture was done when needed.

Result: Clinically the prevalence of dermatophytosis was 13%, it was observed more in males. *T.rubrum* was the commonest species of dermatophyte isolated, which presented as *Tinea corporis.*

Conclusion: This study focused on the variations in dermatophytosis presentation and the species involved and found that *Trichophyton rubrum* was the most common affecting the present population.

Keywords: Dermatophytes, Tinea rubrum, Tinea corporis, superficial mycoses

Introduction

Fungi are widely found in the environment and most of them are harmless commensals, contaminants, or nonpathogenic agents. There are at least 100,000 named species of fungi of which less than 500 are associated with human and animal diseases. A major contributor to the emergence of fungal infections is the increasing number of immunocompromised individuals, especially those with Acquired immunodeficiency syndrome.[1] The fungi are now recognized as a significant cause of morbidity and mortality among humans. They have emerged as an important etiological agent of opportunistic infections. Fungal infections are broadly classified as superficial, subcutaneous, and systemic mycosis. Superficial or cutaneous mycosis is caused by fungi that infect only the superficial keratinized tissue (skin, hair, and nails). [3]The most important of these are dermatophytes. [2]Skin infections due to dermatophytes have become a significant health problem affecting children, adolescents, and adults. Accurate diagnosis is important to initiate appropriate treatment and is also essential for epidemiological purposes [4]According to Emmon's morphological

classification, the dermatophytes are classified into three anamorphic genera -Trichophyton, Microsporum, and Epidermophyton based on conidial morphology. Some species of dermatophytes are endemic in certain parts of the world and have limited geographic distribution. [5]The increasing mobility of the world's population is disrupting several epidemiological patterns. Some dermatophytes like E.floccosum, T.rubrum, and T.tonsurans are globally distributed. Dermatophyte infections can be treated effectively if a timely and proper diagnosis is made.[6] The different syndromes of ringworm infections require different treatment regimens. Generally, topical therapies are used for localized or mild infections and oral antifungals for more extensive infections in addition to topical therapy. [7]The newer Azoles such as Fluconazole or Itraconazole and Terbinafine are now the preferred oral drugs to griseofulvin for extensive or severe dermatophytosis. Although Griseofulvin is cheaper it is less effective. Though various topical and systemic agents are available for the treatment of dermatophytosis, chronic dermatophytosis is commonly seen. Chronic Dermatophytosis is a refractory condition that runs for one year with remissions and exacerbations.[8,9]

Material And Methods: This Observational Cross-sectional study was done on 130 suspected dermatophytosis cases who are Attending the outpatient department of Sri Lalithambigai Medical College and Hospital Adayalampattu, Maduravoyal, Chennai in June 2022.

Inclusion Criteria:1. Suspected dermatophyte lesions of the skin, hair, and nails of HIV-infected and uninfected patients were considered for the study irrespective of a) age, b) sex, and c) socioeconomic status. **Exclusion Criteria:**1.All other superficial mycosis like Tinea versicolor, and piedra were excluded from the study.2.Patients with a history of diabetes, malignancy, and patients taking immunosuppressive therapy were excluded from the study. For the clinically diagnosed dermatophytosis, case details regarding age, sex, occupation, educational status, socioeconomic status, and personal hygiene were recorded. The type of lesion and the area of distribution of the lesion was noted. The specimens collected were skin scrapings, hair clippings scalp scrapings, and nail clippings. The affected areas were cleaned with 70% alcohol and specimen of skin, hair, and nail were taken with the help of a sterile scalpel and scissors as the case may be. Scraping was done after wearing protective gloves. In the case of skin lesions, with the help of a sterile scalpel, scrapings were taken from the margin of the lesions as the center of the lesion usually heals quicker and the margin had the proliferative and growing fungal elements. In the case of hair, the affected hair shafts were taken including the roots, and scrapings were taken from the surrounding scalp area. Nail clippings were taken using a nail cutter and the subungual area was scraped using a blunt scalpel. Care was taken to obtain nail material from the advancing infected edge closest to the cuticle, where the likelihood of viable hyphae is the greatest. The specimens were collected in individual sterile folded squares of paper that permitted drying of the specimen, reduced the bacterial contamination, and also provided conditions under which specimens may be stored for long periods without appreciable loss in the viability of ringworm fungi. Processing of specimens was done on the same day as the collection of specimens. The samples were studied directly in 10% potassium hydroxide and examined under the microscope for evidence of branching septate hyphae, and arthrospores.KOH preparation was made by emulsifying the specimen in a drop of 10% KOH on a clean microscopic slide. The KOH specimen mixture was allowed to stand for 10 minutes before examination in the case of skin and about 30 minutes in the case of nails and hair at room temperature. A coverslip was applied and the specimen was examined for the presence of narrow regular hyphae that characteristically break up into arthroconidia. In hairs, endothrix and ectothrix types of infection were observed. After examining the KOH mounts, the remaining specimen was used for culture. All specimens were cultured irrespective of the direct KOH mount result. The standard media for primary isolation of dermatophyte namely Sabouraud's dextrose agar, containing chloramphenicol (0.04gms/litre) and cycloheximide (0.5g/litre) was used. The specimens were inoculated onto two sets of Sabouraud's dextrose agar and in each, more than four implants were made for increasing the chances of isolation. One inoculated slant was kept at room temperature and the other was incubated at 370C. The cultures were maintained for 30 days before discarding them as negative. Growth

