EPIDEMIOLOGICAL AND CLINICAL ASPECTS OF DERMATOPHYTOSES IN EASTERN SLOVAKIA: A RETROSPECTIVE THREE-YEAR STUDY

Zuzana Baranová[1], Tomáš Kampe[2], Erik Dorko[3], Kvetoslava Rimárová[3]

[1]Department of Dermatovenerology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovak Republic
[2]Department of Dermatovenerology, Louis Pasteur University Hospital, Košice, Slovak Republic
[3]Department of Public Health and Hygiene, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovak Republic

SUMMARY

Objective: A three-year retrospective study of fungi isolated from samples of patients with suspected fungal skin infections in Eastern Slovakia is presented.

Methods: A total of 11,989 samples were collected and investigated with direct microscopic examination using 20% KOH and cultivated in Sabouraud and Mycosel medium. Identification was based on macroscopic and microscopic characteristics.

Results: Of the total samples, 61.76% (7,405/11,989) were completely negative and 38.24% positive (4,584/11,989). Dermatophytes accounted for 45.88% of isolates (2,103/4,584), yeasts for 26.79% (1,228/4,584), non-dermatophytes for 15.29% (701/4,584), and *Malassezia* sp. for 12.4% (552/4,584). *Trichophyton rubrum* was the most prevalent causative agent (79.08%) implicated in fungal skin infections, followed by *Trichophyton interdigitale* (10.60%). Less frequent isolates included *Trichophyton tonsurans* (5.13%), and *Trichophyton mentagrophytes* (3.14%). Other dermatophytes (*Microsporum audouinii, Microsporum gypseum, Microsporum canis, Trichophyton violaceum, Trichophyton verrucosum*, and *Epidermophyton floccosum*) were very rarely identified (each in less than 1% of all samples). The main clinical form of dermatophytosis in the sample was tinea unguium (42.61%), followed by tinea pedis (30.86%), tinea inguinalis (11.65%), tinea corporis (8.04%), and tinea manus (4.76%). Tinea capitis et faciei (2.08%) was more common among children and adolescents.

Conclusion: The assessment of data has showed the predominance of tinea unguium among adult patients, tinea capitis et faciei among children, and the prevalent aetiological role of *Trichophyton rubrum* in fungal skin infections; findings that are in agreement with recent European studies.

Key words: retrospective study, epidemiology, dermatophytes, tinea

Address for correspondence: Z. Baranová, Department of Dermatovenerology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice and Louis Pasteur University Hospital, Trieda SNP 1, 040 11 Košice, Slovak Republic. E-mail: zuzana.baranova@upjs.sk

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INTRODUCTION

Incidences of fungal infections are increasing throughout the world as a result of the use of broad-spectrum antibiotics, immunosuppressive therapies, cytotoxic agents, chemotherapies and new medical therapeutic methods such as biologics. Other risk groups suffering from fungal infections are most notably in the aged population with increased prevalence of chronic diseases. Socioeconomic status, a warm and humid environment, lifestyle, presence of pets, age and personal hygiene of a patient are also important factors predisposing to dermatophyte infections. Fungal infection risks are highly dependent on a combination of host immune competency and specific exposures of people both within the health care system and their communities (1).

Fungal infection – dermatophytosis – is an infection of the skin, nails and hair caused by dermatophytes. The dermatophytes can use cutaneous keratin as a nutrient because of their keratinophilic and keratinolytic properties. Three ecological groups of dermatophyte species are known: anthropophilic, zoophilic and geophilic. Anthropophilic species are confined as a host and spread from human to human. The zoophilic species' usual hosts are animals, and can spread from animals to human. Geophilic species can spread from soil to humans, but are only occasionally pathogenic for animals or man.

The clinical features of dermatophyte infections result from a combination of keratin destruction and an inflammatory host response. The wide variation in clinical presentation depends on the following factors: species and probably strain of fungus, the size of inoculum, site of body infected, and immune status of host. Anthropophilic species typically produce mild but chronic lesions. Zoophilic and geophilic species tend to produce highly inflammatory reactions in humans (2).

The dermatophytes embrace the three genera *Epidermophyton* (*E.*), *Trichophyton* (*T.*) and *Microsporum* (*M.*). Within those genera are the major anthropophilic species *T. rubrum, T. interdigitale*. *T. tonsurans, T. violaceum, M. audouinii*, and *E. floccosum;* the zoophilic species *M. canis, T. verrusocum, T. mentagrophytes, T. equinum, M. nanum, M. disortum*, and *M. persicolor;* and the geophilic species *T. terrestre, M. gypseum*, and *M. fulvum*.

