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A comparative study of dermatophyte infections in Bursa, Turkey

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A total of 555 specimens from 372 patients with symptoms compatible with superficial mycosis were included in this study. Those from patients clinically diagnosed as having dermatomycosis were thoroughly investigated by mycological examinations in the laboratory, including microscopic studies of KOH mounts and cultivation of the samples in culture. The results of this study and a previous study in our hospital conducted in 1980 were compared with respect to clinical presentation and etiological agents. Onychomycosis was the most common clinical form of dermatomycoses, and *Trichophyton rubrum* was the most common pathogen in this study.

Keywords dermatophytes, epidemiology, superficial fungal infections

Introduction

Superficial fungal infections affect millions of people worldwide, with an estimated lifetime risk of 10–20% [1]. These infections (dermatophytosis or ring-worm) tend to involve the hair, nail, or skin of the host, and are caused by dermatophytes which are comprised of three genera, i.e., *Epidermophyton*, *Trichophyton*, or *Microsporum*.

Dermatophytes may be anthropophilic, zoophilic or geophilic based on their natural habitats. These fungi produce keratinases which are proteolytic enzymes capable of hydrolysing keratin, the major protein constituent of hair, nail and skin. Infections may have mild to severe symptoms depending on the immunological response of the host [2,3]. Cutaneous infections resembling dermatophytoses may be associated with yeast (*Candida* spp, *Malassezia* spp, *Trichosporon* spp) or by unrelated filamentous fungi (dematiaceous or hyaline) that are generally considered saprobes or plant pathogens. Such infections are referred to opportunistic dermatomycoses [4]. Previous studies from Turkey have focused on the changes of dermatophytes associated with infection over the course of time [5,6]. The purpose of this study was to determine the distribution of dermatomycoses and their etiological agents in Bursa, Turkey, as well as to compare the results with those from a previous investigation conducted at our hospital in 1980 [7].

Materials and methods

A total of 372 outpatients were admitted to the Department of Dermatology of Uludag University between March 2005 to February 2006 with clinical symptoms of cutaneous mycoses, including itching, desquamation, vesiculation, discoloration, fissures on skin, maceration on interdigital spaces, thickening and discoloration of the subungual area, yellowish-brown nail plates or desquamation or alopecia on scalp skin. Patients receiving systemic/topical antifungal therapy within the last six months, those who had concurrent cutaneous or nail lesions of psoriasis, lichen planus or other dermatomycoses were excluded. All patients provided informed consent.

All patients completed a survey providing epidemiological data including sex, age, previous treatments, occupation, duration and type of lesion, and personal hygiene (hygienic shower). Samples from skin lesions from patients with preliminary clinical diagnoses of superficial fungal infection were collected and referred to the mycology laboratory from dermatology outpatient clinic. A total of 555 specimens were obtained by clippings or scrapings of the scales at the periphery of the skin lesions and by

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subungual curettage of affected nails. If patients had more than one site of infection, scrapings were collected from each lesion and were subjected to direct microscopy and culture. Direct microscopic examination of the specimens was performed after digestion in 10–20% potassium hydroxide (KOH).

For culture analysis, specimens were inoculated onto slants of Sabouraud's dextrose agar (SDA) containing chloramphenicol, gentamicin and cycloheximide, Potato Dextrose Agar (PDA) and Mycobiotic agar (MA) (Oxoid Ltd., Basingstoke, Hampshire, England).

All cultures were incubated at 25–37°C and examined three times weekly for growth. Samples were declared as negative only if there was no growth after four weeks of incubation [8]. Fungal growth was usually apparent after one or two weeks and identification was based on macroscopic and microscopic morphology of resultant colonies according to standard criteria. Species (dermatophyte and non-dermatophyte) identification was performed on the basis of cultural characteristics, pigment production and urease production. Microscopic features of the isolates were studied by cellotape flag preparation and slide culture techniques using lactophenol-cotton blue dye. All dermatophytes isolated from cultures were considered as pathogens.

