Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Research Paper

Prevalence of Dermatophytoses and Their Antifungal Susceptibility in a Tertiary Care Hospital of North India.

Microbiology

KEYWORDS : Dermatophytes, Mean inhibitory concenteration, Antifungal susceptibility testing.

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ABSTRACT

Dermatophytoses is the infection of keratinised tissues caused by fungal species of genera Trichophyton, Epidermophyton and Microsporum, commonly known as dermatophytes . Various antifungal agents both topical and systemic have been introduced into clinical practice for effectively treating dermatophytic conditions. The present study aims at determining the susceptibility patterns of dermatophyte species recovered from patients in Amritsar to antifungal agents; Itraconazole, Terbinafine and Fluconazole.

Methodology: A total of 140 isolates of dermatophytes recovered from the superficial mycoses were examined. Broth microdilution method M38-A2 approved protocol of CLSI for filamentous fungi was followed for determining the susceptibility of dermatophyte species.

Results: Trichophyton mentgrophyte was found to be the most common species followed by Trichophyton rubrum.Itraconazole was found to be the most effective drug followed by Terbinafine while Fluconazole was found to be the least effective drug in vitro.

Conclusion: The MIC values observed in the present study based on standard protocol might serve as reference for further studies. Such studies also reflect on the acquisition of drug resistance among isolates of dermatophyte species based on MIC values.

Introduction:

Dermatophytes are the fungal etiologic agents causing skin infections commonly referred to as ringworm. They are considered as an important public health problem, though not dangerous but being chronic cutaneous infections they are difficult to treat and cause physical discomfort to the patient[1]. Dermatophytoses is caused by dermatophytes, a group of keratinophilic fungi with long incubation period, causing superficial fungal infection of the keratinised tissue like epidermis, hairs and nails. Hot and humid climate in tropical and subtropical countries like India make dermatophytosis very common superficial fungal infection with an increasing frequency. There are three genera of dermatophytes *Trichophyton, Microsporum , Epidermophytes.* They spread by direct contact from infected human beings (anthropophilic organisms), animals (zoophilic organisms), soil (geophilic organisms) and by indirect way from fomites. Although the clinical signs of dermatophytoses may vary depending on the affected region of the body, pruritis is the most common symptom[2].

Dermatophytoses includes several distinct clinical entities, depending upon the anatomic site and etiological agent involved. The disease process in dermatophytosis is unique as no living tissue is invaded, the keratinised stratum corneum is simply colonised. However, the presence of the fungus and its metabolic products usually induces an allergic and inflammatory eczematous response in the host. Treatment for dermatophytic infection is prolonged which has lead to the emergence of resistance in dermatophytes to antifungal agents or recalcitrance to therapy [3,4,5]. Antifungals used systemically are terbinafine, itraconazole, fluconazole and ketoconazole. Resistance pattern can be of various types either primary or acquired antifungal resistance among the previously susceptible species which has lead to increased incidence of infection with less common species which are intrinsically resistant to the available antifungal agents [6].

Aim:

To isolate the dermatophytic fungus from the various clinical samples skin, nail and hair.

To test their antifungal suspectibility against Fluconazole, Terbinafine and Itraconazole.

Material and methods:

The present study was carried out in patients with the clinical signs and symptoms of dermatophytosis, attending the out-patient department of Skin and Venereology Department at Guru Nanak Dev Hospital attached to Government Medical College, Amritsar ,Punjab after taking approval from institutional ethical committee. This study was conducted over a period from September 2013- July 2015. All clinical samples were processed for the isolation of dermatophyte.

