
PREVALENCE OF DERMATOPHYTOSIS AND ASSESSMENT OF ANTIFUNGAL SUSCEPTIBILITY IN PATIENTS REFERRED TO A MYCOLOGY CLINIC IN TEHRAN

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ABSTRACT

Dermatophytic infection is a common fungal disease which can involve keratinization of skin and hair. *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Epidermophyton floccosum* were isolated in 124 from 272 patients with cutaneous fungal infections referring to the Tehran Mycology Laboratory. The identification was confirmed by direct microscopic examination and culture according to routine mycological methods. Then they were examined for antifungal drugs susceptibility test by using fluconazole, ketoconazole, and itraconazole by using disc diffusion method. Dermatophytosis had a higher prevalence in men. Tinea manuum/pedis showed the highest incidence, whereas tinea unguium, tinea cruris, tinea corporis, tinea capitis, tinea faciei, and tinea barbae were less common. Itraconazole was the most appropriate antifungal agent, while fluconazole showed the least antifungal activity.

Keywords: Fungal infections, dermatophytes, *Trichophyton*, Itraconazole, drugs susceptibility.

REZUMAT

Infecția dermatofitică este o boală fungică comună, care poate implica cheratinizarea pielii și a părului. *Trichophyton mentagrophytes*, *Trichophyton rubrum*, și *Epidermophyton floccosum* au fost izolate la 124 din 272 de pacienți cu infecții fungice cutanate, care s-au adresat Laboratorului de micologie din Teheran. Identificarea a fost confirmată prin examen microscopic direct și în cultură, conform metodelor micologice de rutină. Apoi au fost examinate pentru testarea sensibilității la medicamente antifungice utilizând fluconazol, ketoconazol și itraconazol, prin metoda difuzimetrică. Dermatofitoza a avut o prevalență mai mare în rândul bărbaților. Tinea manuum/pedis a prezentat cea mai mare incidență, în timp ce tinea unguium, tinea cruris, tinea corporis, tinea capitis, tinea faciei și tinea barbae au fost mai puțin frecvente. Itraconazolul a fost cel mai potrivit agent antifungic, în timp ce fluconazolul a prezentat cea mai mică activitate antifungică.

Cuvinte-cheie: Infecții fungice, dermatofite, *Trichophyton*, Itraconazole, sensibilitate la medicamente.

INTRODUCTION

Surface fungal diseases are infections in which the organism is colonized at the surface of the skin and is unable to invade the underlying live tissues. Therefore, the host has no cellular response, or the complication is associated with a brief host reaction, and sometimes a mild hyperkeratosis at the site of the lesion (tinea versicolor). In the invasion of the body,

the organism only affects the superficial parts of the hair (black and white), and the total damage to the tissue in this type of infection is negligible and more related to esthetic problems. Dermatophytosis is a fungal infection that can involve hair, nail, and epidermis stratum corneum. Dermatophytes are a group of keratinophilic fungi that are similar in structure. In general, dermatophytes species belong mostly

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to 3: *Microsporum*, *Trichophyton*, and *Epidermophyton* [1, 2]. Dermatophytes also have a very close relationship with each other in terms of physiological and antigenic properties which can make the serological diagnosis difficult and in some cases impossible. Only differences between species and sex have been described with slight differences in physiological and nutritional properties. Dermatophytosis is spread throughout the world and there is no specific geographic area for this kind of infection [3, 4]. According to reports in the United States, millions of children and adults suffer from dermatophyte diseases annually. Dermatophytes can be isolated from various devices such as coat shoulder strap, towel cloth, underwear, and so on. In the field of ecology, dermatophytes are divided into three types of anthropophilic, zoophilic and geophysical species. The body's response to human dermatophytes is slow and this makes it possible to prolong and develop the infection in order to have enough time to create conidia and contaminating elements and transfer to new hosts. The most important dermatophytes are: *Trichophyton schoenleinii*, *Trichophyton mentagrophytes* of Interdigital Variety, *Trichophyton rubrum*, and *Epidermophyton floccosum*. For the treatment of these fungal infections clotrimazole, fluconazole, and amphotericin B are commonly used. By considering the recent antifungal drug resistance reported in dermatophytes, the aim of this study was to determine the sensitivity of dermatophyte agents isolated from cutaneous fungal infections in patients referred to the Mycology Clinics of Tehran University of Medical Sciences [5–8].

MATERIAL AND METHODS

Isolation and identification of fungi

This study was performed for 6 months in patients suspected of superficial and cutaneous fungal infections referring to the Mycology Clinic of Tehran University for the evaluation of fungal infections and drug sensitivity. Skin samples were taken from patients by scraping. Identification was confirmed by direct microscopic examination and culture according to routine mycological methods.