The dermatophytosis is usually called the „tinea" and classified according to the body part involved (scalp dermatophytosis is known as tinea capitis, face dermatophytosis as tinea faciei,

body dermatophytosis as tinea corporis, pedis dermatophytosis as tinea pedis, etc.).

Sometimes, the lesion caused by dermatophytes is known as "ringworm" because of the anatomical shape.

Dermatophytosis diagnosis is made by direct mycological examination with 10–20% potassium hydroxide (KOH) of biological material obtained from suspected lesions and by cultivation. Molecular methods such as polymerase chain reactions can sometimes be used.

MATERIALS AND METHODS

The aim of this work was to retrospectively analyse the epidemiological and mycological profile of a patient population with presumed dermarophyte diagnosis of fungal infections.

During a three-year period (from January 2014 to December 2016) a total of 11,989 clinical samples with lesions of the skin, nails and hair were collected from patients with suspected fungal infections. Samples were obtained from dermatological inpatients and outpatients departments in eastern Slovakia. All samples were investigated at the Mycological Laboratory of the Department of Medical and Clinical Microbiology, University Hospital in Košice. The study comprised all samples of patients with suspected diagnosis of skin fungal infection, regardless of causative species, site of infection, gender, age, or prior therapy.

All samples were processed by direct microscopic examination using 20% potassium hydroxide (KOH) with Parker ink for the visualization of fungal elements (hyphae and spores). However, microscopy provides no information about the identity of species. Therefore, samples were inoculated onto slants of Sabouraud's dextrose agar (SDA) with chloramphenicol and BD Mycosel Agar (Sabouraud's dextrose agar containing cycloheximide and chloramphenicol). Cultures were incubated for three to four weeks at 25 °C. Samples were identified as negative if there was no fungal growth after four weeks of incubation. The dermatophyte was identified on the basis of macroscopic characteristics such as growth rate, nature of growth, macroscopic appearance, surface texture, colour of colonies, pigmentation on reverse and microscopic morphology (characteristic arrangement, size, shape and type of spores produced, presence of macroconidia, production of chlamydospores and some special characteristics).

RESULTS

In our study, a total of 11,989 samples were examined over a three year period for suspected cases of skin fungal infection. Only positive cultivations of dermatophytes were evaluated, which is the gold standard for fungal infection diagnosis. Of these 11,989 clinical samples 4,584 (38.24%) proved positive for micromycetes (direct microscopic examination and/or culture) and 7,405 (61.76%) were completely negative (direct microscopic examination and cultivation).

The sex and age distributions are shown in Table 1 and 2. Data analysis showed that 6,684 (55.75%) were female and 5,305 (44.25%) male. Study subjects' ages ranged from two to 81 years with a mean age of 42.4 years. Fungal elements (spores and hyphae) were detected in 4,584 (38.24%) of all clinical samples by KOH wet mount of which 552 (12.04%) were *Malassezia sp*., as the causative agent of Pityriasis versicolor. Further investigation of samples by means of cultivation (Table 3) showed the presence of dermatophytes in 45.88% of cases, (such as *Trichophyton sp., Epidermophyton floccosum,* and *Microsporum sp*.,), non-dermatophytes in 15.29% of cases (*Aspergillus sp, Onychocola canadensis, Cladosporium sp., Paecilomyces sp., Scedosporium sp., Scolpulariosis brevicaulis, Fusarium sp*,) and yeasts in 26.79% of cases (*Candida sp*).

We have evaluated the data on dermatophytoses in correlation with age group (Table 4).

Among all the dermatophyte isolates, *T. rubrum* (79.08%) was the most common cause of infection, followed by *T. interdigitale* (10.60%), and *T. tonsurans* (5.14%), whereas *T. violaceum*, *T. mentagrophytes, Microsporum sp*. and *E. floccosum* were the least common. Of the zoophilic species, *T. mentagrophytes* was the most common pathogen (3.04%) followed by *M. canis* (0.90%). Annual occurrences of isolated species showed a constant frequency over the study period (Table 5).

