Original Article

Comparison of the species of dermatophytes isolated from patients responding and not responding to terbinafine treatment

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Abstract

Background: *Trichophyton rubrum* and *Trichophyton mentagrophytes*, which cause infections of skin and nails, are two of the most frequently isolated dermatophytes. Terbinafine is widely used in therapy of dermatophyte infection. Presently clinicians are facing lack of clinical response to terbinafine in some patients. Hence, clinical response to treatment was noted and data was compared between those responding and not responding to terbinafine treatment. **Material and Methods:** A total of the 187 cases were clinically diagnosed as tinea corporis.The skin scrapings were collected two times from each patient, before instituting treatment and one week after completion of terbinafine treatment in clinically resistant cases. A total of 123 patients were responsive to clinical antifungal treatment which were grouped as the Responsive Group; and 64 patients were clinically resistant to antifungal treatment (Resistant Group). **Results:** In all 64/187 (34.2%) patients with *T. rubrum* and *T. mentagrophytes* infections were clinically non responsive to treatment with terbinafine. Patients had persistent lesions and all these were positive by direct microscopy or culture or both in their post treatment clinical specimens. **Discussion:** The clinical resistance of dermatophyte isolates may involve more than one mechanisms which are both fungus related and host related. The low diversity of antimycotic drugs may indicate existence of differences between pathogen and host yet unexplored which maybe used in new drug development.
Key Words: Dermatophytes, terbinafine, resistance, response.

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INTRODUCTION

The dermatophytes are by far the most significant fungi because of their widespread involvement of population at large and their prevalence all over the world[1]. The estimated lifetime risk of acquiring a dermatophyte infection is between 10 and 20 %[2]. Although dermatophytosis does not cause mortality, it does cause morbidity and poses a major public health problem and also is of cosmetic importance[3,4]. The clinical presentation, though very typical of ringworm infection is very often confused with other skin disorders, making laboratory diagnosis and confirmation necessary[5]. Recognition and appropriate treatment of these infections reduces both morbidity and discomfort and lessens the possibility of transmission[6]. Dermatophytosis is a clinical entity caused by members of the imperfect genera *Trichophyton, Microsporum and Epidermophyton*[1]. *Trichophyton rubrum* and *Trichophyton mentagrophytes*, which cause infections of skin and nails, are two of the most frequently isolated dermatophytes[7]. *T. rubrum* among other dermatophytes is a major causative agent for dermatophytoses. Infections due to *T.rubrum* are often associated with frequent relapses following cessation of antifungal therapy[8]. Common therapeutic strategies based on use of terbinafine and griseofulvin are generally considered effective. Terbinafine is very widely used both orally and topically, in therapy of dermatophyte infection[9]. The occurrence of terbinafine resistant clinical isolates is rare one case has been documented and thoroughly investigated. Nevertheless dermatophytes with

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abnormally low susceptibility to terbinafine do exist[10]. Presently clinicians are facing lack of clinical response to terbinafine in some patients. Hence, in the present study isolation and identification of dermatophytes associated with tinea corporis was undertaken. Clinical response to treatment was noted and data was compared between those responding and not responding to terbinafine treatment.

MATERIAL AND METHODS

The present prospective study was carried out in the Department of Microbiology, during period of January 2014 to December 2015. A total of 187 clinically diagnosed and culture positive cases of tinea corporis infection of all age groups of both sexes were taken for the study. After the detailed history, a detailed clinical examination of patient was done. The affected area was swabbed with 70% alcohol and the active edge of lesion scraped with a sterile scalpel. The scrapings were collected from the margins of the lesion without injuring the skin surface. The scrapings were collected in a sterile petri dish. The skin scrapings were collected two times from each patient, before instituting treatment and one week after completion of terbinafine treatment in clinically resistant cases (Terbinafine treatment 250 mg orally daily for minimum 2 and maximum 4 weeks). The samples were examined for dermatophyte hyphae, arthroconidia by using 10% KOH. Specimens were then cultured on Sabouraud's Dextrose Agar (Merck, Germany) containing Chloramphenicol, Gentamycin and Cyclohexamide; and were kept at 26°C for four weeks. fungal growth was identified by slide culture and physiological and biochemical tests[1].