For laboratory diagnosis, routine microscopic examination of samples of skin and nails with KOH 10-20% and for hair samples with the lactophenol solution was performed. To isolate the fungi, samples were cultured into Sabouraud Dextrose Agar with cycloheximide and chloramphenicol. Urease and Corn Meal Agar medium were also applied for characterization and differentiation between *Trichophyton mentagrophytes* and *Trichophyton rubrum* [2, 9].

Preparation of the fungal suspension

A volume of 5 ml sterile saline was poured into a tube containing 10-14 days culture of each fungus and after that the sterile cotton swabs or antiseptic pipettes containing physiological serum were slowly rubbed on colonies until spores were released and the contents of the tube were transferred to a sterile tube. Then it was left for 15-20 minutes until heavy particles were settled, and then the supernatant containing spores was transferred to another sterile tube. For adjusting the number of spores per ml a neobar slide count was used and the concentration of spores was equivalent to 1×10^6 CFU/ml of each species. Regarding *T. rubrum*, the organism was sub-cultured on Oatmeal agar at 30°C for 4 to 15 days. Following growth, conidia were harvested in sterile saline and using a haemocytometer the conidial suspension was adjusted to 1×10^6 conidia/ml.

Antimicrobial susceptibility test

For *in vitro* testing fluconazole, itraconazole, and ketoconazole powders (Sigma-Aldrich) were used. All drugs were dissolved in dimethyl sulfoxide, followed by further dilutions in RPMI 1640 medium and the working concentrations achieved on the discs for fluconazole 25 µg/disc, for itraconazole 30 µg/disc and for ketoconazole 10 µg/disc. To perform the antimicrobial susceptibility test, Mueller Hinton Agar (MHA) (Difco) plates were streaked evenly with a swab dipped into the standardized inoculum suspension. Lids were left open for 5 min in a laminar flow for drying. Then discs containing the tested agents were placed and the plates were incubated at

a temperature of 25°C for 5 to 10 days [10]. At the end of the incubation time, the result was interpreted by using standard breakpoints of antifungal agents according to [11]. In this study, all characteristics of patients such as personal data, visit time, location of the lesion, the results of direct examination and culture, and other complementary diagnoses were introduced in the SPSS program to determine the correlations among the dozens of variables.

RESULTS

According to Fig. 1, out of 272 suspected cases of superficial and cutaneous fungal infections, 158 cases were men and 114 women, indicating that the prevalence of this kind of disorder in men was higher than in women which can be due to many reasons such as wearing the same shoes for a longer time. This figure also reveals the distribution of absolute and relative frequency of superficial and cutaneous fungal infections referring to the Medical Mycology Laboratory of the University of Tehran. According to this table, dermatophytosis with the highest percentages of 45.58% was the most common infection which is much higher in men. Candidiasis was the only infection which was higher in women than in men (Fig. 1). Fig. 2 shows the incidence of various forms of dermatophytosis in patients with superficial and cutaneous fungal infections. According to this figure, among the

various types of tinea, tinea manuum/pedis showed the highest incidence with 41.9%, whereas tinea unguium, tinea cruris, tinea corporis, tinea capitis, tinea faciei, tinea barbae were less frequent (Fig. 2). The most frequent dermatophyte is *Trichophyton mentagrophytes* and the less frequent is *Microsporum canis* (Table 1). Table 2 indicates the antimicrobial susceptibility test of 21 *Trichophyton mentagrophytes*, 12 *Trichophyton rubrum*, and 7 *Epidermophyton floccosum* strains against the three tested antifungals, respectively fluconazole, itraconazole and ketoconazole. According to the disk diffusion result obtained in this study, regarding ketoconazole, the highest sensitivity was observed for *Trichophyton rubrum* with 75%, *Trichophyton mentagrophytes* with 71.6% and *Epidermophyton floccosum* with 57.1%. The highest resistance rate to ketoconazole was observed in *Epidermophyton floccosum* with 28.6%. In the case of fluconazole, the highest sensitivity was also recorded for *Trichophyton rubrum* with 75%, followed by *Epidermophyton floccosum* with 57.1%, and *Trichophyton mentagrophytes* with 19%. *Trichophyton mentagrophytes* exhibited the highest resistance to fluconazole with 16.7%. In the case of itraconazole, the highest sensitivity was obtained for *Epidermophyton floccosum* with 100%, followed by *Trichophyton mentagrophytes* with 85.5% and *Trichophyton rubrum* with 83.33%. The most