Sample Processing[7]:

In case of skin, the lesions in the affected area was cleaned with 5% cetavlon and then with 70% alcohol. The Bard Parker Knife No. 14 was used to collect the skin scrapping from active margin of the lesion with the blunt edge. In case of nail, scrapings were taken from the affected nail plate, the nail bed as well as debris underneath nail plate. In case of hair, hair was plucked out with roots intact using fine forceps. Skin, hair was examined with 10% KOH and nail was examined in 40% KOH. Then it was examined under the low power and then under high power of the microscope for the presence of hyphae and arthrospores. After taking all sterile precautions, the material was inoculated on Sabouraud's Dextrose Agar slant in a test tube containing Chloramphenicol 0.05mg/ml, Gentamycin 0.02mg/ml and cycloheximide 0.5 mg/ml. The test tubes was incubated at 28^{0}C and observed for fungal growth for 2 to 3 weeks. Growth on Sabouraud's Dextrose Medium was taken on a clean glass slide, placed on a drop of lactophenol cotton blue and teased gently. Slide was examined under the low power and then under the high power of the microscope to identify the causative dermatophyte by virtue of their hyphae, type of spores and their arrangement.

Determination of antifungal susceptibility testing[8]:

Antifungal susceptibility was performed as per CLSI M38-A2

Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

guidelines using microbroth dilution technique.

- **Broth microdilution method:**

Broth microdilution method M38-A2 approved protocol of CLSI for filamentous fungi was followed for determining the susceptibility of dermatophyte species.

- **Drug dilutions:**

Stock dilutions of Fluconazole, Itraconazole and Terbinafine were prepared in dimethyl sulfoxide (HiMedia) according to the standard protocol. The two-fold dilutions of the stock solution were further prepared in RPMI 1640 medium with L-glutamine and without sodium bicarbonate (HiMedia). These dilutions were used in the test at a pH of 7.0 ± 0.1 with

3-(N-morpholino) propanesulfonic buffer (HiMedia) along with 1N NaOH. The concentrations of different dilutions of the antifungal drugs ranged from0.12 µg/ml to 128µg/ml.

- **Preparation of inoculums of dermatophyte species:**

Cultures of dermatophyte species (7–8 days old) grown on PDA slants at 30°C were used to prepare inoculums.The fungal growth was covered with 5 ml of sterile normal saline and suspensions prepared by scraping the growth from the surface of the slants with a sterile swab

that contained conidia and hyphal fragments. The heavy particles were allowed to settle down for 10–15 min. The upper clear suspension was transferred to fresh tube, and its optical density was set equal to 0.5 McFarland standards. The final cell density was set between 2×10^3 and 6×10^3 colony forming units per ml which was used in the assay.

- **Test procedure:**

Flat-bottomed, 96 well microtitre plates having 8 rows and 12 columns were used to perform the susceptibility test. Eight test organisms in a volume of 100 µl each was placed in the wells of 8 rows of the plates (one test organism in each row). The dilutions (100 µl) of the drugs were added in the each well of ten columns of the plate from left to right. The concentration of the drug was highest in the first column and decreases from left to right. The contents were incubated at 35°C for 4–5 days. The 11th and 12th columns contained un-inoculated negative control and inoculated positive controls respectively.

- **Incubation of the microtitre plate:**

Incubate the microtitre plate at 35˚C without agitation. Evaluate the microtitre plate containing dermatophyte isolates after four days of incubation for recording MIC.

- **Reading of mean inhibitory concenteration (MIC):**

For MIC reading we compare the growth in each MIC well with that of the positive growth control well with the help of a reading mirror. For Fluconazole, Terbinafine and Itraconazole end points are defined as, MIC is taken as 80% or more reduction in growth compared to the growth in the positive control well (drug free medium).

