

Clinico-mycological study of dermatophytic infections and their sensitivity to antifungal drugs in a tertiary care hospital

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ABSTRACT

Background: Dermatophytosis is a very common superficial fungal infection prevalent in tropical and subtropical countries. Decreased drug responsiveness and non-compliance of the patient is responsible for its chronicity. As there is increased incidence and resistance to antifungals, mycological study and antifungal sensitivity testing is the need of the day.

Methods: This was a six months cross sectional observational study which included 50 patients, clinically suspected cases of dermatophytic infection. A detailed history with general physical examination and sample collection for mycological examination was done. Skin scrapings were examined under direct microscopy by KOH mount and culture was done on Sabouraud's dextrose agar. The isolated species were subjected to antifungal susceptibility testing by agar-based disc diffusion method.

Result: Out of 50 cases of dermatophytosis, 24 (48%) were males and 26 (52%) were females. Tinea corporis was the most common type observed (found in 21 cases). Out of isolated dermatophytes, *Trichophyton mentagrophytes* was the most common isolate that is 50%, followed by *Trichophyton tonsurans* (26%), *Microsporum gypseum* (11%), *Trichophyton rubrum* (7%), *Trichophyton schoenleinii* (4%). Antifungal susceptibility testing showed the drug Itraconazole to be the most sensitive, while fluconazole the least sensitive drug.

Conclusion: Inadequate use of antifungal and non-compliance of patients has led to the emergence of resistance, causing poor treatment outcomes. So, for proper treatment, it is very important to detect the prevalent fungal species and performing antifungal sensitivity to find the most effective antifungal.

KEY WORDS: Dermatophytosis, Tinea, Antifungal, Sensitivity

INTRODUCTION

Dermatophytes are the most commonly encountered fungi in humans and other vertebrates that spread through direct contact with infected humans, animals, and soil.¹ Infections due to these agents are usually restricted to the stratum corneum and are generally referred as 'tinea' or 'ring-worm' (tinea capitis; tinea barbae; tinea corporis;

tinea cruris; tinea manuum; tinea pedis and tinea unguium).^{2,3}

Recalcitrant dermatophytosis refers to relapse, recurrences, reinfection, persistence, or chronic infections, and possibly microbiological resistance.⁴

The study was done to identify the clinico-mycological pattern of dermatophytic infections in our region and to obtain sensitivity of isolated derma-

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tophytes against five antifungals (Itraconazole, Fluconazole, Amphotericin B, Ketoconazole, Clotrimazole)

AIM AND OBJECTIVES

To identify the clinico-mycological pattern of dermatophytic infections in patients attending the outpatient department of dermatology of a tertiary care center and to obtain sensitivity of isolates against five antifungals (Itraconazole, Fluconazole, Amphotericin B, Ketoconazole, Clotrimazole).

METHODS

The study was conducted on 50 patients with dermatophytic infections, attending Dermatology OPD of a tertiary hospital, in Jaipur, Rajasthan from 1 Jan 2019 to 30 June 2019. Detailed history and clinical examination were carried out. Scrapings obtained from skin/nail/hair were taken for KOH examination and culture on Sabouraud's dextrose agar. All KOH and culture positive samples were evaluated for further data analysis. Specimens were inoculated on Sabouraud's dextrose agar with cycloheximide and chloramphenicol. Test tubes were incubated at 25°C for four weeks irrespective of their KOH results. Species were identified through colony morphology and microscopy on lactophenol cotton blue mount. Isolated dermatophytes were subjected to Antifungal Sensitivity Testing by agar-based Disc Diffusion method. (Tables 1 & 2)

Table 1

Methods of Antifungal Drug Susceptibility testing
Macro- Broth Dilution
Micro- Broth Dilution
Agar Dilution
Disc Diffusion Method

Table 2

Technique	Advantages	Disadvantages
Macro broth dilution	Accurate reproducible results	Technically laborious
Micro broth dilution	Gold standard technique according to standard guideline, but till now only CLSI technique has been more evaluated against dermatophytes	Costly
Agar Dilution	Easy to perform Relatively Cheaper	Not recommended to test dermatophytes using standard guidelines
Disc Diffusion	Easy to perform Relatively Cheaper	Not recommended to test dermatophytes using standard guidelines

RESULTS

Out of 50 suspected cases of dermatophytosis, 24 (48%) were males and 26 (52%) were females. Females were more affected than males with male to female ratio of 1:1.08. Most of the affected patients, belonged to the age group of 21-30 years (16 patients; 32%), followed by 41-50 years is (15 patients; 30%) (shown in Table 3).

Table 3

Age group (years)	Males	Females	Total
1-10	0	1	1
11-20	5	1	6
21-30	7	9	16
31-40	7	4	11
41-50	4	11	15
51-60	1	0	1
	24	26	50
	48%	52%	100%

Forty seven out of 50 samples were positive for KOH mount, while only 26 were culture positive,

rest were culture negative (shown in Table 4).

Table 4

Culture	Males	Females	Total
Positive	14	12	26
Negative	10	14	24

Most common clinical finding was Tinea corporis (21 cases), followed by Tinea cruris (18 cases), Tinea Pedis and faciei (8 cases) and Tinea manu-

um (5 cases).

Out of isolated dermatophytes, *Trichophyton mentagrophytes* was the most common isolate that is 50%, followed by *Trichophyton tonsurans*, 26%, *Microsporum gypseum*, 11%, *Trichophyton rubrum*, 7%, *Trichophyton schoenlenii*, 4%.