RESULTS

A total of the 187 cases were clinically diagnosed as tinea corporis. The maximum number of patients 66 (35.3%) were found in the age groups of 21 – 30 years. There were 109 males and 78 females showing more prevalence in males. The study included patients who were positive for dermatophyte culture. It was observed that 123 patients were responsive to clinical antifungal treatment which were grouped as the Responsive Group; and 64 patients were clinically resistant to antifungal treatment (Resistant Group). *T. rubrum* was isolated from 136 patients (72.7%) and *T.mentagrophytes* from 51(27.3%). Among patients with *T. rubrum* infection, 67.6% were responsive to treatment and 32.4% were resistant to treatment. The findings with *T. mentagrophytes* were in concordance with those with *T. rubrum* in that 60.8% of *T. mentagrophytes* infected patients were responsive to treatment and 39.2% were resistant (Table 1).

Table 1: Culture Results in Patients

Total no. Of culture positive patients (n=187)	Responsive group (n=123)		Resistant group (n=64)		Total	
	No.	%	No.	%	No.	%
T. rubrum (N=136)	92	67.6	44	32.4	136	72.72
T.mentagrophytes (N=51)	31	60.8	20	39.2	51	27.28
Total	**123**	**65.8**	**64**	**34.2**	**187**	**100**

There was no significant difference in the species isolated from responsive group and resistant group. The clinically resistant patients were 64 out of 187 (34.2%). The study revealed that 29.4% of the patients of age group 31-40 with *T. rubrum* infection showed response to treatment which was significantly more than the patients with *T.rubrum* in the same age group who were resistant to treatment. The same was not true with *T.mentagrophytes* where the difference was not significant. Twenty nine percent of the cases among *T.mentagrophytes* Responsive group belongs to age group 31 – 40 yrs which was less as compared to 35% of the cases among *T.mentagrophytes* Resistant group but the difference was not significant. The highest 79.7% of patients were in the age group of 21-50 years (149/187). Lesser number of patients were seen in age group <20 years and > 50 years. 55.4% of the cases among *T.rubrum* Responsive group were male which was less as compared to 72.7% of the cases among *T.rubrum* Resistant group but the difference was not significant. 51.6% of the cases among *T.mentagrophytes* Responsive group were male which was comparable to 50% of the cases among *T.mentagrophytes* Resistant group and the difference was not significant. In the responsive group 54.5% were males and 45.5% were females whereas in the resistant group there was a predominance of males 65.6% as compared to females 34.4% and the difference was not significant. In the patients infected with *T.rubrum*, 9 out of 44 patients (20.4%) showed severe lesions which was higher than seen in the responsive group in whom only 5 out of 92 (5.4%) showed severe lesions. Thus, the severity of lesions in resistant cases due to *T.rubrum* infection was significantly high. While in patients with *T.mentagrophytes*infection, the type of lesions were mild or severe was observed in responsive and resistant groups. It was seen that in 10 out of 20 resistant cases, (50%) the lesions were severe. Whereas, in only 9.6%, the lesions were severe in responsive group. The severity

of lesions in resistant cases was significantly higher than in responsive cases. Multiple lesions were seen in both responsive and resistant group but difference was not significant. Multiple sites were affected in some of the 187 patients.It was observed that 25% of the patients in the resistant group with *T.rubrum* infections had lesions on face as compared to 9.8%of the patients in the responsive group and the difference was significant indicating that T. faciae caused by *T.rubrum* was more likely to be resistant than responsive. Overall abdomen was the commonest site of infection in both groups, followed by back. Face was the least commonly affected site. Above table reveals that 9.8% of the cases among *T.rubrum* Responsive group had lesion on face which was significantly less as compared to 25% of the cases among *T.rubrum* Resistant group.9.7% of the cases among *T. mentagrophytes* Responsive group had lesion on face which was less as compared to 20% of the cases among *T. mentagrophytes* Resistant group but the difference was not significant. All the 187 patients of tinea corporis had typical signs and symptoms of scaling, raised border of lesion and itching. Papulovesicular rash and pain were complained by 3.7% of patients. Satellite lesions were observed in 4.8% of patients.100% of the cases had Erythema among *T.rubrum* Responsive group and *T.rubrum* Resistant group which were same and difference was not significant.96.8% of the cases had Erythema among *T. mentagrophytes* Responsive group which was comparable to 95% of the cases among *T.rubrum* Resistant group and difference was not statistically significant. There was no statistically significant difference in the signs and symptoms of *T.rubrum* infection and *T.mentagrophytes* infections, so also, between responsive and resistant groups. After the completion of the antifungal therapy, repeat mycological assessment was done by collecting scrapings for microscopy and culture. In *T.rubrum* infected patients KOH positivity was seen in 31 out of 44 patients who were clinically resistant (70.45%). Culture was positive in all the 43 patients. Only KOH positivity with culture negativity was seen in one patient.