Tinea unguium caused by dermatophytes was the most prevalent form of infection at 42.61% (896/2,103), followed by tinea pedis (30.86%), tinea inquinale (11.65%), tinea corporis (8.04%), tinea manus (4.76%) and tinea capitis et faciei (2.08%) (Table 6). Tinea capitis et faciei mostly affected children and adolescents

Table 1. *Annual frequency and percentage distributions by gender (N = 11,989)*

Year	Female n (%)	Male n (%)	Total n (%)
2014	2,094 (53.78)	1,800 (46.22)	3,894 (32.48)
2015	2,265 (54.91)	1,860 (45.09)	4,125 (34.41)
2016	2,325 (58.56)	1,645 (41.44)	3,970 (33.11)
Total	6,684 (55.75)	5,305 (44.25)	11,989 (100.00)

Table 2. *Gender distribution according to age group (N = 11,989)*

Age group	0–20 years		21–60 years		≥ 61 years	
	Female	Male	Female	Male	Female	Male
2014	32	40	1,196	1,039	866	721
Total	72		2,235		1,587	
2015	48	59	1,525	1,288	692	513
Total	107		2,813		1,205	
2016	46	52	1,594	1,141	685	452
Total	98		2,735		1,137	

Table 3. *Annual distribution of all micromycetes (N = 4,584)*

Years	Dermato-phytes	Nonderma-tophytes	Yeasts	Malassezia sp.	Total
2014	731	195	472	223	1,621
2015	736	295	396	189	1,616
2016	636	211	360	140	1,347
Total n (%)	2,103 (45.88)	701 (15.29)	1,228 (26.79)	552 (12.40)	4,584 (100.00)

Table 4. *Frequency of clinical presentation of dermatophytoses according to age group (N = 2,103)*

Clinical manifestations	0–20 years	21–60 years	≥ 61 years	Total	%
Tinea capitis et faciei	29	15	0	44	2.08
Tinea corporis	28	123	18	169	8.04
Tinea manus	8	59	33	100	4.76
Tinea inguinalis	0	118	127	245	11.65
Tinea pedis	21	320	308	649	30.86
Tinea unguium	4	556	336	896	42.61
Total n (%)	90	1,191	822	2,103	100.00

Table 5. *Annual prevalence of dermatophytes (N = 2,103)*

Dermatophytes	2014	2015	2016	Total	%
T. rubrum	579	584	500	1,663	79.08
T. interdigitale	67	80	76	223	10.60
T. mentagrophytes	34	17	13	64	3.04
T. tonsurans	39	38	31	108	5.14
T. violaceum	2	0	1	3	0.14
T. verrusocum	1	0	0	1	0.05
M. audouinii	1	5	3	9	0.43
M. canis	5	7	7	19	0.90
M. gypseum	3	4	3	10	0.48
Epidermophyton	0	1	2	3	0.14
Total	731	736	636	2,103	100.00

under 20 years of age, while the other forms of tinea, mainly tinea pedis et unguium, were more frequent in adulthood. A significant proportion of patients had onychomycosis and tinea pedis in the adult age range ($p < 0.001$), whereas tinea capitis was more common among children and adolescents ($p = 0.010$).

Mainly zoophilic species such as *T. mentagrophytes, M. canis* and antropophilic *M. audouinii* were associated with tinea capitis in the under 20 years age group. *T. rubrum* was the predominant species isolated from adults with tinea unguium (37.95% − 798/2,103) and tinea pedis (27.15% − 571/2,103). *T. tonsurans* was the predominant species isolated (5.13%) mostly with tinea corporis, while other forms of tinea were less frequent (Table 6).

DISCUSSION

Dermatophytoses are a worldwide problem. The increasing prevalence of fungal infections may be attributed to an ageing population and the use of immunosuppressive therapies, cytotoxic agents, chemotherapies, and biologic drugs. Other factors may be related to lifestyle, occupation or immune status. The prevalence of fungal infections varies according to geographical location, environmental conditions and cultural and socio-economic factors. The incidence of superficial fungal infections in the global human population is assumed to be 20 to 25% (3).

In the period between 2014 to 2016, 11,989 samples were obtained from skin, hair and nails and examined in a mycological laboratory. Direct microscopic investigation and/or cultivation gave positive results in 38.24% (4.584/11,989) of samples, while 61.76% of samples were completely negative. Dermatophytes were detected in 45.88% (2,103/4,584) of all positive samples, yeasts in 26,79% (1,228/4,584), nondermatophytes in 15.29% (701/4,584) and *Malassezia* sp in 12.04% (552/4,584) of cases.

Berenji et al. (4) found that among 3,804 patients with cutaneous and superficial fungal infections, 2,212 (58.1%) were positive for *Malassezia* infections. Furthermore, 1,257 (33.1%) cases of dermatophytes, 258 (6.8%) cases of *Candida* infections, 62 (1.6%) cases of aspergillosis and 15 (0.4%) cases of other fungal infections were identified. Miklic et al. (5) reported the frequency of superficial fungal infections based on aetiological agents isolated during a ten-year period in Zagreb, Croatia. The results showed that dermatophytes were responsible for 63% of all superficial fungal diseases, followed by yeasts (36%) and moulds (1%).

