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

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Abstract

Background: Worldwide, dermatophytic infections are running a chronic course either due to ineffective treatment or emerging drug resistance. In the past three decades, there has been an increase in incidence and non-responsiveness to conventional antifungals, which suggests that there is a need of antifungal sensitivity testing.

Aims: This study was aimed at identifying clinico-mycological pattern of dermatophytic infections in patients attending the dermatology outpatient department of a tertiary care hospital, and to obtain the sensitivity pattern of isolates against six commonly used oral antifungals (fluconazole, terbinafine, itraconazole, ketoconazole, griseofulvin and voriconazole).

Methods: Patients with suspected dermatophyoses attending the outpatient department of Sir Sunderlal Hospital, Varanasi, were enrolled in the study. A detailed history, clinical examination and sample collection for mycological examinations was done. In vitro antifungal sensitivity testing was done on species isolated from culture as per the Clinical and Laboratory Standard Institute M38-A standards, with broth microdilution method.

Results: There were 256 patients recruited in the study, with a male: female ratio of 3:1. The most commonly affected age group was 20–40 years (52.4%). Tinea corporis et cruris was the most common type observed (27.2%). Potassium hydroxide positivity was seen in 211 samples (79.6%) and culture positivity was found in 139 samples (52.4%). The most common species identified was *Trichophyton mentagrophytes* (75.9%). Sensitivity testing was done on fifty isolates of *T. mentagrophytes*. Minimum inhibitory concentrations of itraconazole, ketoconazole, terbinafine and voriconazole were comparable, while griseofulvin showed the highest minimum inhibitory concentration. Itraconazole was found to be the most effective drug, followed by ketoconazole, terbinafine and fluconazole. Griseofulvin was the least effective drug among the tested antifungals.

Limitations: This is a hospital-based study, and may not reflect the true pattern in the community. Sensitivity pattern of only one species *T. mentagrophytes* was carried out.

Conclusion: Inadequate and irregular use of antifungal drugs has led to the emergence of resistant strains, which cause poor treatment outcomes. Thus, it is very important to test for antifungal sensitivity to check for resistance to antifungals.

Key words: Antifungal, dermatophytes, sensitivity

Introduction

The dermatophytes are hyaline septate molds, 42 species in three genera, *Trichophyton*, *Microsporum* and *Epidermophyton*, known to infect keratinized tissues. Ajello, in 1960, said “species not only differs from region to region but may change with the passage of time.” By the end of 20th century, fungi were reported to be developing drug resistance. Resistance to griseofulvin...
was detected for the first time in 1969 by Lenhart.2 Mukherjee et al. in 2003 from Cleveland, USA, first reported resistance to terbinafine. In this background of increasing resistance of dermatophytic infections to therapy, this study was conducted mainly to obtain the sensitivity pattern of dermatophytes for commonly used systemic antifungals.

Methods
The study was conducted on patients with dermatophytic infection attending the dermatology outpatient department of Sir Sunderlal Hospital, Banaras Hindu University, from January 2014 to October 2014. Detailed history and clinical examination was carried out. Scales obtained by skin/nail/hair scrapings were taken to the laboratory for potassium hydroxide examination and culture on Sabouraud’s dextrose agar. All potassium hydroxide-positive and/or culture-positive samples were included for further data analysis.

Specimens were inoculated on culture media (Sabouraud’s dextrose agar) with cycloheximide (0.05 g/L) and chloramphenicol (0.005 g/L). Test tubes were incubated at 28°C for 4 weeks before labeling it negative. Isolation was done by further subculture on Potato dextrose agar for the preparation of conidial suspension for antifungal sensitivity testing. Most fungi grew within 1 week of incubation. Species identification was done by colony morphology and microscopy on lactophenol cotton blue mount. Antifungal sensitivity testing was done with broth microdilution test according to the Clinical and Laboratory Standard Institute M38-A standards.4

Stock solutions of concentration 1 mg/ml were made by dissolving powdered drugs in normal saline for fluconazole and in 100% dimethyl sulfoxide for terbinafine, itraconazole, griseofulvin, ketoconazole and voriconazole. Drug double dilutions were prepared from 0.125 to 64 µg/ml for fluconazole and 0.03 to 16 µg/ml for rest of the drugs with the help of RPMI-1640 ([HiMedia] with L-glutamine but without sodium bicarbonate and buffered at pH 7.0 with 3-[N-morpholino]propanesulfonic acid, monosodium salt).