Out of the 5 antifungals, Itraconazole was found to be the most sensitive and fluconazole least sensitive drug (Table 5)

Table 5

Antifungal drugs		<i>T. tonsurans</i>	<i>T. schoenlenii</i>	<i>M. gypseum</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
Itraconazole	S	6	1	3	12	2
	R	1	0	0	1	0
Fluconazole	S	1	1	0	0	0
	R	6	0	3	13	2
Amphotericin B	S	2	0	0	3	0
	R	5	1	3	10	2
Ketoconazole	S	1	0	0	2	0
	R	6	1	3	11	2
Clotrimazole	S	0	0	0	4	0
		7	1	3	9	2

DISCUSSION

Dermatophytes belong to 3 closely related genera- *Trichophyton*, *Microsporum* and *Epidermophyton*.⁵ In India, we are presently noticing a significant rise in number of dermatophytosis cases with chronic recalcitrant disease, atypical presentations, frequent relapses, and treatment failures.⁶⁻⁹

Dermatophytosis is considered to be recurrent when there is recurrence of the disease (lesions) after 4 weeks of completion of approved systemic therapy.¹⁰ Relapse denotes the occurrence of dermatophytosis (lesions), after a longer period of infection-free interval (6–8 weeks) in a patient who has been cured clinically.¹¹ Dermatophytosis is considered to be chronic when the patients who have suffered from the disease for more than 6 months

to 1 year, with or without recurrence, in spite of being adequately treated.¹¹

Though the reason for this phenomenon is not yet clear, it is assumed that unchecked availability of cheap and irrational fixed-dose corticosteroid–antifungal–antibacterial combinations sold over the counter in India and in-vitro resistance to common antifungals (to some extent) is playing a pivotal role. Due to recent increase in the reports of antifungal drug resistance in dermatophytes, many groups have suggested to perform the antifungal drug susceptibility testing especially for the dermatophytes isolated from chronic/recurrent/recalcitrant cases or those with atypical presentations.¹²

Clinical and Laboratory Standards Institute (CLSI) has approved a micro broth dilution method for antifungal susceptibility testing of molds,

but these tests are cumbersome and difficult to be performed in routine laboratory set up. The agar based disc diffusion (ABDD) is an easy method to determine the antifungal susceptibility of dermatophytes, but data regarding these methods are scarce and not standardized.^{13,14} The application of in vitro antifungal susceptibility testing for guidance of antifungal drug therapy has been limited due to uncertain correlation between in vitro and in vivo action of drugs.^{15,16} Antifungal susceptibility testing of the isolated dermatophytes was performed by agar based disc diffusion method for 5 antifungal drugs.^{13,14} The antifungal discs were procured in readymade form from Hi-Media (Mumbai, India). They are as follows: Ketoconazole (KT): 10µg/disc, Fluconazole (FLC): 10µg / disc, Clotrimazole (CC): 10µg/disc, Itraconazole (IT): 10µg/ disc, Nystatin (NS): 100U/ disc. The isolates were sub-cultured on Potato Dextrose Agar (PDA) at 28 °C for 7 days to enhance sporulation. The growth was harvested in sterile saline and the suspension was adjusted to 1 x 10⁶ /ml using a hemocytometer. Plates of Mueller Hinton Agar (MHA) of 4mm depth were inoculated. The surface of the MHA plate was streaked in 4 different directions (90°) to cover the entire surface, then kept aside for a few minutes for drying and discs were applied using sterile forceps. *Trichophyton mentagrophytes* ATCC 9533 and *Trichophyton rubrum* ATCC 28188 strains served as control. The plates were incubated in a BOD incubator at 25°C for 5-10 days. After sufficient growth occurred, the diameters of zones of inhibition surrounding the antifungal discs were measured and results interpreted.^{13,14,17}

Morbidities of tinea infection are not only because of its frequent relapses but also due to increasing resistance to antifungal drugs, that has become a

major concern of dermatologists and patients. In this study, majority of patients were adults (20–40 years) which is the norm in previous studies too.^{18,19,20} In this study, majority of patients were 20-30 years old and male: female ratio was 1:1.08; with female preponderance like in some earlier studies with females having *T. pedis* and manuum due to kitchen and household work.^{21,22}

All cases in this study were selected on the basis of treatment taken earlier, that is if a person has used any topical application over tinea unknowingly, they were excluded from the sample.

A history of fungal infections in family members was elicited in 44% of cases of which, 36.36% were conjugal. Transmission by direct contact occurs in tinea infection also in family members, it can be due to fomites or de novo infection.²³⁻²⁷

Most common clinical finding was Tinea corporis (21 cases), followed by Tinea cruris (18 cases), Tinea Pedis and faciei (8 cases) and Tinea manuum (5 cases).

Out of 50 samples, 47 samples were positive by microscopy on KOH mount and 26 cases were culture positive that is 52%.

In our study, *T. mentagrophytes* was found most common being 50%, followed by *T. tonsurans*. According to the studies conducted in last two decades, *T. rubrum* was the most common isolate. In our study, *T. mentagrophytes* (50%) was the most common, followed by *T. tonsurans* (26%). Similar findings were observed by Sahai, Mishra and Bhatia and Sharma.^{28,29}

In present study, Antifungal Susceptibility testing was performed by agar-based disc diffusion method for 5 antifungal drugs: Itraconazole, Fluconazole, Amphotericin B, Ketoconazole and Clotrimazole.

Itraconazole was found most sensitive followed

by Amphotericin B, whereas Fluconazole was found least sensitive.

To conclude, antifungal sensitivity testing may be helpful in proper treatment of these recalcitrant tinea infections. The treatment of the disease would be more effective and meaningful when antifungal agents are prescribed based on culture and antifungal susceptibility results. A greater number of patients and long-term study is required for a better conclusion.

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