Malassezia genus yeast belongs to normal skin microflora and is the causative agent of pityriasis versicolor (PV). Clinical diagnosis is simply confirmed by direct microscopical examination with KOH and demonstration of pseudohyphae and blastoconidia in the typical "spaghetti and meatballs" pattern. Cultivation is not necessary for routine diagnosis. Prevalence is high in regions with hot and humid climates with over 15% of the population

Table 6. *Causative agents of dermatophytosis in correlation with clinical forms*

	Tinea capitis, faciei	Tinea corporis	Tinea manus	Tinea inguinalis	Tinea pedis	Tinea unguium	Total n (%)
T. rubrum	0	45	62	187	571	798	1,663 (79.08)
T. interdigitale	0	4	2	54	69	94	223 (10.60)
T. mentagrophytes	14	23	22	0	5	0	64 (3.04)
T. tonsurans	5	85	8	2	4	4	108 (5.13)
T. violaceum	0	2	1	0	0	0	3 (0.14)
T. verrusocum	0	0	1	0	0	0	1 (0.05)
M. audouinii	9	0	0	0	0	0	9 (0.43)
M. canis	12	7	0	0	0	0	19 (0.90)
M. gypseum	4	2	4	0	0	0	10 (0.48)
Epidermophyton	0	1	0	2	0	0	3 (0.14)
Total n (%)	44 (2.08)	169 (8.04)	100 (4.76)	245 (11.65)	649 (30.86)	896 (42.61)	2,103 (100.00)

potentially affected (6). In Italy, the prevalence of this superficial yeast infection was 2.1% among 1,024 young Italian sailors (7). We observed a higher incidence of Pityriasis versicolor. This may be explained by the characteristics of our patient population because we have evaluated samples of patients with presumed skin fungal infection.

Of the 2,103 dermatophytes isolates in the present study, 79.08% was accounted for by *T. rubrum*, followed by *T. interdigitale* (10.60%), and *T. tonsurans* (5.13%). *Microsporum sp*, *Epidermophyton floccosum*, *T. violaceum*, and *T. mentagrophytes* were rare as causative agents. Tinea ungium predominated in our study (42.61%), followed by tinea pedis (30.86%) and tinea inguinalis (11.65%). The most common sites of involvement were nails and feet in adults, and scalps in children, which is consistent with the literature. Other recent epidemiological surveys of skin fungal infections have confirmed the increased prevalence of tinea unguium, as well as the predominant etiological role of *T. rubrum* (8−12).

In a survey of over 96,000 patients in 20 European countries (known as the Achilles Project), onychomycosis was diagnosed in 29.6% of the population. In over 70% of diagnoses confirmed by cultivation dermatophytes were the fungi causing infection (13).

According to Vena et al. (14), the most prevalent clinical form was tinea ungium (39.2% of total dermatophytoses) followed by tinea corporis (23%), tinea pedis (20.5%), and tinea cruris (8%). *T. rubrum* was the most prevalent causative agent, implicated in 64% of total cases.

In Sweden (15), onychomycosis had the highest overall prevalence of 14.1%, followed by tinea pedis (4.4%). *Trichophyton rubrum* was the predominant pathogen isolated from these cases (83.2%), followed by *T. mentagrophytes* (7.4%).

According to the retrospective analysis from 2006 to 2009 by Simonnet et al. (16), the most prevalent clinical form was onychomycosis (28.2% of total dermatophytoses) and *T. rubrum* was the most common dermatophyte recovered from cases of onychomycosis (67.4%), tinea pedis (70.6%) and tinea corporis (52.4%).

Our data showed that tinea capitis and tinea faciei were dominant clinical types in the 0−20 years age group (65.91%, 29/44 cases). These infections were not observed in adults aged over 61 years. Aetiological agents identified with infections were *T. mentagrophytes* (31.82%, 14/44 cases), *M. canis* (27.27%, 12/44 cases), *M. audouinii* (20.45%. 9/44 cases), *T. tonsurans* (11.36%, 5/44 cases) and *M. gypseum* 9.09% (4/44 cases). Increased anthropophilic scalp infections (caused by *T. tonsurans. T. violaceum, M. audouinii*) are reported in western and northern Europe, while zoophilic *Microsporum canis* remains the predominant organism with the highest incidence in Central and Southern Europe (17−21).

CONCLUSION

We conclude that the overall epidemiological and clinico-mycological profile of fungal skin infections in eastern Slovakia is in agreement with most published studies.

The assessment of data has showed the predominance of tinea unguium among dermatophytosis and the prevalent aetiological role of *T. rubrum*.

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Conflict of Interests

None declared

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