Result

Out of 254 cases included in our study males were 162 (63.78%) and females were 92 (36.22%). Most common age group was found to be 21- 30 years with 75(29.53%) cases, followed by 31-40 years with 71 (27.95%) cases. Least common age group was more than 70 years with 5 (1.97%) cases, followed by less than 10 years with 9 (3.54%) cases. Youngest patient was 10 months old and oldest patient was 77 years old.Maximum number of cases belonged to Tinea corporis 90 (35.43%) followed by Tinea unguium 77 (30.31%) and Tinea capitis 34 (13.39%). Least number of cases were of Tinea manuum 2 (0.79%) and Tinea pedis 3 (1.19%).[Table 1]

Table 1:AGE AND SEX DISTRIBUTION OF CLINICAL TYPES AMONGST SUSPECTED CASES OF DERMATOPHYTOSES

Clinical Types	<10yrs		11-20yrs		21-30ys		31-40ys		41-50yrs		51-60yrs		61-70yrs		>71yrs		Total (n)
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
TCR	1	0	5	4	21	16	22	12	3	5	0	0	1	0	0	0	90
TU	0	0	5	2	13	8	13	5	11	6	2	3	5	2	2	0	77
TCA	3	5	13	4	3	3	0	2	0	1	0	0	0	0	0	0	34
TCU	0	0	1	0	3	3	8	3	0	2	3	0	1	1	0	0	25
TCC	0	0	0	0	3	0	4	0	4	1	1	1	2	1	1	0	18
TF	0	0	1	0	2	0	2	0	0	0	0	0	0	0	0	0	05
TP	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	03
TM	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	02
																	254

TCR- Tinea corporis, TU- Tinea unguium, TCA- Tinea capitis, TCU- Tinea cruris, TCC- Tinea corporis and Tinea cruris, TF- Tinea faciei, TP- Tinea pedis, TMA- Tinea manuum , M-male, F- female

Among the clinically suspected case of dermatophytoses fungal elements was seen in 172 (67.72%) cases either by direct microscopy or by culture. Out of 172 cases, 135(53.15%) cases were positive by both microscopy and culture, 32(12.60%) cases were KOH positive and negative on culture while 5 (1.97%) cases were KOH negative and positive on culture. Out of all the samples 82 (32.28%) cases were both KOH and culture negative. [Table 2]

Table 2: KOH AND CULTURE FINDINGS AMONGST CLINICALLY SUSPECTED CASES OF DERMATOPHYTOSES

	Number	Percentage (%)
Total number of cases	254	100.00
KOH or Culture positive	172	67.72
KOH & Culture positive	135	53.15
KOH positive & Culture negative	032	12.60
KOH negative & Culture positive	005	01.97
Total culture positive	140	55.12
KOH & Culture negative	82	32.28

KOH-Potassium hydroxide

In the present study out of 254 clinical samples 140 cases were culture positive. Most common dermatophytic species isolated was *Trichophyton mentagrophytes* 65(46.43%) followed by *Trichophyton rubrum* 34 (24.29%).Other fungal species isolated was *Trichophyton verrucosum* 17(12.14%), *Trichophyton schoenlleinii* 16(11.43%) , *Trichophyton violaceum* 5 (3.57%) and *Microsporum gypseum* 3 (2.14%).[Table 3]

Table 3: VARIOUS DERMATOPHYTIC FUNGAL SPECIES ISOLATED FROM CLINICAL SPECIMEN

S.No	Species	Number	Percentage (%)
1	Trichophyton mentagrophytes	65	46.43
2	Trichophyton rubrum	34	24.29
3	Trichophyton verrucosum	17	12.14
4	Trichophyton schoenlleinii	16	11.43
5	Trichophyton violaceum	05	3.57
6	Microsporum gypseum	03	2.14
	Total	140	100

On comparing three antifungal drugs Itraconazole was found to be most potent drug followed by Terbinafine and Fluconazole was found to be least effective drug.[Table 4]

Table 4: MEAN MIC VALUE OF THREE ANTIFUNGAL DRUGS

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Fluco	51.86	140	20.017	1.692
	Terb	4.51193	140	3.761651	0.317918
Pair 2	Fluco	51.86	140	20.017	1.692
	Itra	0.66689	140	0.582354	0.049218
Pair 3	Terb	4.51193	140	3.761651	0.317918
	Itra	0.66689	140	0.582354	0.049218

Fluco-Fluconazole,Terb-Terbinafine,Itra-Itraconazole.