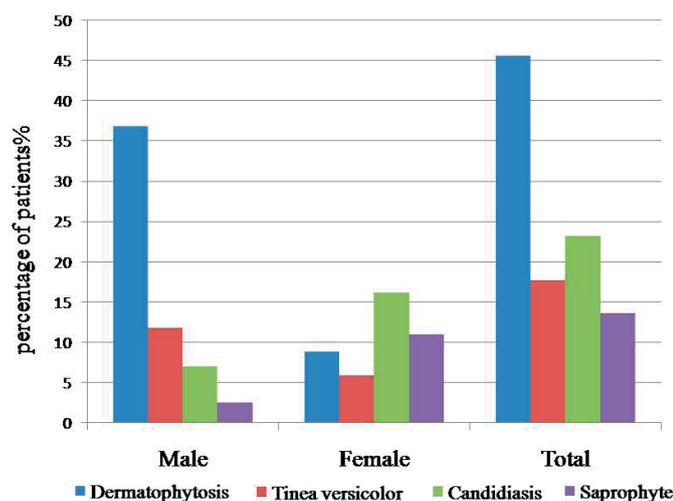


Fig. 1 - Distribution of superficial and cutaneous fungal infections

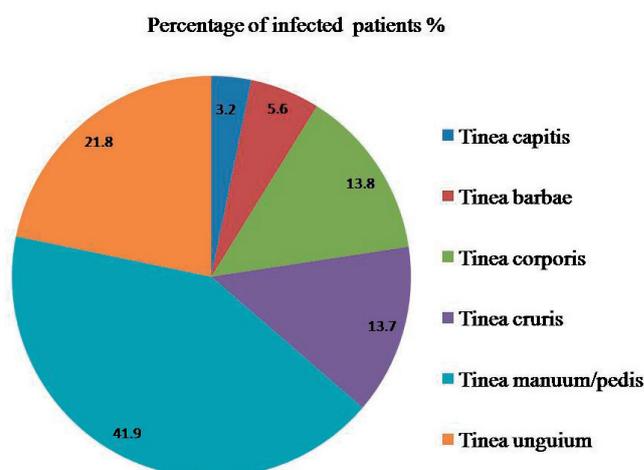


Fig. 2 - Prevalence of different types of dermatophytosis in patients.

Table 1 - Distribution of absolute and relative abundance of different species in terms of dermatophyte species in patients

| | Tinea capitis | | Tinea barbae | | Tinea corporis | | Tinea cruris | | Tinea manuum/pedis | | Tinea unguium | | Total | |
|------------------------------------|---------------|------|--------------|------|----------------|-------|--------------|-------|--------------------|-------|---------------|-------|--------|--------|
| | N* | P** | N | P | N | P | N | P | N | P | N | P | N | P |
| <i>Trichophyton mentagrophytes</i> | 0.00 | 0.00 | 0.00 | 0.00 | 3.00 | 2.42 | 4.00 | 3.23 | 28.00 | 22.58 | 13.00 | 10.48 | 48.00 | 38.71 |
| <i>Trichophyton rubrum</i> | 0.00 | 0.00 | 1.00 | 0.81 | 6.00 | 4.84 | 3.00 | 2.42 | 15.00 | 12.10 | 8.00 | 6.45 | 33.00 | 26.61 |
| <i>Trichophyton tonsurans</i> | 3.00 | 2.42 | 4.00 | 3.23 | 1.00 | 0.81 | 0.00 | 0.00 | 3.00 | 2.42 | 2.00 | 1.61 | 13.00 | 10.48 |
| <i>Trichophyton verrucosum</i> | 1.00 | 0.81 | 1.00 | 0.81 | 2.00 | 1.61 | 0.00 | 0.00 | 5.00 | 4.03 | 2.00 | 1.61 | 11.00 | 8.87 |
| <i>Epidermophyton floccosum</i> | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.81 | 9.00 | 7.26 | 0.00 | 0.00 | 0.00 | 0.00 | 10.00 | 8.06 |
| <i>Trichophyton violaceum</i> | 0.00 | 0.00 | 1.00 | 0.81 | 1.00 | 0.81 | 0.00 | 0.00 | 3.00 | 2.42 | 2.00 | 1.61 | 7.00 | 5.65 |
| <i>Microsporum canis</i> | 0.00 | 0.00 | 0.00 | 0.00 | 2.00 | 1.61 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.00 | 1.61 |
| Total | 4.00 | 3.23 | 7.00 | 5.65 | 16.00 | 12.90 | 16.00 | 12.90 | 52.00 | 41.94 | 27.00 | 21.77 | 124.00 | 100.00 |

