Original Article

Clinico-mycological pattern of dermatophyte infection and their sensitivity to antifungal drugs

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Abstract

Background: Most of the existing antifungal drugs failing to produce satisfactory responses against dermatophyte infection and making it difficult to cure. By assessing in vitro antifungal sensitivity of currently available antifungal drugs will help to select appropriate medicine.

Objective: This study was aimed at identifying the clinical and mycological pattern of dermatophyte infection in patients and to obtain the sensitivity pattern of the dermatophytes against five commonly used antifungals (fluconazole, itraconazole, terbinafine, griseofulvin, ketoconazole).

Methods: Patients attending the outpatient department of dermatology at Bangabandhu Sheikh Mujib Medical University (BSMMU) clinically diagnosed with dermatophytosis were enrolled in the study. The sample was collected for mycological examination and in vitro antifungal sensitivity testing was done on species isolated from culture.

Results: Tinea corporis was the most common clinical type of human dermatophyte infection. Trichophyton rubrum was the commonest (96%) dermatophyte followed by Trichophyton mentagrophyte (3%) and Epidermophyton floccosum (1%). Terbinafine (97.9%) and itraconazole (88.5%) was most sensitive, followed by ketoconazole (59.4%), fluconazole (22.9%) and griseofulvin (15.5%) against T. rubrum. Terbinafine (100%), itraconazole (66.7%), ketoconazole (66.7%) and griseofulvin (33.3%) were sensitive, and fluconazole (100%) was resistant against all cases of T. mentagrophyte species. Against E. floccosum species Terbinafine, Itraconazole, Ketoconazole and Griseofulvin were sensitive (100%) and Fluconazole was resistant (100%).

Conclusion: Terbinafin and itraconazole are sensitive against all types of dermatophytes whereas griseofulvin and fluconazole are mostly resistant.

Keywords: antifungal, resistance, sensitivity, dermatophytes.

Introduction

Dermatophytosis is one of the most common causes of human skin disease.[1] According to the World Health Organization (WHO) about 25% of the world population is affected by dermatophytes.[2] The causative species dermatophyte vary with geographic regions, some species are distributed worldwide others have partial geographic restrictions but no age or racial group is spared. Recently dermatophytes have been reclassified by multilocus phylogenetic study into seven genera: Arthroderma, Epidermophyton, Lophophyton, Microsporum, Nannizzia, Paraphyton, and

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Trichophyton.[3] Epidermophyton, Microsporum, and Trichophyton are the major cause of superficial mycosis.[4] Most commonly isolated dermatophytes in Asia are Trichophyton rubrum and Trichophyton mentagrophytes.[5]

Although dermatophytosis is not life-threatening but often causes significant morbidity and cost to society because of its chronic nature and relapse after cessation of therapy.[6] Superficial dermatophytosis is often confused with many other skin diseases e.g. eczema, atopic dermatitis, psoriasis, and lupus erythematous due to similar clinical presentation.[7]

The infection begins by attachment of arthroconidia to corneocytes with fast germination and production of germ tubes (within 4–6 hours), which grow through layers of keratin in both a horizontal and vertical direction.[8] Dermatophytes have mechanisms to defeat the host response of reducing inflammation and phagocytosis by fungal mannans, which also prevent the multiplication of keratinocytes, favoring the establishment of a persistent chronic infection.[9]

Though a good number of antifungal drugs are currently available they have only a few cellular targets. Some fungi have developed multidrug resistance (MDR) due to the overlapping mechanisms of action of the commonly used anti-fungals and also some patient factors such as negligence, discontinuation of treatment for long-term use, and the associated side effects.2 Antifungal resistances may be classified as microbiological and clinical. Microbiological resistance can be primary (intrinsic), or secondary (acquired).[10] Clinical resistance refers to therapeutic failure to eradicate a fungal infection by any antifungal agent which is found susceptible in vitro against that organism.[11]

Dermatophytes can be detected in the laboratory by direct microscopy of clinical samples and in vitro culture. Direct microscopic detection of fungal elements from the clinical samples is a rapid diagnostic technique having less specificity and sensitivity with false-negative results in up to 15% of cases.[12] In vitro culture is a specific diagnostic test but it is a slow technique and may take up to 8 weeks to give the results.[13] In vitro antifungal sensitivity test helps to predict the ability of a given antifungal agent to eradicate dermatophytes.[14]

This study was aimed to identify the clinical and mycological pattern of dermatophyte infection and to obtain the sensitivity pattern of the isolates against commonly used five antifungal drugs (fluconazole, itraconazole, terbinafine, griseofulvin, ketoconazole)by disc diffusion method.