Volume 5, Issue 6; November-December 2022; Page No 132-139

was relatively slow, usually, seven days to three weeks were required. When growth became evident on the primary isolation media, fungi were identified macroscopically based on colony appearance, pigmentation, consistency [granular, gritty, or velvety nature of the colony] and microscopically by the appearance of conidia – both micro and macro, that were variable from one species to another. For observing the microscopic appearance, using a teasing needle, mounts from the culture were made in Lactophenol cotton blue [LCB]. With a pair of dissecting needles, a small portion of the colony was taken and placed on a microscopic slide in a drop of LCB. Then the colony was teased apart with a needle. A cover slip was placed over the specimen and gentle pressure was applied on the surface of the coverslip to disperse the mount. The preparation was then examined under 10 x and 40 x objectives.

Culture Method: A sterile Petri dish of 135 mm size was taken and labeled with the specimen number and date of inoculation. A grease-free sterile glass slide was placed above and from the already prepared Sabouraud's agar, a square block of agar was cut and placed on the glass slide and from the primary inoculation of the growth of the concerned fungus, the sides of the agar block was inoculated. This was covered with sterile cover glass. 20% glycerol was kept in a sterile screw cap of the universal container inside the Petri dish to keep the atmosphere humid. This was incubated at 26oC for one to two weeks by which time adequate growth of the fungus was obtained. The slide culture allowed observation of the fungus while it was growing. When spores were evident, a lactophenol cotton blue mounting was made on the coverslip after gently lifting it with sterile forceps. Then the agar block was removed and the slide was stained with LCB which served as the second mount. The primary fungal growth was further subcultured on Potato dextrose agar for bringing out better pigmentation and improving conidiation. The urease agar media was used to differentiate between T.rubrum and T.mentagrophyte species. Descriptive statistics like mean and percentages were used to infer results with spss 19.0.

Results:

Table – 1 Age-Wise Distribution

AGE IN YEARS	Cases in Non-HIV	%	Cases in HIV	%
0 - 10	5	3.84	-	-
11 – 20	11	8.46	-	-
21 - 30	21	16.15	11	44
31 – 40	**53**	**40.76**	**14**	**56**
41 - 50	28	21.54	-	-
51 - 60	12	9.23	-	-

Table :1 shows It was observed that the highest number of dermatophytosis was seen in the age group of 31-40 years in both non-HIV and HIV cases. 130 samples from non-HIV cases were analyzed, sex-wise and it was found that 81 were males (62.3%) and 49 were females (37.7%). Out of 25 HIV cases, 18 (72%) were males and 7 (28 %) were females.

Table – 2 Cd4 Count In Plha Cases With Dermatophytosis

CD4 count	No.of cases	Percentage
0 – 99	5	20

Volume 5, Issue 6; November-December 2022; Page No 132-139

100 – 199	6	24
200 – 299	6	24
300 – 399	4	16
400 – 499	4	16

Table:2 For the 25 HIV cases with dermatophytosis, CD4 counts were analyzed. 5 cases (20%) were with CD4 count 0 - 99 and 6 cases (24%) were with CD4 100-199 and CD4 200-299 each and 4 cases (16%) were with CD4 count 300-399 and 400 to 499 each.

Table – 3 Clinical Presentation

DIAGNOSIS	Non-HIV	%	HIV +	%
Tinea corporis	**74**	**56.9**	**15**	**60**
Tinea cruris	37	28.5	9	36
Tinea facei	7	5.4	1	4
Tinea capitis	5	3.8	-	-
Tinea ungium	7	5.4	-	-
Total	130	100	25	100

Table :3 It was observed that the commonest clinical lesion in this study was tinea corporis (56.9%) followed by tinea cruris(28.5%) in both non-HIV and HIV + patients. It was observed that tinea corporis was the predominant clinical presentation in both HIV and non-HIV, the common age group being 21- 40 years. Tinea capitis was the only clinical presentation of dermatophytosis seen in the age group 0-10 years.