The fungal colony grown on Potato dextrose agar after 7 days was used for in-vitro sensitivity testing. The slant was flooded with 1 ml of sterile normal saline and few drops of 1% Tween 80. Colony was scraped gently with the help of sterile loop. The heavy particles were allowed to settle for 3–5 min. The upper homogeneous suspension containing mixture of nongerminated conidial and hyphal fragments was mixed for 15 s with vortex. The turbidity was measured using a spectrophotometer at 530 nm and adjusted to final optical density range of 0.09–0.11 or visually containing standard 1,000,000 cells/ml of fungi counted on Neubauer’s chamber. Stock inoculum suspension was diluted at 1:50 in RPMI-1640 medium. This test was performed in round-bottomed 96-well microdilution trays. Columns 1–9 were filled with double dilutions of 100 µL of respective antifungal drugs in rows in each well. Column 10 was a stability control, containing 200 µL of RPMI-1640 and column 11 acted as a growth control (drug free), having 200 µL of pure conidial suspension. Now, 100 µL of conidial suspension was filled in 1–9 well of serially diluted drugs. This tray was incubated at 30°C for 48–96 h of incubation and minimum inhibitory concentrations were determined, read visually. The growth in each well was compared with that of drug-free growth control and negative control. For most of the drugs, the minimum inhibitory concentration end point criterion for fungi was the lowest drug concentration, showing 50% inhibition and 90% inhibition of growth.

Antifungal sensitivity testing was done on fifty strains of T. mentagrophytes. Minimum inhibitory concentration of fluconazole ranged from 0.25 to >64 µg/ml. It was 0.25 µg/ml in 13 (26%) and 16 µg/ml in 12 (24%) strains. Minimum inhibitory concentration ≥64 µg/ml was considered resistant.4,5 Hence, in the present series, 11 (22%) strains were found resistant to fluconazole.

Minimum inhibitory concentration of terbinafine ranged from 0.03 to >16 µg/ml. More than two-third (70%) of the strains had minimum inhibitory concentration of 0.03 µg/ml. Sensitive strains of terbinafine had minimum inhibitory concentration ranged from 0.01 to 1 µg/ml. Minimum inhibitory concentration >1 µg/ml was found in 9 out the 50 (18%) strains, considered resistant to terbinafine.4,5

Minimum inhibitory concentration of itraconazole ranged from 0.03 to >16 µg/ml and 31 (62%) strains showed minimum inhibitory concentration of 0.03 µg/ml. According to the Clinical

Results
Out of 265 patients, 199 (75.1%) were males and 66 (24.9%) were females. Male:female ratio was approximately 3:1. Age of the patients ranged from 2 to 70 years with mean of 29.08 ± 13.46 years in males and 37.03 ± 13.70 years in females. The common age group involved was 20–30 years in 76 males (38.2%), while it was 30–40 years in 24 females (36.4%). Multiple site involvement (mixed type of infection) was the common presentation in 124 (46.8%), followed by tinea corporis in 55 (20.8%) and tinea cruris in 50 (18.9%). Most of the cases presented with prolonged duration of illness, 107 (40.4%) patients had a history of intermittent or continuous infection which varied from 1 to 6 months, even longer duration of up to 2 years in 95 (35.8%) patients. Associated conditions such as diabetes were seen in 2.6% patients, hyperhidrosis in 2.2%, immunosuppression in 1.5% and atopy in 0.3%. Family history was positive in 82 (30.9%), out of which 25 (9.4%) patients had conjugal transmission. Application of topical steroid alone or in combination with antifungal or antibacterial was reported by 187 (70.6%) patients. Only 15 (5.7%) patients applied antifungal creams as azoles ( clotrimazole/miconazole/sertaconazole) or terbinafine. Half of the patients (54.7%) did not take any systemic treatment. One-fourth (26.8%) of the patients gave a history of taking fluconazole 150 mg weekly (37, 20, 6 and 8 patients took this drug for ≤4, 5–8, 9–12 and >12 weeks, respectively). History of taking terbinafine 250 mg daily was given by 8.7% (n = 23) patients (2, 14 and 7 patients took for ≤1, 2–3 and ≥4 weeks, respectively). Griseofulvin was taken by 2.3% (n = 6) patients for 10–25 days. There was no history of taking multiple antifungals orally simultaneously. Treatment regimens in succession were followed by 20 (7.5%) patients, in which fluconazole, terbinafine or itraconazole were commonly administered.
and Laboratory Standard Institute standards, sensitive strain has minimum inhibitory concentration between 0.01 and 8 µg/ml. Only three strains (6%) had minimum inhibitory concentration ≥8 µg/ml which were resistant to itraconazole.4

