Prevalence and Antifungal Susceptibility Patterns of Dermatophytes Isolated from Patients with Neoplastic Diseases: A Case Control Study

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ABSTRACT Patients with neoplasia who are severely immunocompromised have a higher risk of fungal infections. There are limited data in the literature regarding the frequency of dermatophyte infections and efficacy of antifungals in patients with malignancies.

Objective was assessment of the incidence of dermatophyte infections and antifungal susceptibility, determination of dermatophyte species isolated from patients with neoplastic diseases. 138 patients diagnosed with various malignancies and 160 immunocompetent patients who were referred to the Department of Dermatology in Brasov, Romania, for suspicion of dermatophyte infections were included in the study. Nail clippings or skin scrapings were examined by direct microscopy and cultures in Sabouraud agar medium. Susceptibility tests for antifungals were conducted *in vitro* using a method of broth microdilution. Infections with dermatophytes were identified in 30.4% of patients with neoplastic diseases and in 29.37% in the control group. There was a significantly higher frequency of dermatophyte infections in patients with hematologic malignancies (52%) compared to those with solid cancers (25.66%) (P=0.01). The clinical aspects of dermatophyte infections in patients with neoplastic diseases were not different from those of patients without cancer; though in some cases the infections were more extensive. There were no statistically significant differences between mean values of minimum inhibitory concentration of antifungals compared with controls. Terbinafine had the highest antidermatophyte activity for all tested dermatophyte species isolated from patients with neoplastic diseases.

There were no differences in frequency of dermatophyte infections and antifungal susceptibility to dermatophytes between patients with neoplastic diseases and immunocompetent patients.

KEY WORDS: dermatophyte; neoplasia; antifungals; *in vitro* susceptibility

INTRODUCTION

The prevalence of fungal infections has increased significantly in recent decades mainly due to the increasing number of immunocompromised patients. Risk factors such as population aging or increased incidence of certain diseases such as human immunodeficiency virus (HIV) infection, diabetes mellitus, malignancies, or immunosuppressive therapies has led to an increasing incidence of fungal infections.

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Patients with malignancies who are severely immunocompromised have a particular risk of these infections.

In this case-control study we aimed to identify which species of dermatophyte are causing infections in patients diagnosed with neoplastic diseases and also to assess *in vitro* susceptibility of isolated dermatophytes in these patients to itraconazole (ITZ), ketoconazole (KTZ), fluconazole (FLZ), voriconazole (VCZ), and terbinafine (TBF). We compared the findings with those in immunocompetent patients.

PATIENTS AND METHODS

138 patients (71 men and 67 women, sex ratio M/F 1.06) diagnosed with various malignancies that were referred for dermatophyte infections to the Department of Dermatology of the Clinical County Emergency Hospital in Brasov, Romania were included in this study. Only patients who had not recieved any immunosuppressive therapy or radiotherapy in the last 6 months were included in the study. Malignancies diagnosed in study group are shown in Table 1. The mean age of this group (expressed as mean±standard deviation, SD) was 58.84±13.08 years, with a range between 8 and 83 years. The mean duration of the evolution of neoplasia at time of diagnosis was 3.17±1.96 years. In parallel, a control group consisting of 160 immunocompetent patients (84 men and 76 women, sex ratio M/F 1.10) aged between 18 and 78 years (average age 56.42±11.70 years) who showed signs of fungal infections was also analyzed. Pathological material was collected by clipping the nail or scraping the skin lesions from each patient from both groups.

Table 1. The frequency of dermatophyte infections in neoplastic patients with neoplastic diseases

Neoplasia	No. of patients	Dermatophyte infection rate
Colorectal carcinoma	23	26.1%
Breast cancer	22	22.7%
Gastric adenocarcinoma	17	35.3%
Leukemia	16	68.7%
Prostate adenocarcinoma	13	23.1%
Lymphoma	11	36.4%
Cervical cancer	11	18.2%
Ovarian cancer	8	12.5%
Lung carcinoma	7	28.6%
Squamous cell carcinoma	4	25.0%
Melanoma	3	33.3%
Hepatocellular carcinoma	2	0.0%
Esophageal cancer	1	0.0%

The specimens taken from infected areas were examined by direct microscopy. Positive specimens were then cultured in a Sabouraud dextrose agar medium with addition of cycloheximide, chloramphenicol, and gentamicin (Bio-Rad, France) and incubated at 30°C for 2-4 weeks. Dermatophyte species were identified according to macroscopic and microscopic criteria of colonies developed in the culture medium.