For yeast identification, germ tube tests, chlamydospore formation studies and assimilation tests were performed. The involvement of a non-dermatophyte fungus was judged clinically significant only in the presence of positive direct microscopy (hyphae, spores, or yeast cells) and isolation of the non-dermatophyte fungus in pure culture from a significant portion of clinical samples. A non-dermatophyte was considered to be the primary pathogen only after being repeatedly isolated from two separate samples [8,9].

Descriptive statistics were performed and the results were expressed as mean, standard deviation and percentage. In addition, results of this and the previous study were compared by Pearson Chi-square Test using SPSS (Statistical Package for Social Sciences) for Windows version 17.0 software.

Results

The most prevalent clinical manifestation was onychomycosis, followed by tinea pedis, tinea inguinalis, tinea corporis, tinea manuum, tinea barbae and tinea capitis. Comparison of the observed clinical conditions between present and the previous study and their statistical significance are shown in Table 1. Increases in the prevalence of onychomycosis and decreases in tinea pedis, tinea inguinalis and tinea capitis were found to be statistically significant.

The most common etiological agent of dermatomycoses was *Trichophyton rubrum,* followed by *T. mentagrophytes* and *T. tonsurans*. Comparison of etiological agents recovered in the two studies and their statistical significance are shown in Table 2.

Table 1 Clinical pictures, number of patients and percentages in previous and present studies.

	Number of patients and percentages			
Clinical picture	Previous study	Present study	*P*	OR
Tinea pedis	145 (51.78%)	208 (37.48%)	< 0.001	1.79
Tinea inguinalis	60 (21.43%)	28 (5.05%)	< 0.001	5.13
Onychomycosis	28 (10.00%)	281 (50.63%)	< 0.001	0.11
Tinea unguium pedum	ND	240 (43.24%)		
Tinea unguium manum	ND	41 (7.39%)		
Tinea capitis	18 (6.43%)	1 (0.18%)	< 0.001	38.06
Tinea corporis	15 (5.36%)	25 (4.50%)	0.586	1.20
Tinea manum	0	10 (1.80%)	0.036	0
Tinea barbae	0	2 (0.36%)	0.553	0
Kerion celsi	9 (3.21%)	0	< 0.001	-
Favus	5 (1.79%)	0	0.004	-
Total	280 (100%)	555 (100%)		

ND, not differentiated.

During the present study period, a total of 555 specimens from 372 patients were evaluated for the presence of superficial fungal infection (Table 3). Among these, 307 (55.32%) were identified as positive by direct KOH wet mount examination and 149 (26.85%) were culture positive (Table 4). In total, 318 (57.30%) specimens were confirmed by direct microscopy and/or culture. While fungi were isolated from only 11 direct microscopy negative specimens, no growth was obtained in culture with 55.32% of clinical specimens which were positive by direct microscopic examination.

Tinea unguium pedum was the most frequent clinical diagnosis, followed by tinea pedis (Table 5). When toenail and fingernail infections were combined, onychomycosis accounted for half of the dermatomycoses (50.63%).

Table 2 Number and percentage of isolated strains in previous and present studies.

Dermatophyte spp.	Previous study	Present study	*P*	OR
T. mentagrophytes	40 (30.07%)	5 (3.36%)	< 0.001	12.39
E. floccosum	34 (25.56%)	1 (0.67%)	< 0.001	50.83
T. rubrum	20 (15.04%)	130 (87.25%)	< 0.001	0.03
M. canis	16 (12.03%)	0	< 0.001	-
T. violaceum	15 (11.28%)	0	< 0.001	-
T. verrucosum	5 (3.76%)	0	0.022	-
T. tonsurans	3 (2.26%)	2 (1.34%)	0.67	1.70
Candida spp.		9 (6.04%)	0.001	-
Alternaria spp.		2 (1.34%)	0.50	-
Total	133 (100%)	149 (100%)		

Table 3 Accordance with direct microscopy and culture results.