Table –4 Direct Koh Mount And Culture Positivity

Diagnostic Methods	No.of Samples (Non-HIV)			No. of Samples (HIV)		
	Collected	Positive	%	Collected	Positive	%
KOH Mount Positive	130	112	86.15	25	21	84

Volume 5, Issue 6; November-December 2022; Page No 132-139

Culture Positive	130	100	76.92	25	17	68

Table:4 KOH positivity was more than culture positivity in HIV and non-HIV cases.

Table –5: Dermatophyte Species Isolated

SPECIES	Non-HIV	%	HIV	%
T. rubrum	**74**	**74**	**13**	**76**
T. mentagrophytes	22	22	4	24
T. violaceum	2	2	-	-
E. floccosum	1	1	-	-
M. gypseum	1	1	-	-
Total	100	100	17	100

Table: 5 On analyzing the species involved in various dermatophytes in both non-HIV and HIV cases, it was seen that in non-HIV cases T.rubrum was isolated from 47 tinea corporis cases,(63.5%), 22 tinea cruris cases (59.5%), 1 from tinea facei and 4 from tinea unguium. T.mentagrophytes was isolated from 14 tinea corporis cases, 3 tinea cruris, 1 tinea capitis and 4 tinea facei. T. violaceum was isolated from 2 tinea capitis cases. E. floccosum was isolated from one tinea cruris case and M.gypseum from 1 tinea facei case.

Table – 6 Species Involved In Various Dermatophytosis (N)

Species	T.corporis	T.cruris	T.capitis	T.facei	T.unguium
T.rubrum	47(63.5%)	22(59.5%)	_	1(14%)	4(57%)
T.mentagro phytes	14(18.9%)	3(8.1%)	1(20%)	4(57%)	_
T.violaceum	_	-	2(40%)	_	_
E.floccosum	_	1(2.7%)	_	_	-

Volume 5, Issue 6; November-December 2022; Page No 132-139

M.gypseum	_	_	_	1(14%)	_

Table:6 shows that tinea corporis and tinea cruris are predominantly caused by T.rubrum in non-HIV cases. All tinea unguium cases are caused by T.rubrum. T.violaceum was involved in T.capitis and E.floccosum in T.cruris only. Of the 17 isolates from HIV patients, T.rubrum was isolated from 9 (60%) tinea corporis cases and 4 (44%) tinea cruris cases.T.mentagrophytes was isolated from 2 (22%) of tinea cruris and 1 (7%) of tinea corporis cases.

Table – 7 Species Involved In Various Dermatophytosis (Hiv)

Species	T.corporis	T.cruris	T.capitis	T.facei	T.unguium
T.rubrum	9 (60%)	4 (44%)	-	-	-
T.mentagro Phytes	1(7 %)	2(22%)	-	1	-
T.violaceum	-	-	-	-	-
E.floccosum	-	-	-	-	-
M.gypseum	-	-	-	-	-

Table: 7 In antifungal susceptibility testing, Terbinafine was put up in dilution from 0.002 µg to 1 µg. In this study, the MIC of T.rubrum and T.mentagrophytes against terbinafine in both HIV and non-HIV was in the 0.002 to 0.03 µg range. Fluconazole was put up in dilutions from 0.06 µg to 32 µg. The MIC of T.rubrum and T.mentagrophytes against Fluconazole was in the 0.5 to 4 µg range

Table – 8 Antifungal Susceptibility Testing

A.Fluconazole MIC Sensitivity Range (0.5 to 4 µg)

	Non-HIV					HIV				
Species	No.	S	%	R	%	No.	S	%	R	%
T. rubrum	13	11	85	2	15	13	13	100	-	-
T. mentagrophytes	4	4	100	-	-	4	4	100	-	-

S = Sensitivity, R = Resistant

B.Terbinafine MIC Sensitivity Range (0.002 to 0.03 µg)

	Non-HIV					HIV				
Species	No.	S	%	R	%	No.	S	%	R	%
T. rubrum	13	13	100	-	-	13	13	100	-	-
T. mentagrophytes	4	4	100	-	-	4	4	100	-	-

Volume 5, Issue 6; November-December 2022; Page No 132-139

S = Sensitivity, R = Resistant

TABLE:8 It was seen that all isolates of T.rubrum and T.mentagrophytes were sensitive to Terbinafine and the MIC range of Terbinafine is less than fluconazole.