*number of people

**percentage of people

Table 2 - Antimicrobial susceptibility test

| | Ketoconazole | | | | | | Fluconazole | | | | | | Itraconazole | | | | | | | | | | | |
|--------------------------|--------------|------|---|------|----|------|-------------|-----|----|------|---|------|--------------|------|----|-----|---|-----|---|-----|----|------|----|-----|
| | R | | I | | S | | T* | | R | | I | | S | | T | | R | | I | | S | | T | |
| | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P |
| <i>T. mentagrophytes</i> | 4 | 19 | 2 | 9.8 | 15 | 71.4 | 21 | 100 | 14 | 66.7 | 3 | 14.3 | 4 | 19 | 21 | 100 | 2 | 9.5 | 1 | 4.8 | 18 | 85.5 | 21 | 100 |
| <i>T. rubrum</i> | 2 | 16.7 | 1 | 8.3 | 9 | 75 | 12 | 100 | 1 | 8.3 | 2 | 16.7 | 9 | 75 | 12 | 100 | 1 | 8.3 | 1 | 8.3 | 10 | 83.3 | 12 | 100 |
| <i>E. floccosum</i> | 2 | 28.6 | 1 | 14.3 | 4 | 57.1 | 7 | 100 | 1 | 14.3 | 2 | 28.6 | 4 | 57.1 | 7 | 100 | 0 | 0 | 0 | 0 | 7 | 100 | 7 | 100 |
| Total | 8 | 20 | 4 | 10 | 28 | 70 | 40 | 100 | 16 | 40 | 7 | 17.5 | 17 | 42.5 | 40 | 100 | 3 | 7.5 | 2 | 5 | 35 | 87.5 | 40 | 100 |

Susceptibility expressed as R (resistant), I (intermediate), and S (susceptible). N: number of people. P: percentage of people.

*Total number and percentage

frequent resistance to itraconazole was seen in *Trichophyton mentagrophytes* with 9.5%.

DISCUSSION

The aim of this study was to determine the prevalence of superficial and cutaneous fungal diseases and to determine the drug's sensitivity among the population that had superficial and cutaneous fungal infections during the last 6 months and were referred to the Tehran Medical College of Mycology. Dermatophytosis with the highest percentages of 45.58% was the most common infection in this study. In a similar study by Koksall *et al* in Turkey, performed between 2000 and 2007, from a total of 8200 investigated patients, with 5720 fungal infection cases were diagnosed, with dermatophytosis (74%) having the highest percentage, whereas candida yeast (21%) and tinctoric (3%) were less frequent [10]. According to a study of 124 cases of positive dermatophytosis, tinea manuum/pedis was the most common type of disease (41.9%). Tinea manuum/pedis has a worldwide spread and is increasing in frequency. This disorder is more common in men than in women. However, there are differences between the various statistics presented in Iran and other parts of the world. The prevalence of this disorder in men can be attributed to wearing socks and shoes without hermetically sealed shoes, wearing shoes for a long time, the type of shoes and socks that men tend to wear more, participation in sports and military activities, travel, sweating. Most males are more likely to use public places, such as swimming pools and sports fields, due to occupational situations. Therefore, tinea unguium and tinea cruris were more common in men than in women. The increase in onychomycosis in this study may be due to many factors, including greater accuracy in sampling and diagnosis, as well as the greater interest of people for individual health condition and beauty. The incidence of head tinea among other fungal infections varies from country to country. Tinea barbae has a relatively small incidence comparing to the others. In a recent study, the incidence of bruising was 15.1% in all types of dermatophytosis, which

is much lower than the figures presented in other studies. Onychomycosis, the fungal infection of the nail in the recent study was reported pretty high (18.9%). According to a recent study, *Trichophyton mentagrophytes* was the most common cause of dermatophytes infections, followed by *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *Epidermophyton floccosum*, and *Microsporum canis*. The results in this study revealed that for infections with *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*, fluconazole is not a suitable antifungal agent. In another study by Rich *et al*, they evaluated antifungal drug resistance of 35 dermatophytes strains, including *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Trichophyton tonsurans* against four antifungal agents, ketoconazole, midazanol, clotrimazole, and fluconazole. Clotrimazole was reported as an effective antifungal agent as compared to fluconazole [12]. However, itraconazole in this study and many other studies has shown the appropriate antifungal activity that makes it a suitable for treatment.

CONCLUSIONS

According to the result obtained in this study, for infections with *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*, itraconazole was the most appropriate antifungal agent, while fluconazole showed the least antifungal activity. On the other hand, regarding *Epidermophyton floccosum* infections, surprisingly, itraconazole indicated the most appropriate antifungal activity with 100% sensitivity.

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