Minimum inhibitory concentration of griseofulvin ranged from 0.03 to 8 µg/ml. In 19 (38%) strains, it was 4 µg/ml and 11 (22%) had lowest minimum inhibitory concentration, i.e., 0.03 µg/ml. Minimum inhibitory concentration of 0.06–3 µg/ml was considered a limit of effectiveness.6 Accordingly, 25 strains (50%) were found resistant to griseofulvin.

Minimum inhibitory concentration of ketoconazole ranged from 0.03 to >16 µg/ml. In 14 strains (28%), it was 0.03 µg/ml and 9 (18%) strains had 0.5 µg/ml. The Clinical and Laboratory Standard Institute guideline for filamentous fungi is that minimum inhibitory concentration ≥8 µg/ml is resistant to ketoconazole.4 According to this guideline, only four strains (8%) had shown resistance to ketoconazole.

Minimum inhibitory concentration of voriconazole ranged from 0.03 to >16 µg/ml. Ninety percent of the isolates had minimum inhibitory concentration ≤8 µg/ml, only 2 (4%) isolates had minimum inhibitory concentration >16 µg/ml [Table 1].

There was a statistically significant difference in the sensitivity of itraconazole as compared to terbinafine, fluconazole and griseofulvin (P = 0.12, 0.04 and <0.001, respectively). Although fungi were more sensitive to itraconazole than ketoconazole, the difference was not statistically significant (P = 1.0). There was higher sensitivity to terbinafine than to griseofulvin and the difference was statistically significant (P = 0.002). Terbinafine was less effective than ketoconazole, but the difference was statistically insignificant (P = 0.23). Sensitivity of dermatophytes to fluconazole, as compared to terbinafine and ketoconazole was low, but the difference was not statistically significant (P = 0.8 and 0.09, respectively). Griseofulvin was found to be significantly less effective than fluconazole (P = 0.007) and it was found to be the least effective among all the tested drugs.

Discussion

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.8,11 Male:female ratio was 3:1; a male preponderance has been seen in some earlier studies.8,15–16 However, others have showed female predominance, with females mainly having tinea pedis and manuum and onychomycosis due to kitchen and household work.7,14

A prolonged duration of illness of 6 months and above was found in 53.9% of the patients. The reason behind such chronicity may be due to inadequate doses of anti-fungal medication, irregular treatment and application of topical steroids, which only reduce inflammation and pruritus, but help in proliferation of fungi by modifying their microenvironment. In an earlier study, Kumar et al. had found the duration of symptoms to be greater than 3 months in 53.3% of the patients, 1–3 months in 33.7% cases and less than 1 month in 13% of the cases.12

A history of fungal infections in family members was elicited in 30.9% of cases, of which 9.4% were conjugal. Transmission by direct contact occurs in tinea infection, explaining the conjugal cases, while transmission in family members might be due to fomites or de novo infection.12,14,15,19,20