Susceptibility to antifungals was tested using a broth microdilution method according to the criteria of M38-P of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi (1). Five antifungal substances were used for testing *in vitro* susceptibility of isolated dermatophytes: itraconazole (Janssen Research Foundation, Beerse, Belgium), ketoconazole (Janssen Research Foundation, Beerse, Belgium), fluconazole (Pfizer Inc., New York, USA), voriconazole (Pfizer Inc., New York, USA), and terbinafine (Novartis, Basel, Switzerland). ITZ, KTZ, FLZ, and VCZ were dissolved in a 100% dimethyl sulfoxide solution, and TBF was prepared in dimethyl sulfoxide solution with 5% Tween 80 according to the protocol of Jessup *et al.* (2). To reduce the initial concentration of each antifungal 100 times, ten serial twofold dilutions were performed. Concentrations of serial drug dilutions ranged from 0.0078 to 4 µg/mL for VCZ and TBF, to 0.0625 to 32 µg/mL for KTZ and ITZ, and 0.125 to 64 µg/mL for FLZ. The initially obtained colonies of dermatophytes were recultured in Sabouraud agar medium at 30°C for 7-15 days, the time necessary to achieve sporulation. The plates with Sabouraud agar medium were covered with sterile normal saline (0.9%), after which the dermatophyte colonies were gently scraped with a sterile loop. The suspension consisting of fragments of conidia and hyphae was transferred to a sterile tube. For sedimentation of heavy particles, the tube was left at room temperature for 15-20 minutes and then the upper suspension was centrifuged at 2000 rpm for 15 seconds. The supernatant was further diluted to 1:50 in buffered RPMI 1640 solution and standardized spectrophotometrically to approximately 0.5 McFarland units at a wavelength of 520 nm and a transmission of 70-80% to the desired concentration of 0.5-5×10^4 colony-forming units (CFU)/mL for final test inoculums. RPMI 1640 medium (Sigma-Aldrich) buffered at pH 7.0 with 0.165 mol/L 3-(N-morpholino) propanesulphonic acid (MOPS) was used for sensitivity tests for antifungals. The sensitivity tests were performed in sterile, round-bottomed, 96 U-shaped well microplates, each well with a nominal capacity of 300 µL. Alliquots of 100 µL of the serial 2-fold dilutions of the antifungal substance and then 100 µL of the diluted inoculums suspensions were added into each well in columns 2 to 11. The first column wells

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Table 2. Etiological agents and clinical forms of dermatophyte infections in both groups

	T. rubrum		*T. mentagrophytes*		*M. canis*		*E. floccosum*		*Total*	
	Neoplasia	**Control**	**Neoplasia**	**Control**	**Neoplasia**	**Control**	**Neoplasia**	**Control**	**Neoplasia**	**Control**
Tinea unguium	18	19	1	2	-	-	-	-	45.2%	44.7%
Tinea pedis	7	11	2	2	-	-	1	1	23.8%	29.8%
Tinea cruris	3	4	1	1	-	-	-	1	9.5%	12.8%
Tinea corporis	2	2	-	-	2	1	-	-	9.5%	6.4%
Tinea manum	2	1	-	-	-	-	-	-	4.8%	2.1%
Tinea faciei	-	-	-	-	1	1	-	-	2.4%	2.1%
Tinea capitis	-	-	-	-	1	-	-	-	2.4%	0%
Tinea barbae	-	-	-	-	1	1	-	-	2.4%	2.1%
Total strains	32	37	4	5	5	3	1	2	42	47
%	76.2%	78.7%	9.5%	10.6%	11.9%	6.4%	2.3%	4.2%	100.0%	100.0%

were filled with 200 µL of RPMI 1640 medium serving as sterility control. 100 µL of inoculum solution and 100 µL of RPMI 1640 medium were distributed in the twelfth column's wells, serving as growth control. *T. mentagrophytes* (ATCC MYA-4439), *Candida parapsilosis* (ATCC 22019), *T. rubrum* (ATCC MYA-4438), and also *Candida krusei* (ATCC 6258) were included as quality control. Microdilution plates were incubated at 30°C for 7 days. Rate of growth in each well was visually assessed daily, starting 48 hours after inoculation, based on comparison with growth in sterility controls and growth controls, respectively.