Table 2: Mycological Assessment after Completion of Antifungal Treatment in Resistant Infected with *T. rubrum*

	KOH positive (N=31)(%)	KOH negative (N=13)(%)	TOTAL (N=44)(%)
Culture positive	30 (68.18)	13 (27.27)	43 (97.73)
Culture negative	1(2.27)	-	1(2.27)
Total	**31 (70.45)**	**13 (29.54)**	**44**

p>0.05 - not significant

Table 3: Mycological Assessment after Completion of Antifungal Treatment in Resistant Infected with *T. mentagrophytes*

	KOH positive (N=15) (%)	KOH negative (N=5) (%)	TOTAL (N=20) (%)
Culture positive	13 (65)	5 (25)	18 (90)
Culture negative	2 (10)	-	2 (10)
Total	**15 (75)**	**5 (25)**	**20**

p>0.05 - not significant

A total of 20 patients infected with *T.mentagrophytes* were resistant to antifungal treatment. In these, KOH positivity was seen in 15 (75%) and culture was positive in18 (90%) patients. Thus, in all the 20 patients either microscopy or culture or both were positive even after end of treatment.

DISCUSSION

Tinea corporis is a common but distressing cutaneous fungal infection of the skin of the body. Since many cutaneous conditions resemble tinea corporis, a correct diagnosis is essential for instituting appropriate therapy. The treatment of Tinea corporis involves use of topical antifungal creams and Terbinafine is the latest addition to the antifungal armamentarium. Following oral administration of terbinafine for treatment of Tinea corporis a clinical cure rate of 71–100% has been reported[11]. In our study, out of 187 patients studied 123 responded clinically favourable to treatment (65.8%) and 64 did not respond clinically to treatment (34.2%). Thus, the clinical cure rate was little less than reported by Newland *et al*[11]. Despite the extensive use of terbinafine for treatment of dermatophytosis, the first clinically confirmed case of resistance to terbinafine in dermatophytes was reported in 2003[9]. The author isolated *Trichophyton rubrum* from 6 clinically resistant cases who had failed oral Terbinafine therapy. Most of the studies indicate that Terbinafine resistance is encountered in *Trichophyton rubrum* whereas *Trichophyton mentagrophytes*has not been studied for its Terbinafine resistance. Infections due to *T.rubrum* are often associated with frequent relapses when antifungal therapy is stopped but these relapses have not been related to resistance to antifungals[9]. Research has revealed that failure of patients with *T.rubrum* infection to antifungal therapy is not related to development of resistance to the drug and that failure of Terbinafine therapy maybe dependent on host related factors[9]. In a recent study, 71 culture positive cases of T.corporis was studied and the fungal isolates were *T.rubrum* in 59 (83%), *T.mentagrophytes* in 10 (14%) and *Epidermophytonfloccosum* in 2 (3%)[5]. In another study, culture results of 50 cases of tinea corporis revealed