	Positive with direct microscopy	Negative with direct microscopy	Total
Culture positive	138 (24.86%)	11 (1.98%)	149 (26.85%)
Culture negative	169 (28.83%)	237 (42.70%)	406 (73.15%)
Total	307 (55.32%)	248 (44.68%)	555 (100%)

Among 372 patients with a mean age 42.7 ± 16.2 years, 166 were male and 206 were female. Demographic data of these patients are presented in Table 5. Distribution of cutaneous mycoses according to sex is shown in Table 6.

As seen in Table 2, *T. rubrum* was the most frequently isolated species (87.25%). Eleven isolates of *Candida* and *Alternaria s*pecies were recovered in culture. Seven out of nine *Candida* species isolated from fingernails indicate the predominance of yeasts in tinea unguium manum. The highest isolation rate was noted in cases of tinea pedis.

Discussion

Superficial fungal infections continue to be a worldwide problem, constituting a large number of cases admitted to dermatology outpatient clinics. As dermatophytes are keratinophilic fungi, the disease is restricted to the superficial keratinised tissues. The increasing prevalence of mycotic infections may be attributed to an increase in the number of people living in close proximity to each other. Other factors may be related to the patient's family history, occupation, lifestyle, underlying physiology or immune status. Prevalence of different types of fungal infections varies according to race and geographical location, environmental conditions, migration, and socioeconomic and cultural factors [9–11]. Thus, results from population-based clinicomycological studies of onychomycosis are expected to assist in selection of appropriate treatment. The severity of infections depends on location and host immunity, as well as hormonal abnormalities [12]. Population characteristics of our study are shown in Table 5.

Although new laboratory methods for the confirmation of infection exist, mycological examination is still the most commonly used diagnostic technique. Our results emphasize that samples from lesions should be divided into two parts and analysed using both direct microscopy and culture. Direct microscopic examination allows visualisation of fungal elements in the samples within 20–30 min. Microscopy may be used to distinguish dermatophyte hyphae, pseudohyphae of yeast and non-dermatophyte hyphae, but provides no information about the identity of the species. Therefore, microscopy must be combined with culture to enable species identification. Accurate diagnosis depends on the expertise of laboratory staff and the quality of the samples. Samples should always be collected from the appropriate infected areas. Direct microscopic examination yields positive results more often than the use of cultures. Approximately half of clinically suspected diagnoses can be confirmed by direct microscopy [7]. In this study, 55.32% of the samples were found to be positive by direct microscopic examination, while only 26.85% of the samples were culture positive (Table 3). Data regarding direct microscopy were not reported in the previous study; therefore we were not able to compare these results.

Similar to other studies, 169 KOH-positive specimens failed to yield a fungus when a portion of the specimen was inoculated in culture. This might be the results of the fact that the specimens contain dead and non-viable hyphae as a result of chronic disease or insufficient previous treatment before the patient was admitted [9].

Onychomycosis is a common condition observed in primary care settings. It accounts for up to 50% of nail diseases and 30% of all superficial fungal infections [13]. The diagnostic accuracy may be as low as 50–70% [12,14]. Dermatophytes have been reported as the major etiologic agents in all types of onychomycosis (62.1–87.5%) [15]. Clinical presentation often does not offer any useful clues regarding the identification of the fungal agent. The pathogens that are involved in onychomycosis may vary according to age and gender of patients, as well as sites of involvement and presence of paronychia [16]. Madhuri

Table 4 Relative frequencies of dermatophytic species observed and distribution according to the anatomical site affected.

