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Expression profile of ABC and MFS multi-drug transporter genes in terbinafine- and fluconazole-resistant *Trichophyton* spp.

Dipika Shaw[1], Sunil Dogra[2], Tarun Narang[2], Anup Ghosh[1], Harsimran Kaur[1], Arunaloke Chakrabarti[1], Shivaprakash M Rudramurthy[1]

[1]Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

[2]Department of Dermatology, Venerology and Leprology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

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Objectives: Over the past few decades, an unprecedented increase in recalcitrant and chronic dermatophytosis with atypical clinical presentations have been noted in the Indian subcontinent. Recent studies have reported *Trichophyton mentagrophytes/interdigitale* complex as predominant etiological agent of chronic/recurrent dermatophytosis followed by *T. rubrum*. Multiple factors, including unregulated use of antifungal drugs, poor compliance to treatment, and irrational fixed-drug combinations are associated with emerging resistance. However, molecular studies investigating the underlying mechanisms related to terbinafine and fluconazole resistance in dermatophytes are sparse.

The present study was designed to explore the role of multidrug efflux transporters in terbinafine- and fluconazole-resistant *T. mentagrophytes/interdigitale* complex and *T. rubrum*.

Methods: The expression of MDR transporter genes was evaluated in 36 isolates with terbinafine resistance (with or without F397L or L393F mutation in squalene epoxidase gene, SE) of *T. mentagrophytes/interdigitale* complex ($n = 26$), and *T. rubrum* ($n = 10$) isolates along with 16 susceptible wild-type isolates of *T. mentagrophytes/interdigitale* complex ($n = 10$), and *T. rubrum* ($n = 6$) isolates. In addition, 19 fluconazole-resistant *T. mentagrophytes/interdigitale* complex ($n = 11$), and *T. rubrum* ($n = 8$) isolates along with 13 susceptible *T. mentagrophytes/interdigitale* complex ($n = 9$), and *T. rubrum* ($n = 4$) were also included. Quantitative real-time PCR (qRT-PCR) was used to evaluate the expression of ABC transporter (MDR1, MDR2, MDR3, MDR4, and MDR5) and MFS transporter genes (MFS1 and MFS2).

Results: In terbinafine-resistant T. mentagrophytes/interdigitale complex, the mean expression of MDR1 and MDR2 was 4.98 and 5.27-fold in isolates with wild-type SE, compared with 0.64 and 1.04-fold in isolates with non-wildtype SE gene ($P < .0001$) and susceptible isolates at 0.4, and 0.88-fold ($P < .0001$), respectively. For MFS1 gene, 3.4-fold-change expression was noted in isolates with wild-type SE compared with 1.51-fold in isolates with non-wild type SE ($P < .001$). In resistant isolates with non-WT SE, the upregulation of the SE gene was noted at 6.3-fold compared with 1.8-fold in isolates with wild-type SE ($P < .001$), and 0.63-fold in susceptible isolates ($P < .0001$). However, there was no significant upregulation of MDR3, MDR4, MDR5, and MFS2 genes among the three groups. In terbinafine-resistant *T. rubrum*, the mean inducible expression of MDR1 was significantly higher in isolates with wild-type SE at 3.88-fold compared to isolates with non-wild type SE at 0.5-fold ($P < .05$) and susceptible isolates at 0.33-fold ($P < .01$). Only resistant isolates with wild-type SE showed significant upregulation of MDR2 ($P < .01$) compared to susceptible isolates. Other genes, MDR3, MDR4, MDR5, MFS1, MFS2, and SE, did not show substantial overexpression among the groups (Resistant WT vs. Resistant NWT vs. susceptible WT). In fluconazole-resistant and -susceptible isolates of *T. mentagrophytes/interdigitale* complex, and *T. rubrum*, similar findings were observed. In both the species, the mean inducible expression of MDR1, MDR2, and MDR3 was significantly higher ($P < .05$) in resistant isolates compared to susceptible isolates.

Conclusion: In conclusion, this study demonstrates the overexpression of MDR1 and MDR2 in terbinafine-resistant *Trichophyton* spp. isolates lacking drug-target gene mutation, suggesting the role of multidrug transporters in resistance. This study also suggests the importance of MDR2, and MDR3 transporter genes in imparting fluconazole resistance in *Trichophyton* spp.