Potassium hydroxide examination for fungal elements was positive in 79.6% of the patients. Previous studies had reported similar findings for potassium hydroxide positivity.16,21–25 In the present study, culture positivity was 52.4 per cent; previous reports show a variance of this ranging from 24 to 87 per cent.9,12,16,19,22–29 On the basis of these findings, sensitivity of potassium hydroxide examination, considering culture to be the gold standard, was 94.2% and its specificity was 31.8 per cent Sensitivity and specificity of culture, if one were to consider potassium hydroxide as the gold standard was 68.6% and 77.8%, respectively. Hence, we can say that potassium hydroxide is highly sensitive and less specific and culture is highly specific and less sensitive. Similar results were found in other studies.30–32

In studies conducted between 2002 to 2011, T. rubrum was the most common isolate. In the present study, the most common species identified was T. mentagrophytes (75.9%) followed by T. rubrum (11.3%). Similar findings were also observed by Sahai and Mishra and Bhatia and Sharma.16,34 Ajello, in 1960, said “species not only differ from region to region but may change with the passage of time.”11

Resistance of dermatophyte infections to all antifungals (except voriconazole) has been reported in literature. Minimum inhibitory concentration of fluconazole in the present study ranged from 0.25 to >64 µg/ml. Similar observations were noticed in other studies, in which the minimum inhibitory concentration range of fluconazole for T. mentagrophytes varied from 0.06 to >64 µg/ml.35–39 In previous studies, resistance to fluconazole in dermatophytes is well documented.35,40,41

Resistance to terbinafine was first reported in 2003, in which minimum inhibitory concentration of terbinafine for T. rubrum strains was >4 µg/ml, whereas it was <0.0002 µg/ml for the susceptible reference strains.7 In the present study, minimum inhibitory concentration of terbinafine ranged from 0.03 to 16 µg/ml. Only two studies had similar minimum inhibitory concentration which ranged from 0.003 to 16 µg/ml.38,42

Resistance to griseofulvin was found in 50% of strains, as they had a minimum inhibitory concentration >3 µg/ml which is considered as the limit of effectiveness.8 Similar findings have been reported previously.7,35,40,42–44

**Table 1: Results of in vitro sensitivity testing of fifty strains of Trichophyton mentagrophytes (µg/ml)**

| Drugs       | MIC range   | Mode MIC<sub>25</sub> | MIC<sub>50</sub> | MIC<sub>90</sub> |
|-------------|-------------|-----------------------|-----------------|-----------------|
| Fluconazole | 0.25–>64    | <0.25                 | 0.25–16         |                 |
| Terbinafine | 0.03–>16    | <0.06                 | 0.03            |                 |
| Itraconazole| 0.03–>16    | <0.06                 | 0.03            |                 |
| Griseofulvin| 0.03–8      | 2                     | 4               |                 |
| Ketoconazole| 0.03–>16    | <0.06                 | 0.03            |                 |
| Voriconazole| 0.03–>16    | <0.06                 | 0.03            |                 |

MIC: Minimum inhibitory concentration
In a study conducted by Magagnin et al., resistance to itraconazole was observed in 42.3% and resistance to ketoconazole was observed in 53% of the strains. Minimum inhibitory concentration of ketoconazole in the present study was found in the range of 0.03–16 μg/ml. None of the studies showed such wide range of variation in minimum inhibitory concentration of ketoconazole.

Minimum inhibitory concentration of itraconazole in the present study ranged from 0.03 to >16 μg/ml. Ataides et al. (2012) also reported similar results (0.062–15 μg/ml). Most of the other studies had a narrow range of minimum inhibitory concentration (0.01–4 μg/ml). Gupta et al. observed a wider range of minimum inhibitory concentration (0.06–32 μg/ml). Resistance of voriconazole in dermatophytooses has not yet been reported.

Thus, itraconazole was found to be the most sensitive drug among the tested antifungals. The second most sensitive drug was found to be ketoconazole, followed by terbinafine and fluconazole. Griseofulvin was the least effective drug among the tested antifungals.

To conclude, treatment of this menacing tinea infection can be helpful with the help of antifungals sensitivity testing.

**Limitations**

The limitations of the study were: a) a small sample size on which sensitivity testing was done and b) minimum inhibitory concentration ranges of only T. mentagrophytes were calculated.

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**Conflicts of interest**

There are no conflicts of interest

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