Methods:

This study was conducted on patients having dermatophyte infections attending the dermatology outpatient department of Bangabandhu Sheikh Mujib Medical University (BSMMU) from January 2017 to January 2018. In this laboratory-based experimental study samples (skin/nail/hair) were collected from 136 clinically suspected cases of dermatophytosis for microscopic examination of potassium hydroxide preparation and culture in sabouraud dextrose agar medium. Among them, 100 samples those were positive in both microscopy and culture were included for further data analysis. The area of the skin lesion was cleaned with 70% alcohol to remove surface contamination. Gentle scraping was done from the erythematous, peripheral, actively growing margins of the lesions (scale, crust, vesicle, or pustule). An open sterile dry petri dish was held immediately below the sampling area and skin scales were flaked into it. In the case of multiple lesions, the most recent affected area was chosen for sample collection and in the vesicular lesion, the tops of fresh vesicles were taken as specimens. In the case of nails; clinically abnormal nails were cleaned with 70% alcohol and nail clipping was collected from several nails when more than one nail was affected. In the case of hair; plucked hair was taken. The samples were first examined under the microscope following the addition of a drop of potassium hydroxide. Dermatophyte was identified under the microscope by the presence of segmented hyphae and spores formed directly from hyphae (arthroconidia). All the samples were cultured in a screw-capped test tube containing Sabouraud dextrose agar with supplements for primary isolation of fungus. For removal of contamination chlortetracycline and gentamicin were used to inhibit bacteria and cycloheximide to inhibit saprophytic fungi. To observe anti-fungal sensitivity patterns against dermatophytes, five (05) commonly prescribed antifungal drugs (fluconazole, itraconazole, ketoconazole, griseofulvin, and terbinafine) were tested. These drugs were available in powder form which was convenient for use in the drug sensitivity tests. The criteria of sensitivity and resistance of antifungal disks are mentioned in table I.[15]

Table I: Criteria of sensitivity and resistance of antifungal discs[15]

Antifungal drugs	Potency	Zone diameter in mm		
		Sensitive	Intermediate	Resistance
Fluconazole	25µg	≥ 22	21-15	≤ 14
Griseofulvin	25µg	≥ 10	-	No zone
Itraconazole	8µg	≥ 15	14-10	≤ 10
Ketoconazole	15	≥ 22	29-23	≤ 30
Terbinafine	30µg	≥20	19-12	≤ 11

For medium preparation, 32.5 grams of dehydrated SDA powder was dissolved in 500ml of distilled water (DW) and heated to dissolve completely. After autoclaving, the medium was allowed to cool for 50-55°C and dispensed aseptically on sterile petri dishes. All antifungal discs were obtained from commercial sources. The isolates were transferred from DW stocks to potato dextrose agar to enhance sporulation (subcultured). Species identification was done by colony morphology and microscopy on lactophenol cotton blue mount. Seven days old culture was covered with 1 ml distilled water, and the colonies were probed with the tip of a sterile wire loop to obtain a mixture of mycelium and conidia. The suspensions were transferred to sterile tubes and allowed to sediment for 30 minutes. The inoculums were evenly spread on the surface of 10cm petri dishes containing Sabouraud dextrose agar medium and exposed to air dry. Then, the anti-fungal discs were placed on the plates after which the plates were incubated at 25°C for 5-10 days. After the colonies had grown, the zones of inhibition around the discs were measure and recorded.

Results:

Out of 136 patients, 59% were male and 41% were female. The age of the patients ranged from 1 to 70 years with a mean age in male was mean 32.2 ± 14.6 years and in female mean 36.7 ± 13.9 years. Commonly diagnosed clinical types were tinea corporis (62%), tinea cruris (28%), and onychomycosis (6%). Potassium hydroxide examination for fungal elements was positive, and culture growth was present in 100 samples. The most frequently identified species of dermatophyte was T.rubrum (96%) followed by T. mentagrophyte and E. floccosum. T.rubrum was identified in 100% of tinea corporis and 96.4% tinea cruris (Table.II). Against T. rubrum terbinafine and itraconazole was sensitive in 94(97.9%) and 85(88.5%) followed by ketoconazole 57(59.4%), fluconazole 22(22.9%) and griseofulvin 12(15.5%). Terbinafine (100%), itraconazole (66.7%), ketoconazole (66.7%) and griseofulvin (33.3%) was sensitive and fluconazole was resistant against all cases of T. mentagrophyte species. Against E. floccosum species, Terbinafine, Itraconazole, Ketoconazole and Griseofulvin ere sensitive in 1(100%) case and Fluconazole was resistant in that case. Overall antifungal sensitivity of terbinafine was 98% followed by itraconazole which was 89%. Griseofulvin (87.5%) and fluconazole (77.1%) were the most resistant antifungal drugs (Table III).

Table II: Distribution of identified species of dermatophytes in relation to clinical types of dermatophytosis (n=100).

Clinical type	N	*T. rubrum* (n=96) No (%)	*T. mentagrophyte* (n=3) No (%)	*E. floccosum* (n=1) No (%)
T. corporis	62	62(100)	0(0.0)	0(0.0)
T. cruris	28	27(96.4)	1(3.6)	0(0.00)
Both T. corporis and T. cruris	6	6(100.0)	0(0.00)	0(0.00)
T. facie and T. corporis	3	3(100.0)	0(0.00)	0(0.00)
T. capitis	1	1(100.0)	0(0.00)	0(0.00)
T. pedis	3	2(66.7)	1(33.3)	0(0.00)
Onychomycosis (T. unguium)	6	4(66.8)	1(16.7)	1(16.7)

Table III: Sensitivity pattern of isolated different species of dermatophytes to antifungal drugs (n=100).