Discussion

Impaired cell-mediated immunity is one of the various factors that play a role in the acquisition of dermatophytosis. Rook's textbook of dermatology says, there is strong evidence that the development of cellular immunity via sensitized T lymphocytes is a key factor in immunological defense against dermatophytosis. HIV is one disease that shows definite defective cell-mediated immunity. So in this study, the prevalence of dermatophytosis in non-HIV and HIV patients was compared. [10] A similar study by G.Venkatraman et al also revealed that there is no relationship between HIV infection or reduced CD4 count and the prevalence of dermatophyte infection. In this study, though the prevalence was the same clinically in HIV and non-HIV cases, severe and invasive lesions were seen in HIV cases while the lesions were restricted to the stratum corneum in non-HIV cases. [11] This correlates with Rao A et. aL studies where it is given that dermatophytosis in the immunocompromised host is more severe than in the immunocompetent host. In non-HIV cases, nonspecific host defense mechanism like activation of serum inhibiting factors and complement prevents invasiveness of dermatophytes.[12] In HIV, this immune mechanism is impaired which leads to the invasiveness of dermatophytes. In this study, it was observed that 40.76% of non-HIV and 56.25% of HIV cases were affected with Dermatophytosis in the age group 31-40 years. [14]A similar study by Rinaldi MG. also showed that the common age group involved in Dermatophytosis is 21-40 yrs. their study also reported that the mean age of dermatophytosis in HIV cases was 30.7 years. The present observation correlates with previous publications. The mean age of 30 years is the period where the laborers exert more physically, resulting in increased perspiration which produces a hot, humid, environment in the body, favoring the growth of Dermatophytes.[15] Excessive perspiration also washes away fungus-killing oils in the skin making it more prone to dermatophyte infection. In the present study, non-HIV males [62.3%] were more affected than non-HIV females [37.7%]. [16]. In the present study, the male cases were mostly laborers and coolies working in sunlight most of the time leading to profuse sweating which in turn resulted in increased dermatophyte infection. In this study, tinea capitis was seen in 3.8% of non-HIV cases and no tinea capitis was seen in HIV cases. [17]All tinea capitis cases were seen in the age group of 0 - 10 years. This corresponds to the study by Philpot, in which he reported that tinea capitis was a disease in children. He proved that post-pubertal changes in hormones resulted in acidic sebaceous gland secretions which were responsible for the decrease in incidence with age.[18] In the present study, there were no HIV-positive children in the age group 0-10 years and so no T.capitis cases were seen in HIV cases.[19,20]

Conclusion:

In this study, the diagnosis of dermatophytosis in non-HIV and HIV cases was made by demonstrating dermatophytes under a microscope by KOH mount and culturing the specimen on SDA with cycloheximide media and proved that direct KOH mount was found to be a good screening test for dermatophytosis because 86.2% of non-HIV and 84% of HIV samples were positive in KOH mount while 76.9% of non-HIV and 68% of HIV cases only were positive in culture. The isolation of fungus by culture depends on the quality of media, the pH maintenance, the time of collection and processing of specimens, and certain other environmental factors. In the present study, the media used was Hi Media's Cycloheximide agar and processing was done on the same day itself, which contributed to the increased isolation by culture. Culture positivity also depends on the viability of the organism, sufficiency of the sample, site of sample collection, and proper processing of samples. As all these were followed in this study the isolation rate was high. In the present study, the commonest species isolated from both HIV and non-HIV cases was T.rubrum followed by T.mentagrophytes [22%]. The Antifungal susceptibility testing of fluconazole and Terbinafine revealed that fluconazole was effective with a MIC

Volume 5, Issue 6; November-December 2022; Page No 132-139

range of 0.5 to 4 µg and terbinafine was effective with a MIC range of 0.002 to µg. All T.rubrum and T.mentagrophytes isolates from HIV and non HIV samples were sensitive to terbinafine. Two T.rubrum isolates from non HIV sample were resistant to fluconazole. So in this study Terbinafine was found to be more sensitive than fluconazole. Terbinafine inhibits squalene epoxidase thereby suppressing ergosterol biosynthesis and causing toxic accumulation of squalene in the fungal cell wall. Fluconazole inhibits cytochrome-dependent 14 alpha methylation and affects cell membrane synthesis. Because of the toxin accumulation, the fungus is killed by Terbinafine, whereas fluconazole inhibits the multiplication of the fungus by affecting cell membrane synthesis. Hence Terbinafine can be considered a more effective drug than fluconazole in the treatment of dermatophytosis

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Volume 5, Issue 6; November-December 2022; Page No 132-139