Mean minimum inhibitory concentrations (MIC), MIC_{50}, and MIC_{90} were determined for each antifungal agent for each dermatophyte species isolated among control group subjects and in the patient group. In accordance with the broth microdilution method proposed in CLSI M38-P protocol (1), the minimal inhibitory concentration was defined as the lowest concentration that showed 100% growth inhibition for TBF and 80% for azoles compared with the growth controls (3,4). A dermatophyte species was considered resistant to an antifungal agent when MIC was ≥4 µg/mL for ITZ, VCZ, and TBF, ≥8 µg/mL for KTZ, and ≥64 µg/mL for FLZ, according to the criteria of CLSI M38-P (1). Statistical differences in the frequency patterns of dermatophyte species between patients with malignancy and the control group was assessed using Fisher's exact chi-square test. Student's t-test was used for calculate the significance of the difference between the mean values of MICs of the five antifungal substances tested in the abovementioned groups. A value of *P* less than 0.05 was considered significant.

RESULTS

Following the mycological investigations among the 138 patients with neoplastic diseases, positive samples for dermatophyte infections were found in 42 patients (30.4%), for *Candida spp.* in 14 patients (10.14%), and for *Aspergillus* in one patient (0.72%).

Frequency of dermatophyte infections according to type of neoplasia is shown in Table 1. The gender distribution of dermatophyte infections in patients with neoplastic diseases was approximately equal: 29.85% for women and 30.98% for men. Tinea unguium was the most frequent clinical form of dermatophytosis encountered in 19 of the 42 patients (45.2%) with malignancies and dermatophyte infection, followed by tinea pedis in 10 patients (23.8%), tinea cruris and tinea corporis in 4 patients each (9.5%), tinea manum in 2 patients (4.8%), and tinea faciei, tinea capitis, and tinea barbae in one patient each (2.4%).

Dermatophyte and candidal infection rates in the control group were 29.37% (47 patients) and 5.6% (9 patients), respectively. Tinea unguium was also the most frequent clinical form of dermatophytosis in the control group, found in 21 cases (44.7%), followed by tinea pedis in 14 patients (29.8%), tinea cruris in 6 patients (12.8%), tinea corporis in 3 patients (6.4%), and tinea manum, tinea barbae and tinea faciei in 1 patient each (2.1%).

Etiological agents depending on the clinical form of dermatophytosis in both groups of patients are shown in Table 2. *T. rubrum* was the most frequently isolated agent in both groups. There were no statistically significant differences in the frequency patterns of *T. rubrum*, *M. canis*, *T. mentagrophytes* and *E. floccosum* between the two groups ($p > 0.05$).

The MIC statistics including mean MIC, MIC_{50}, and MIC_{90} values of ITZ, KTZ, FLZ, VCZ, and TBF for *T. rubrum*, *M. canis*, *T. mentagrophytes*, and *E. floccosum* in patients with neoplasia and in the control group are shown in Table 3. Mean MIC values of all the antifungal

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Table 3. Mean minimum inhibitory concentration (MIC) values of antifungals for all of the isolated dermatophyte species in both groups

Antifungal	Dermatophyte species	Mean MIC		MIC_{50}		MIC_{90}	
		Neoplasia µg/mL	Control µg/mL	Neoplasia µg/mL	Control µg/mL	Neoplasia µg/mL	Control µg/mL
Ketoconazole	*T. rubrum*	0.19	0.172	0.15	0.062	1	1
	T. mentagrophytes	0.75	0.65	0.5	0.5	1	1
	M. canis	0.8	0.67	1	0.5	1	1
	E. floccosum	0.5	0.375	0.25	0.25	0.5	0.25
Itraconazole	*T. rubrum*	0.118	0.08	0.062	0.062	0.5	0.5
	T. mentagrophytes	0.156	0.0875	0.125	0.062	0.25	0.125
	M. canis	0.275	0.33	0.25	0.062	0.5	0.5
	E. floccosum	0.125	0.094	0.125	0.0625	0.125	0.125
Fluconazole	*T. rubrum*	6.1	6.6	4	4	8	8
	T. mentagrophytes	26	10.4	16	8	64	16
	M. canis	12.8	10.6	16	8	16	16
	E. floccosum	4	3	4	2	4	4
Voriconazole	*T. rubrum*	0.15	0.11	0.062	0.062	0.5	0.25
	T. mentagrophytes	0.312	0.275	0.25	0.25	0.5	0.5
	M. canis	0.225	0.208	0.25	0.25	0.25	0.25
	E. floccosum	0.125	0.0625	0.125	0.0625	0.125	0.0625
Terbinafine	*T. rubrum*	0.009	0.008	0.007	0.007	0.031	0.015
	T. mentagrophytes	0.023	0.021	0.015	0.015	0.031	0.031
	M. canis	0.034	0.041	0.031	0.031	0.062	0.062
	E. floccosum	0.062	0.046	0.062	0.015	0.062	0.062

drugs studied for all of dermatophyte species isolated from the neoplastic patients were similar to mean MIC that was obtained in the control group ($p>0.05$). TBF had the lowest, and FLZ the highest mean MIC, MIC_{50}, and MIC_{90} values for all dermatophytes tested in the two groups. One case of resistance to FLZ of a *T. mentagrophytes* strain isolated from a patient with neoplasia was recorded. The patient had previously received repeated treatments with fluconazole for recurrent candidal infections. *In vitro* activity of KTZ, ITZ, VCZ, FLZ, and TBF against dermatophytes in patients with neoplasia was similar to that of immunocompetent patients.