T.rubrum in 33 (66%) and *T.mentagrophytes* in 17 cases (34%)[12]. Most of the reports published so far from India unequivocally report *T.rubrum* to be the commonest dermatophyte isolated from various types of dermatophytosis followed by *T.mentagrophytes*[13]. Tinea corporis is the commonest clinical presentation of dermatophytosis as shown by several authors[4,5,12]. In our study which included only culture positive Tinea corporis patients, *T.rubrum* and *T.mentagrophytes* were the species isolated from 72.7% and 27.3% patients respectively. Thus, our findings were in conformity with other authors[5,12]. At present very little is known concerning drug resistance mechanisms in dermatophytes. It is suggested that since Terbinafine resistant *T.rubrum* isolates are cross resistant to other classes of squalene epoxidase inhibitors. It is suggested that resistance is associated with changes in target enzymes rather than over expression of efflux pumps[14]. Most of the studies on antifungal resistance in clinical settings incriminate *T.rubrum* among the resistant cases. However studies on *T.mentagrophytes* are sparse. In vitro resistance studies of *T.rubrum* and *T.mentagrophytes* have shown that the activities of Terbinafine and Itraconazole were higher than Fluconazole and Griseofulvin against *T.rubrum* and *T.mentagrophytes*[15]. Most of the studies have shown that the in vitro susceptibility to various antifungal drugs including Terbinafine exhibited good activity against dermatophytes with exception of Fluconazole. It has been shown in one study that Fluconazole has a higher activity for *T.rubrum* than *T.mentagrophytes*, *M.gypseum*and*M.canis*[16]. In spite of the good in vitro activity of terbinafine against dermatophytes which was demonstrated in some studies[16], other studies like Favre *et al* have demonstrated a clinical as well as in vitro resistance to terbinafine[14]. In our study we found that 32.4% of patients infected with *T.rubrum* and 39.2% of patients infected with *T.mentagrophytes* were clinically resistant to treatment but this difference was not significant. In our study**,** the increased incidence of dermatophytosiswas found in the young adults i.e., 79.7% were in age group of 21-50 years. The prevalence reported by other workers in the age group of 21-50 years are 78%[17], 62.5%[18], 70%[19]. In the report of terbinafine resistant *T.rubrum*[14], 6 sequential clinical isolates of *T.rubrum* from a single patient who failed the therapy on oral terbinafine were found to be resistant to terbinafine in vitro. In our study, no age related significant difference was found in resistant and responsive patients so also between *T.rubrum* and *T.mentagrophytes* infected patients. In our study, it was seen that 29.4% of patients in age group of 31-40 with *T.rubrum* infection showed response to treatment which was significantly less than patients with *T.rubrum* in same age group which was resistant to treatment. This finding indicates that among young adults with *T.rubrum* infection there is more likelihood of infection being responsive to treatment than resistant. It is possible that the host factors maybe playing a major role in this age group, contributing to response to treatment. In our study, the ratio of male: female was 1.9:1. Other authors also reported male preponderance in dermatophyte infections. Male to female ratios of 1.6:1[5] and 2:1[13] have been noted by other authors. It was found that there was no significant difference between the number of males and females affected in the responsive and resistant groups. There was found to be a very high prevalence of multiple lesions in our study (97.9%).The maximum number of lesions were > 9 found in 0.8% of responsive and 9.4% of resistant group with no significant difference between the two groups. There is a significant correlation between number of lesions and chronicity as stated in a study by Prasad *et al*[21]. In our study, almost all patients exhibited erythema, scaling, itching as clinical signs and symptoms and raised borders of the lesion. Similar findings were reported in their study by Gupta *et al*[22]. They have reported pain in 6.7%. In our study it was seen in 3.7% of patients. There was no significant difference between signs and symptoms in patients infected with T.*rubrum* and *T.mentagrophytes* or between responsive and resistant groups when first presented. Persistence of symptoms though was observed in the resistant group even after complete treatment. It was observed that in our study, after completion of antifungal treatment, 64 patients still complained of persisting symptoms like pruritis. All these patients infected with *T.rubrum* (44) and *T.mentagrophytes* (20) were positive in direct microscopy or culture or both,when repeat assessment was done post treatment. Terbinafine is extensively used for treatment of dermatophytosis and resistance both clinically and in-vitro has only recently been demonstrated[14]. At present very little known concerning drug resistant mechanisms in dermatophytes. Target enzyme changes could be the reason[14]. Persistence and relapses are often not related to related to resistance to antifungals[9]. It is also proposed by some authors that drug resistance and failure to terbinafine treatment may not be related to development of resistance to the drug but maybe dependent on host related factors[9]. The clinical resistance of dermatophyte isolates may involve more than one mechanisms which are both fungus related and host related. Comprehensive studies on events that promote resistance are essential for development of structural modifications in antifungal drugs which will serve as further avenues for research. The low diversity of antimycotic drugs may indicate existence of differences

between pathogen and host yet unexplored which maybe used in new drug development.

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