Discussion

Dermatophytoses is responsible for large number of cases attending dermatology department. Dermatophytic infection tends to be more common in the tropical region and India being a tropical country it a cause of concern. . Due to the increasing number of immunosuppressive patients the incidence of dermatophytic infection is increasing and their presentation is also atypical[2].

In the present study total of 254 cases was examined out of these 162 (63.78%) were males and 92(36.22%) were females. Male to female ratio was 1.76:1 .Males outnumbered females. [Table 1]This is in concordance with study done by Veer et al who reported incidence in males was 65% and male to female ratio was 1.8:1 [9]. Male predominance could be due to increased outdoor physical activity and increased opportunity for exposure to infection than females. Males could be more at risk of acquiring dermatophytic infection also due to close contact with the surrounding, soil and animals due to their work requirement[10] .Female hormones also play a role in protecting them against dermatophytic infection leading to lower number of cases in them[11]. However the lower numbers of female is not a true representation of the proportion of females infected. This could due to fewer females attending the clinics because of financial constraints, gender bias, neglect and social stigma attached with the disease, while male report more to the hospital, hence reported prevalence among males is more[12].

Most common age group being 21-30 years with 75(29.53%) cases followed by 31-40 years with 71(27.95%).[Table 1] Almost similar finding were shown by **Amodkumar Yadav** et al who reported most common age group to be affected was 21-30 years with 20 cases (23%) while least common age group affected was 61-70 years with 3 cases (4%)[13]. Another study done by Hanumanthappa et al also reported most common age group to be 21-30 years with 24% cases[14].However study done by Mahale et al reported that the most common age group was 41-50 years with 33 (35.02%) cases[15]. More number of cases was reported in the age group 21-30 years and 31-40 years as this is the active age group involved in outdoor physical activity, more prone for minor trauma and increased sweating that increases their chances of exposure to the fungus. They could also be more at risk of acquiring dermatophytic infection also due to close contact with the surrounding, soil and animals due to their work requirement and sharing of clothes[10].

In our study Tinea corporis 90(35.43%) was the most common clinical type followed by Tinea unguium 77(30.31%), Tinea capitis 34(13.39%), Tinea cruris 25(9.84%), Tinea corporis with Tinea cruris 18(7.08%), Tinea faciei 5(1.97%), Tinea pedis 3(1.19%), and Tinea manuum 2(0.79%) [Table 1]. This was in concordance with study done by Agarwal et al also reported Tinea corporis was the most common clinical pattern in 37.3% cases, followed by mixed clinical pattern in 44 (14.7%), Tinea cruris in 13.7% cases, Tinea capitis in 39 (13%), Onychomycosis in 33 (11%), Tinea pedis in 11 (3.7%), Tinea faciei in 9 (3%), Tinea manuum in 6 (2%) and Tinea barbae in 5 (1.7%) cases[16]. A study done by Hanumanthappa et al who reported Tinea corporis (33.3%) to be the most common clinical type[14]. But this is contrary to results of other studies by Vyas A et al[17] who found Tinea capitis to be most common clinical type with 50% cases, Abu elteen et al[18] who reported Tinea pedis as the commonest clinical type with 35.2% cases and Karamkar S et al[19] who reported Tinea cruris as the commonest clinical type with 34.4% cases.