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Clinical and microbiological spectrum of dermatophytosis from a tertiary care institute

Sukanya Sudhaharan[1], Umabala Pamidimukkala[1], Saba Naaz[1], Savitha Kovida, Naga Appala Raju Sundarapu[1], Dipika Shaw[2], Shivaprakash M Rudramurthy[2]

[1]Nizam's Institute of Medical Sciences, Hyderabad, India

[2]Post Graduate Institute of Medical Sciences, Chandigarh, India

Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: There is an increasing incidence of recalcitrant dermatophytosis in India due to irrational use of antifungals, inappropriate treatment, and also *in vitro* resistance of the organism by itself. This study is done to determine the clinic-mycological profile, antifungal susceptibility, and outcome of patients with dermatophytosis in our institute.

Methods: All patients with culture-proven dermatophytosis attending the outpatient department of our hospital from January 2019 to December 2019 were included in the study. Detailed clinical data of all the patients were collected.

Morphological Identification of the dermatophytes was done by conventional mycological methods. The isolates were sent to PGIMER Chandigarh for further identification by MALDI-TOF and antifungal susceptibility testing. Antifungal susceptibility testing was done for 47 isolates of *Trichophyton* species.

Results: Of the 155 clinical suspected cases, growth of dermatophytes was observed in 55 (35.4%) of the cases. Tinea corporis 39/55(70.9%) was the predominant clinical type The duration of infection was less than 6 months in 22/55(40%) of cases and >6 months in 33(60%) of the cases. Majority of the patients were in the age group of 20-30 years and were male. A total of 36/55 (65.4%) of the patients belong to middle socio-economic status and 19/55 to lower socioeconomic status (34.5%). In all, 10/55(19.2%) of the patients were students and 10/55 (19.2%) housewives; others include auto drivers, mechanics, teachers, cashiers, etc. All except 4 patients were from urban areas. Comorbid conditions noted were diabetes mellitus in 7/55 (12.7%), hypertension in 6/55 (10.9%), systemic steroid usage in 3/55 (5.4%), post-renal transplant status in 1/55 (1.8%), and SLE in 1/55 (1.8%). Sharing of personal use items was found in 12/55 (21.8%) of the patients and 9/55 (16.3%) of patients complained of excessive sweating.

Previous therapy with topical and systemic antifungals was given in 32/55 (58.8%) of the patients, other modes of treatment like homeopathy, and ayurveda in 4/55 (7.2%). Topical steroids were given to 4 patients and 19/55 (34.5%) of the patients were not treated for the infection.

Trichophyton mentagrophytes complex (69%) was the predominant species complex isolated followed by *T. rubrum*, *M. gypseum*, *M.canis*, and *T. tonsurans*.

Of the 47 *Trichophyton* isolates subjected to AFST, all the isolates showed MIC >1ug/ml for fluconazole and griseofulvin. Majority of the isolates showed MIC of <1 ug/ml for other antifungals; high MICs (MIC >1) were exhibited by 5 isolates for terbinafine and naftifine, 2 isolates for sertoconazole, and 1 isolate for voriconazole. Molecular detection of terbinafine resistance done in 15/55 isolates showed mutation in the squalene epoxidase (SE) gene leading to F397L substitutions in 2 isolates. In the present study, the patients were treated with both oral and topical antifungals

Of the 55 cases, complete cure was observed in 21 (38%), partial cure in 9 (16.3%), and relapse in 5 (9%) on 2 years follow-up. However, 20/55 (36.3%) of the cases were lost to follow-up.

Conclusion: *Trichophyton* mentagrophytes complex was the predominant species isolated and *Tinea corporis* was the commonest clinical presentation. Resistance to terbinafine, griseofulvin, and fluconazole has been noted. Dermatophytosis has become a difficult to treat disease due to antifungal resistance, and chronicity/recurrence of the lesions. Early diagnosis followed by rational antifungal therapy are essential for improved outcome.

