Evaluation of Effects of Fluconazole and Terbinafine Against 35 Isolates of *Trichophyton rubrum* Isolated from Dermatophytosis’ Patients in Tehran

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Abstract: Dermatophytosis is an infection of the nail and skin caused by dermatophytes species. In this research the antifungal effects of the fluconazole and terbinafine was studied *in vitro* against isolates of *Trichophyton rubrum* collected from dermatophytosis’ patients referred to laboratories in Tehran. The Sabouraud’s Dextrose Agar culture medium was prepared with different concentrations of fluconazole (1 to 200 mg/l) and terbinafine (0.0001 to 1 mg/l) and their effects on *Trichophyton rubrum* were studied by noting the mean diameter of fungal colony growth after seven and fourteen days. Data were analyzed using SPSS statistical software. Total (100%) fungal growth inhibition was brought about by fluconazole concentration in a range of 50 to 200 mg/l, while complete inhibition of the growth of isolates by terbinafine occurred at 0.1 to 1 mg/l. It can be concluded that the antifungal effect of terbinafine on *Trichophyton rubrum* was stronger than fluconazole.

Key words: *Trichophyton rubrum* • Fluconazole • Terbinafine

INTRODUCTION

Dermatophytes are fungi that belong to the gymnoascomycetaceae family of soil keratinophiles that thrive on keratinized structure such as skin and nails [1, 2]. The disease is usually restricted to the stratum corneum of the epidermis [3]. Dermatophytosis ranks among the most common and widespread infectious diseases world wide and *Trichophyton rubrum* is one of the most frequently isolated dermatophytes [4]. It is an anthropophilic dermatophyte common in Iran and among other dermatophytes, is a major causative agent for superficial dermatophytosis and is known to account for as many as 70% of all dermatophyte infections. Infection due to *Trichophyton rubrum* are often associated with frequent relapses following cessation of antifungal therapy [5-7]. The incidence of dermatophytosis has increased over recent years, particularly in immunocompromised patients [8-11].

The treatment of these cutaneous infections is based on the use of topical and systemic antifungal agents [12].

The number of agents available to treat fungal infections has increased by 30% since the year 2000, yet still only 15 agent are currently approved for clinical use [13]. Fluconazole remains one of the most frequently prescribed triazoles because of its excellent bioavailability, tolerability and side effect profile. Fluconazole also exert effects within the fungal cell membrane. The inhibition of cytochrome P450(cyp)-dependent 14α-demethylase prevents the conversion of lanostrol to ergosterol [14].

Also Terbinafine, an allylamine derivative, represents the most effective of this new chemical class of antymycotic compounds. The antifungal activities of allylamine are based on the inhibition of fungal ergosterol biosynthesis at the point of squalene epoxidation [15].

Because of little data about resistance and sensitivity of *Trichophyton rubrum* so the purpose of this study was to evaluate the impact of terbinafine and fluconazole *in vitro* on the growth of *Trichophyton rubrum* isolated from dermatophytosis patients.

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MATERIALS AND METHODS

Isolates: Thirty five *Trichophyton rubrum* strains isolated from dermatophytosis patients referred to the Medical Mycology Laboratory of Public Health Tehran University of Medical Sciences and some specialized Mycology Laboratory in Tehran, were tested. Each isolate was cultured on Sabouraud Dextrose Agar at 30°C for 14 days and then fresh cultures were used to study the effects of drugs.

Identification of the isolates was based on colony characteristics and microscopic morphology of their micro and macroconidia and accessory structures. Urease test, pigment production in corn meal agar and perforate hair test were used [15]. Preparation of pharmaceutical dilutions:

In order to obtain the desired dilutions of the fluconazole, acetone 50% solvent was used to make dilutions dilution 10mg/ml was prepared as drug stock and then dilutions of 1, 6.25, 12.5, 25, 50, 75, 100 and 200 mg/l were prepared in sterile Sabouraud's Dextrose Agar culture media. Also the terbinafine, dilution 1 mg/ml was prepared as drug stock and then dilutions of 0.0001; 0.0005; 0.001; 0.005; 0.01; 0.05; 0.1; 0.5 and 1 mg/l were also prepared in sterile Sabouraud's Dextrose Agar culture media. The test tube components were uniformly mixed with vortex and subsequently transferred to sterile plates. Sabouraud Dextrose Agar culture medium with acetone 50% as a soluble control medium with out drug and Sabouraud Dextrose Agar with out drug and solvent were used as controls.

Test Method: Using a sterile puncher, 6mm diameter circles were cut from the fresh fungal colony plaques and subsequently transferred to the culture media using a sterile scoop-headed needle in the vicinity of a flame.

In order to control the accuracy of the tests, two plaques were grown in each plate. The plates were incubated at 30°C for two weeks. The diameters of the colonies were measured after seven and fourteen days and the mean diameter of the colonies was considered as organism growth. The degree of colony growth inhibition was estimated by comparison with the mean diameters of the control colonies and reported as the percentage of growth inhibition according to the following formula [15].

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\text{Percentage of growth inhibition} = \frac{\text{Colony diameter in control} - \text{Colony diameter in control colony}}{\text{Colony diameter in control}} \times 100\%
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Statistical Analysis: Data derived by t-test in similar and independent groups were analyzed using SPSS,V.13.

RESULTS AND DISCUSSION

The results showed that the concentration which completely inhibited the growth of isolates was in the range 75-200 mg/l and no growth was observed in any of the isolates (p<0.01). Out of a total of 35 isolates, the growth of 12 isolates (34%) was completely inhibited at 50 mg/l concentration of fluconazole (p<0.05) (Table 1).

Table 2 illustrates that the concentration that completely inhibited growth of isolates was observed in the range 0/5 to 1 mg/l and no growth was observed in any of the isolates (p<0.01). Out of a total of 35 isolates, the growth of 15 isolates (42%) was completely inhibited at 0/1 mg/l of Terbinafine (p<0.05).

Dermatophytes have been known for a long time to cause infection, the many cases reported every year all over the world are still a matter of concern for modern medicine. Several antifungal drug have demonstrated activity against the various type of dermatophytoxes,
but no suitable treatment have been established thus far [16, 4]. Trichophyton rubrum most commonly causes chronic disease with remissions and relapses and infections caused by this organism are generally regarded as difficult to manage [4]. On the other hand standardized reference method for dermatophytes in vitro susceptibility testing is lacking [17]. Moreover, the activity of antifungal drugs against fungal strains is not tested routinely [4]. Begin new paragraph The results of this study showed that complete inhibition of growth of all isolates was observed at concentrations of $\geq 75$ mg/l of the fluconazole. In this research, sensitivity of 12 isolates (34%) against the fluconazole (50 mg/l) were higher than other isolates. Regarding terbinafine, complete inhibition of growth of the tested organisms was observed at $\geq 0.5$ mg/l and in a total of 35 isolates, 15 (42%) were sensitive. So, our results showed that terbinafine was more effective than fluconazole ($\geq 75$ mg/l of fluconazole compared with $\geq 0.5$ mg/l of terbinafine). This result is quite consistent with results of other researchers [4, 5, 7-10, 13]. So replacing terbinafine to fluconazole is recommended in the treatment of dermatophytosis patients caused by Trichophyton rubrum.

REFERENCES