In the present study, out of 254 clinically diagnosed cases of dermatophytoses, 172 (67.72%) were positive for fungal elements either by KOH or culture .Out of all the samples 135(53.15%) samples were positive for fungal elements both on KOH and culture. In our study 32 (12.60%) samples were KOH positive but culture negative .Five (1.97%) samples were negative on KOH and positive by culture. However 82 (32.28%) samples were both KOH and culture negative. Thus a total 140 (55.11%) samples were culture positive in our study. In our study when the KOH findings were compared with culture [Table 2], KOH findings were statistically highly significant (p value < 0.001). These findings are compatible with other studies done by Hanumanthappa et al who reported KOH or culture positive were 79%.Both KOH and culture positive were 36%. Only culture positive and KOH negative were 12.6%.Only KOH positive and culture negative were 30.6%.Both KOH and culture negative were20.6%[14]. Another study done by Veer et al reported total KOH positive rate to be 81.8% and total culture positive rate to be 48.8%.Culture positive and KOH negative rate reported were 5.6% . KOH positive and culture negative reported rate was 38.6% .Both KOH and culture negative reported rate was 12.5%[9]. Bindu V et al reported KOH positivity rate to be 64.00% and culture positive rate to be 45.30%[20]. Vyas A et al also reported KOH positive rate to be 67.50% and culture positive rate to be37.50%[17].

The difference in these rates among different studies may be due to factors involved in the collection and processing of sample. Culture results could also depend on severity, type and stage of the clinical disease. In our study group 5 (1.97%) specimens were negative on direct microscopy with KOH, but were positive on culture. This could be due to non visualization of hyphae on direct microscopy because of severe inflammatory reaction which obscures them or this could be attributed to the inactive sporulating phase of the fungi which is difficult to be seen on microscopic examination. So it is recommended that all KOH negative samples should be cultutred. Microbiological confirmation of species causing dermatophytoses is a very important in diagnosing superficial fungal infections. This also helps in guiding therapy towards the causative fungal agent[15].

Out of 140 isolates obtained of dermatophytes *Trichophyton mentagrophytes* 65 (46.43%) was found to be the most common isolate obtained followed by *Trichophyton rubrum* 34(24.29%), *Trichophyton verrucosum*17 (12.14%), *Trichophyton schoenlleinii*

16 (11.43%),*Trichophyton violaceum* 5(3.57%) and least was *Microsporum gypseum* 3(2.14%).[Table 3] This is in concodarance with the study done by Parvaneh Adimi et al[1] reported *Trichophyton mentagrophytes* 136 (42.5%) to be the most common followed by *Trichophyton rubrum* 89 (27.8%) while study done by Agarwal et al [16]also reported *Trichophyton mentagrophytes* 37.9% also to be the most common isolate . However Veer P et al [9]reported *T.rubrum* 15 (57.6%) to be the most common followed by *T.mentagrophytes* 11(42.3%) and Hanumanthappa et al[14] also reported *Trichophyton rubrum* 58.9% to be the most common isolate followed by *Trichophyton mentagrophytes* 24.6%.

On comparing the efficacy of three antifungal drugs against the isolates obtained using mean MIC[Table 4] of the three drugs Itraconazole, Terbinafine and Fluconazole. Itraconazole mean MIC was found to be the lowest 0.66µg/ml, while Terbinafine mean MIC was found to be 4.51µg/ml and Fluconazole mean MIC was found to be the highest 51.86µg/ml. Thus Itraconazole was found to be the most effective drug *invitro* followed by Terbinafine while Fluconazole was found to be least effective drug both clinically and on statistical analysis (p value <0.05). Studies done by Adimi et al[1] and Ebrahim et al[21] have also reported Itraconazole to be the most effective drug. However Jha et al[22] and Torres et al[23] have reported Terbinafine to be the most effective drug.

Medical progress has led to an expanding population of susceptible hosts with impaired immunological defenses against infection in the community and hospitals .Over the past quarter of a century, invasive fungal infections have emerged as an important cause of morbidity and mortality especially in immunocompromised patients. The incidence of fungal infections has also increased in the recent past. Although several new antifungal drugs have been licensed in recent years, antifungal drug resistance is becoming a major concern during treatment of such patients. The resistance may be intrinsic or acquired. The understanding of the mechanism of resistance and clinical impact is important while planning treatment strategies[24].

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