	Onychomycosis								
	TUP*	TUM*	Tinea pedis	Tinea inguinalis	Tinea corporis	Tinea manuum	Tinea barbae	Tinea capitis	Total (%)
T. rubrum	51	-	68	7	3	1	-	-	130 (87.25)
T. mentagrophytes	2	-	3	-	-	-	-	-	5 (3.36)
T. tonsurans	-	-	2	-	-	-	-	-	2 (1.34)
E. floccosum	-	-	-	-	1	-	-	-	1 (0.67)
Candida spp**	-	7	1	-	-	1	-	-	9 (6.04)
Alternaria spp	1	-	1	-	-	-	-	-	2 (1.34)
Total	61 (40.94)		75 (50.34)	7 (4.70)	4 (2.68)	2 (1.34)	-	-	149 (100)

*TUP, Tinea unguium pedum; TUM, Tinea unguium manum; ** Four *C. albicans*; one for each of *C. tropicalis*, *C. parapsilosis* and *C. inconspicua*; two non-identified *Candida* species.

Table 5 Study population characteristics with dermatophytosis.

Characteristics		% (n = 372)
Gender	Male	44.62 (166)
	Female	55.38 (206)
Age	0–14 year	2.42 (9)
	15–50 year	64.25 (239)
	> 50 year	33.33 (124)
Previous treatment	Yes	37.63 (140)
	No	62.37 (232)
Illness period	< 1 year	36.30 (135)
	1–5 year	39.78 (148)
	> 5 year	23.92 (89)

et al. [17] reported that onychomycosis prevalance rate is highest in the third decade of life. Sahin *et al*. [18] found significant differences in the prevalence of onychomycosis between the 15 year and over age group and those 0–14 years of age. In addition, Gunduz *et al*. [19] have emphasized that prevalance rate of onychomycosis among children living in rural areas is higher to that noted in urban areas. In the present study, the prevalance of cutaneous mycosis was highest (64.25%) in patients aged between 15 and 50 years and 33.33% were aged 50 years and older (Table 5). Poor peripheral circulation, diabetes, repeated nail trauma, longer exposure to pathogenic fungi, defects in immune function, inactivity or the inability to cut the toenails and maintain adequate foot care may explain the age-related increase in the prevalance of onychomycosis [16,20–22].

Garg *et al*. [23] reported the rate of fingernail and toenail onychomycosis as 60% and 26.76%, respectively, whereas Ertam and Aytimur [24] demonstrated rates of 13.3% and 86.7%, respectively. In our study, the rates of tinea unguium manum and tinea unguium pedum were found to be 7.39% and 43.24%, respectively (Table 1).

Vijaya *et al*. [25] noted that toenail infections were more predominant in males, while fingernail infections were more predominant in females. In our study, prevalence of onychomycosis was found to be higher in females as compare to males (58.20% vs. 43.20%) and tinea pedis was more common in males than in females (42.20% vs. 32.70%). In females, prolonged contact with water and detergents may be the main predisposing factor for onychomycosis. Tinea pedis may be more prevalent in males due to their occupations and as a result, lengthy periods of time in enclosed footwear often result in infections. Closed shoes constructed of non-breathable materials are particularly problematic. Indoor shoe use is estimated to affect 10% of the world population [26,27]. High temperature and humidity are also predisposing factors for tinea pedis. Tight shoes make the feet more susceptible to disease and may cause damage to the superficial veins. Cutaneous fungal infections are as highly prevalent in the general population as onychomycosis and tinea pedis. Although these disorders are not serious in terms of mortality or physical and/or psychological sequelae, they have significant clinical consequences in terms of aesthetics, chronicity and therapeutic difficulties.

Yeasts may colonize glabrous skin, hair and nails and become pathogenic only in association with loss of epidermal barrier function due to pre-existing infection or trauma. In this study, *Candida* spp accounted for 6.05% of all isolates.

The increase in the prevalence of *T. rubrum* and decreases of *T. mentagrophytes* and *E. floccosum* were statistically significant. While all isolated species belonged to the anthropophilic group in our study, there were also some zoophilic species such as *M. canis* and *T. verrucosum* in the previous study. The absence of chronic diseases like kerion celsi and favus was another difference between two investigations. The epidemiological differences between dermatomycoses and dermatophytes may be attributed to changes in living habits, improvement in general sanitary conditions, personal hygiene, and socioeconomic development. Although agricultural activities were widely practiced in our region 30 years ago, industrial activities have become more intense in recent years.