DISCUSSION

Although fungal infections do not cause epidemic or pandemic outbreaks, their incidence has increased significantly, mainly due to the increasing number of patients with compromised immune system. Dermatophyte infections in immunocompromised patients are often disseminated, asymptomatic, and refractory to treatment. In this context, accuracy and precocity of diagnosis become essential for epidemiological purposes and to establish proper treatment, and susceptibility testing is mandatory although the correlation between *in vitro* tests and *in vivo* therapeutic response has not been completely defined.

In this study, we investigated the dermatophyte species causing dermatophyte infections, and the *in vitro* susceptibility of those dermatophytes to ITZ, KTZ, FLZ, VCZ, and TBF in patients with neoplasia and compared the results with those in immunocompetent individuals. We have not noticed any difference in the frequency of dermatophyte infections in patients with neoplastic diseases (30.4%) compared to immunocompetent patients (29.37%). Tinea unguium was the most frequent clinical form of dermatophyte infection encountered in 19 of the 42 patients (45.2%) with neoplasia and dermatophytic infection. Our findings indicate that, similar to the immunocompetent population, *T. rubrum* was the principal agent responsible for dermatophyte infection in patients with neoplastic diseases, followed by *M. canis*. Moulds and yeast infections were diagnosed in 0.72% and 10.14% of patients with malignancies, respectively. In one case there was a mixed infection of the nail with *Candida albicans* and *T. rubrum*. In exceptional circumstances mixed fungal infections may exist, their archetype being the association of *Scopulariopsis brevicaulis* and *T. rubrum* in onychomycosis.

The combination *of Mucor spp.* and *Candida spp.* in with patients neoplastic diseases has also been reported in the literature (5).

Patients with lymphoma or leukemia are more susceptible to deep fungal infections in periods with severe neutropenia following chemotherapy or bone marrow transplantation (6,7). In our study, there was a significantly higher frequency of dermatophyte infections in patients with hematological malignancies (55.5%) compared to those with solid cancers (24.3%), similarly to Altay *et al.* who also found a significantly higher incidence of fungal infections of 70% in patients with hematologic malignancies compared with healthy patients, but with *Candida albicans* being the prevalent mucosal infection (8). Patients with solid tumors who are treated with radiation or chemotherapy in small doses have a lower risk of developing dermatophytosis (9).

The patients' age and comorbidities, such as diabetes mellitus (10), psoriasis (11), Cushing syndrome, malnutrition, alcoholism, drugs abuse, and genetic diseases with immune deficiencies, may favor the emergence of dermatophytosis, especially of onychomycosis (12).

Systemic antifungal treatment is the therapy of choice for dermatophytosis (13). Although the therapeutic efficacy of itraconazole (ITZ), ketoconazole (KTZ), fluconazole (FLZ), voriconazole (VCZ), and terbinafine (TBF) is well known for the general population with dermatophytosis, there are limited data for patients with neoplastic diseases.

The main difficulty in treating patients with neoplastic diseases is selecting of the most effective antifungal drug.The use of this therapy may result in undesirable side-effects that can be particularly dangerous in patients with malignancies. Although most clinical trials on immunocompetent patients show that TBF has higher cure and lower relapse rates (14), less data are available about its efficacy in patients with neoplastic diseases. Our data reveal that all tested antifungal drugs are effective against *T. rubrum*, *M. canis*, *T. mentagrophytes* and *E. floccosum* in patients with neoplastic diseases, but TBF has higher antidermatophyte activity with much lower mean MIC than other antifungals, and FLZ was the least efficient antifungal drug, in addition to a strain of *T. mentagrophytes* resistant to FLZ being identified in a patient with neoplasia.

CONCLUSION

Since TBF was the most active antidermatophyte drug *in vitro*, and considering that its higher antidermatophyte activity compared with azoles and its drug interactions do not typically cause problems, we believe TBF should be the therapy of choice for dermatophytosis in patients with neoplastic diseases. However, larger studies are required to assess the safety of antifungal drugs administration in patients with malignancies.

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