Name of the drugs	*T. rubrum* (n=96)		*T. mentagrophyte* (n=3)		*E. floccosum* (n=1)	
	S	R	S	R	S	R
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Terbinafine	94(97.9)	2(2.1)	3(100.0)	0(0.0)	1(100.0)	0(0.0)
Itraconazole	85(88.5)	11(11.5)	2(66.7)	1(33.3)	1(100.0)	0(0.0)
Ketoconazole	57(59.4)	39(40.6)	2(66.7)	1(33.3)	1(100.0)	0(0.0)
Fluconazole	22(22.9)	74(77.1)	0(0.0)	3(100.0)	0(0.0)	1(100.0)
Griseofulvin	12(15.5)	84(87.5)	1(33.3)	2(66.7)	1(100.0)	0(0.0)

Discussion:

Frequent relapses of dermatophyte infection and increasing resistance to antifungal drugs have made it a major concern for dermatologists. In this study majority of patients were in the age group 21-30 years, male: female ratio was 1.4:1. The increased incidence of dermatophytosis in this age group may be due to the fact that they take part in maximum outdoor activities such as agriculture and manual labor.[16-17]

Clinically frequently diagnosed dermatophyte infection was tinea corporis (62%), followed by tinea cruris (28%) and onychomycosis (6%) (Table II). Gansesan et al. (2017) reported tinea corporis was the commonest accounting for 23% of the cases followed by tinea capitis and tinea cruris.[21] Similar findings have been shown by Venkatesan et al.[22] In the present study, 9% of patients showed multiple site involvements. The increased prevalence of multiple site involvement may be due to associated systemic diseases such as diabetes mellitus, changes in climate, poor hygiene, and delay treatment-seeking behavior.

The commonly infecting fungal species was Trichophyton rubrum(96%) followed by Trichophyton mentagrophyte and Epidermophyton floccosum (Table II) . A similar study in Bangladesh by Rahim et al. where frequency of dermatophyte was Trichophyton rubrum 86.6%, Trichophyton mentagrophyte 8.2% and Epidermophyton floccosum 5.2%.18 In another similar study by Lavanya in India the frequency of causative agents were Trichophyton rubrum 51.3%, Trichophyton mentagrophyte 43.2% and Epidermophyton floccosum 5.4%.[19] A closely similar pattern was observed in a Brazilian study where the distribution of T. rubrum was higher in males (65.9%) than females (49.1%).[20] So, in most of the studies T.rubrum was the predominant isolate.

Overcrowding and sharing of clothes and towels are important factors in the household transmission of dermatophytes. Here family history of dermatophytosis was present in 65.6% cases of T. rubrum infected patients. Noronha et al. reported positive family in 20% cases and Bindu et al. found history of contact with infected family members in 16.6%.[23]

In present study, Terbinafine and itraconazole were found highly sensitive to T. rubrum at 97.9% and 88.5% respectively. Ketoconazole, Fluconazole and Griseofulvin were sensitive in 59.4%, 22.9%, 15.5% cases respectively. Against T. mentagrophyte terbinafine was sensitive in 100% cases; itraconazole and ketoconazole was sensitive in 66.7% cases and griseofulvin in 33.3% cases. Against E floccosum all four drugs were sensitive except fluconazole (table III). Ganesanet al. reported terbinafine having the highest sensitivity and most effective drug, which also comparable with Fernandez-Torres et. al.[21,24] Kansra et. al. (2016) found Itraconazole was the most effective drug followed by terbinafin whereas fluconazole and griseofulvin were the least effective drug.[25] Faruqiet. al. observed that terbinafine is the most active drug and also it has perfect in vitro potency and wide spectrum activity against all dermatophyte species.[26] Monitoring the resistance pattern also is useful because detection of resistance for different fungi also gives evidence to emerging threats of fungal infections. Martinez-Rossiet. al. have done an anti-fungal susceptibility test by disc-diffusion method for fluconazole and all the isolates were found sensitive.[27] In our study fluconazole was found mostly resistant against all species of dermatophyte. Mahajan et al. (2017) reported there was a statistically higher sensitivity of itraconazole as compared to terbinafin, fluconazole and griseofulvin.[28]

Finally, terbinafin was the most sensitive followed by itraconazole against all dermatophytes. Fluconazole and griseofulvin were mostly resistant. So to get a desirable treatment outcome against dermatophytosis this pattern of antifungal sensitivity should be considered.

Conclusion:

This antifungal sensitivity pattern study will surely help dermatologists' to choose the effective antifungal medication while treating dermatophytosis. More studies should be done at an interval to detect any change in antifungal sensitivity pattern and thus help the patients from prolonging sufferings.

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Conflict of interest:

No conflicts of interest.

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