The prevalence of the two most common causative agents varies among different regions of our country. The two most common etiologic agents are *T. rubrum* followed by *T. mentagrophytes* [28–30]. Koksal *et al*. have reported that *T. rubrum* was the most common and *Candida* spp. were the second most common pathogens in Istanbul [31]. Metintas *et al*. noted that *T. rubrum* was the most common species but zoophilic and geophilic dermatophyte species were also isolated in another study that was performed in

Table 6 Cutaneous mycoses according to sex in present[a] (n = 372), *previous*[b] *(n = 280)* and the total number of materials (n = 555, number of male 280 and number of female 275).

		Male	(%)		Female	(%)
Tinea pedis[a]	IDA	90	(32.20)		65	(23.60)
	PA	28	(10.00)	25	(9.10)	
Tinea pedis[b]	107	(51.70)	38	(52.10)		
Onychomycosis[a]	TUP	100	(35.70)		140	(50.90)
	TUM	21	(7.50)		20	(7.30)
Onychomycosis[b]	12	(5.80)		16	(21.90)	
Tinea inguinalis[a]	18	(6.40)	10	(3.60)		
Tinea inguinalis[b]	55	(26.60)		5	(6.80)	
Tinea corporis[a]	13	(4.60)		12	(5.80)	
Tinea corporis[b]	10	(4.80)		5	(6.80)	
Tinea manum[a]	7	(2.50)		3	(1.10)	
Tinea barbae[a]	2	(0.07)			(0.00)	
Tinea capitis[a]	1	(0.04)			(0.00)	
Tinea capitis[b]	14	(12.10)		4	(9.60)	
Total (only present[a])		280	(100.00)	275	(100.00)	

IDA, inter-digital area; PA, planter area; TUP, Tinea unguium pedum; TUM, Tinea unguium manuum.

Table 7 Frequency of etiological agents of dermatomycoses in different countries*.

City/country, [Ref.]	*T. rubrum*	*T. mentagrophytes*	*T. schoenleinii*	*T. verrucosum*	*T. violaceum*	*T. tonsurans*	*M. canis*	*Candida* spp.
Delhi/India [34]	1	2						3
Gdansk/Poland [35]	3	1					2	
Creete/Greece [36]	1	3					2	
Prague/Czech Rep. [37]	1	2						
Lanzou/China [38]	1	2						
Hamadan/Iran [39]			2	1				
Tripoli/Libya [40]	2				1			
Estonia [41]	1	3						2
Denmark [42]	1	2			3			
Rome/Italy [43]	3	2					1	
UK [44]	1	2				3		

*First two or three fungal species in order to frequency.

rural areas of Eskişehir, Turkey [32]. Lopez-Martinez *et al*. have emphasized that the prevalence of *T. rubrum* infections is increasing in many parts of the world (USA, Spain, Italy, Peru and UK) while the prevalences of *T. mentagrophytes*, *T. tonsurans* and *M. canis* infections are decreasing [33]. They have attributed these changes to greater adaptive capacity of *T. rubrum* to host conditions, as shown by low inflammatory response and long course of infection whereas the other dermatophyte species, particularly the zoophilic fungi, usually cause a more severe inflammatory process. In other parts of the world, these findings vary according to changes in geographic location, educational status, economic conditions and living habits and data are shown in Table 7 [34–44]. Predominance of some zoophilic species like *M. canis* noted in some countries [34,35,42] may be attributed to widely practiced pet ownership, which in contrast is very rare in our country.

Studies have reported that different species of dermatophytes may behave significantly different as aetiological agents in different regions and years, as well as socioeconomic status [19,45].

In conclusion, the most significant results of our study are: (1) *T. rubrum* is the most common dermatophyte isolated in our study population, and (2) onychomycosis is the most prevalent dermatomycosis. This differs from the findings of the previous study, and it has been attributed to the changes in socioeconomic conditions.

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