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Reversed-phase high-performance liquid chromatography as rapid and simple method for quantifications of terbinafine drug in human serum from dermatophytosis cases

Sheetal Thakur[1], Dipika Shaw[2], Tarun Narang[1], Sunil Dogra[1], Harsimran Kaur[2], Anup K Ghosh[2], Shivaprakash M. Rudramurthy[2]

[1]Department of Dermatology, Venereology, and Leprology, Postgraduate Institute of Medical Education and Research, Chandigarh, India, Chandigarh, India

[2]Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India, Chandigarh, India

Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: The alarming situation of treatment failure cases in dermatophytosis and resistance to the first-line drug (terbinafine) is a worrisome condition for the management of tinea cases. However, studies also reported non-responders to terbinafine treatment even when the isolates are susceptible to this drug *in vitro*. Thus, evaluating the pharmacokinetic profile of terbinafine might help better manage dermatophytosis. This study was conducted to standardize and validate a rapid and simple reversed-phase high-performance liquid chromatography (HPLC)-based protocol for terbinafine in serum/plasma of dermatophytosis cases.

Methods: HPLC analysis was standardized for terbinafine drug on an Agilent 1290 infinity system (Agilent Technologies InC., USA). Chromatographic parameters including mobile phase [acetonitrile (A), methanol (M), and water (W)], flow rate (0.7-1.5 ml/min), injection volume (20 μl), and various wavelengths ranging from 220 to 265 nm under isocratic conditions were assessed and optimized. The mobile phase consisted of a filtered and degassed mix of A: W and M: W with various ratios of 85:15, 60:40, 50:50, and M-100%, respectively. Quality control samples were prepared in drug-free serum by spiking with the terbinafine at 0.0312-100 μg/mL concentrations. An equal volume of serum and acetonitrile (A) were mixed. The mixture was vigorously vortexed for 30 s, followed by high-speed centrifugation at 13 000 rpm at 40°C for 10 min. The supernatant was transferred into the chromatographic vials and placed in the autosampler of HPLC for injection. The standardized method was tested in 6 dermatophytosis patients' serum/plasma samples collected at 3-time points (first, second, and third week of start of antifungal).

Results: Linearity of calibration standard for terbinafine was optimized at 250C at a flow rate of 1.0 ml/min, injection volume 20 μl, 8 minutes run time with the standardized wavelength at 245 nm under isocratic conditions. The best suitable graph was determined by plotting the area under the curve (AUC) and peak height separately against the drug concentrations measured by reversed-phase- HPLC for terbinafine drugs (Fig. 1a and b). The standardized mobile phase consisted of filtered and degassed Methanol (100, v/v). The chromatographic separation was achieved on an Agilent C18 column, and 4.3 ± 1 time represents the peak for terbinafine drug. Based on the standardized protocol, six tinea cases were included for validation, and the therapeutic range achieved for terbinafine in clinical samples was 0.6 to 1.13 μg/ml.

Conclusions: The standardization of HPLC method was successfully applied to quantify terbinafine in spiked samples with terbinafine drug and showed no observable interferences at the standardized parameter. Further evaluation with larger number of samples is warranted.

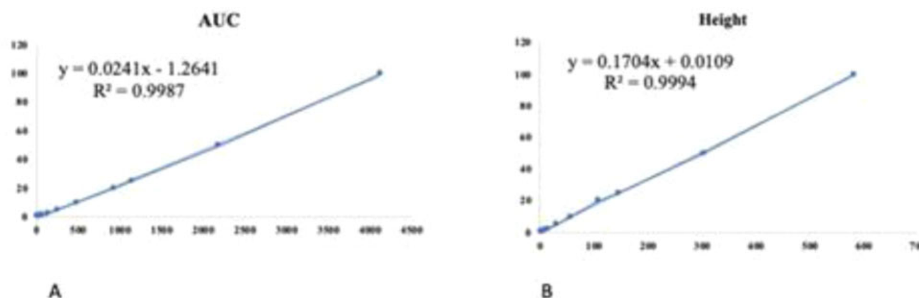

Figure 1. Best fit curve (linearity response R^2[0.99]) of terbinafine drug concentrations on y axis with area under curve (AUC) (A) and peak height (B) on x axis.