Tropical medicine rounds

A twenty-year survey of dermatophytoses in Braga, Portugal

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Abstract

Background Modifications in social habits together with the increase of emigration have contributed not only to increased dermatophytoses but also to an altered etiology. During the last few years, Braga has suffered a radical change from a rural to a cosmopolitan life-style.

Methods A statistical study of dermatophytoses and the etiology of their causative agents was performed by a retrospective survey carried out among patients of Hospital de São Marcos, Braga, Portugal, from 1983–2002. In this study, a total of 10 003 patients were analyzed.

Results Over this period the frequency of dermatophytoses, as defined by the recovery of a dermatophyte in culture, was found to be 23.6%, whereas nondermatophytic infections accounted for 7.0%. Analysis of the clinical forms and the isolated fungi supports that the dermatophyte species have a predilection for certain body areas ($P \leq 0.01$). Age is a very important factor regarding the occurrence of dermatophytoses ($P \leq 0.0001$), with a correlation between increasing age and infection, positive for *Trichophyton rubrum* and negative for *Microsporum canis*. Overall the gender of the patients is not an association factor for the development of dermatophytoses; however, significant differences were detected in the distribution of some etiologic agents ($P \leq 0.05$).

Conclusions The results showed the main etiologic agent of dermatophytoses to be *Trichophyton rubrum* (37.4%). Moreover, dermatophytoses are both decreasing and showing a new profile in Braga, and a pronounced decrease of *Trichophyton megninii* was observed throughout the study.

Introduction

Cutaneous fungal infections have been reported worldwide as one of the most common human infectious diseases in clinical practice.[1] In spite of the therapeutic advances in the last decades, the prevalence of cutaneous mycoses is still increasing and 10–15% of the human population is at risk of developing these infections.[1,2] The main etiologic agents causing cutaneous infections are dermatophytes,[3] which are classified into three genera according to the structure of their conidia, i.e. *Microsporum*, *Trichophyton* and *Epidermophyton*.[4] Even though more than 40 species have been identified, only a few can be identified as responsible for the majority of dermatophytoses.[5] These organisms are characterized by the ability to metabolize keratinized tissues such as the corneous extract of epidermis, nails and hairs.[6] In general, the clinical forms of the disease are designated as tinea corporis, tinea cruris, tinea mannus, tinea pedis, tinea unguium, tinea capitis and tinea barbae.[7] Distribution of the clinical forms has been associated, by several authors, with the gender, age and social status of the patients.[7]

Precise knowledge of the ecology and epidemiology of dermatophytes and the major clinical aspects of the disease are essential for the identification of such infections, and a better understanding of their transmission patterns.[4,8,9] Although a few strains are endemic within specific geographic areas, the increased phenomenon of emigration has altered the world-wide patterns of dermatophyte distribution.[10] The etiology of dermatophytes causative of tinea capitis exemplifies the changes in geographic distribution both in Europe and in the USA.[4,11]

The present work aimed to study the epidemiological profiles by a retrospective analysis of the frequency and etiology of dermatophytoses occurring in the Braga region during the period 1983–2002. This study may also contribute to develop further prospective studies to define new strategies for the management and prevention of cutaneous mycoses.

Materials and Methods

Study population

This retrospective analysis, referring from January 1983 to December 2002, was carried out on 10,003 patients with

suspected skin mycoses observed in the dermatology services of Hospital de São Marcos, Braga (north of Portugal). The population of the Braga district (831,400 habitants) was characterized in respect to our patients' age (ranking from 1–95 years old, with a normal distribution among the age-stratified groups described below), gender (4653 males and 5350 females) and eight clinical variants (tinea corporis, tinea barbae, tinea capitis, tinea pedis, tinea cruris, tinea unguium, tinea manuum and tinea versicolor). For statistical analysis the population was stratified accordingly to age into four groups: up to 14 years (< 14) years, 15 ≥ 25 years, between 25 ≥ 65 years, and patients over 65 years (> 65) years.

Samples

Based on the clinical evaluation, specimens were collected from scales and scrapings taken from the rim of lesions, using a sterile scalpel blade. Sample analysis was carried out by both microscopic examination and fungal growth culture.

Mycological examination

Direct microscopic examination of the samples was performed following treatment with potassium hydroxide (KOH; 20%), during 30 min.[12] In addition to microscopy analysis, all samples, except when tinea versicolor was suspected, were cultured on Mycobiotic agar medium (Difco, Detroit, MI) supplemented with both chloramphenicol (40 μL mL^{-1}) and cycloheximide (0.5 mg mL^{-1}).[7] Cultures were incubated at 24 °C and considered negative at the end of the fourth week. For classification purposes, the growing colonies of positive cultures were studied. Classical methods to differentiate dermatophytes, based on the assessment of their macroscopic and microscopic morphology, were used together with tests for nutrient requirements and colony pigmentation.[13] For patients where bovine contact was known, fungal growth was also screened at 37 °C.

Statistical analysis

Statistical analysis was performed using the Chi-square test to study the association of the selected variables with the occurrence of dermatophytoses and to detect significant differences in the distribution of each species between categories of variables. Values of $P = 0.05$ were considered to be statistically non significant (NS). All tests were performed using the software SPSS version 11.0 for Windows© (LEAD Technologies Inc., Chicago, IL, USA).

Results

Samples were obtained from patients with suspected cutaneous mycosis, during the period January 1983 to December 2002. Among a total of 10,003 clinically suspected samples examined, 41.33% were mycologically positive by direct microscopy and/or culture analysis. In addition, the 3059 positive cultures revealed that 2357 patients were infected with dermatophytes and 702 with nondermatophytic infections, tinea versicolor (19.5%), candidosis (1.0%), and bacterial/saprophytic moulds colonization (2.4%). In the studied period, from the 2357 clinical isolates, 13 different dermatophyte species were obtained (Table 1). The *M. audouinii* (0.4%), *T. schoenleinii*, *M. nanum*, and *M. ferrugineum* were grouped and named 'Others' for further analysis owing to their low frequency and to avoid the occurrence of statistical deviation (Table 1).

The univariate statistical analysis, considering all data collected through the 20 years, showed an association of

Table 1 Distribution of dermatophyte species isolated by the clinical forms

Dermatophyte species	Total	Clinical forms							*P*-values
		Tinea barbae	*Tinea capitis*	*Tinea corporis*	*Tinea unguium*	*Tinea manuum*	*Tinea pedis*	*Tinea cruris*	
Antropophilic									
E. floccosum	71	1	0	19	1	0	14	36	***
T. megninii	201	28	7	144	5	12	3	2	***
T. mentagrophytes interdigitale	196	0	0	8	25	4	156	3	***
T. rubrum	882	22	9	322	105	56	236	132	***
T. tonsurans	56	3	24	27	0	0	2	0	***
T. violaceum	100	2	52	39	0	3	4	0	***
Zoophilic									
T. mentagrophytes granulare	179	9	27	99	5	16	20	3	***
M. canis	590	22	347	196	4	7	10	4	***
T. verrucosum	27	0	4	23	0	0	0	0	**
Geophilic									
M. gypseum	39	1	9	23	0	4	0	2	***
Others	16	0	13	1	0	1	1	0	***
Total	2357	88	492	901	145	103	446	182	***

$^{**}P \leq 0.01$, $^{***}P \leq 0.0001$.

Table 2 Distribution of dermatophyte species isolated by age-stratified groups

Dermatophyte species	Age-stratified groups				*P*-values
	< 14	15–25	26–65	≥ 65	
Antropophilic					
E. floccosum	12	23	32	4	***
T. megninii	26	34	106	35	***
T. mentagrophytes interd.	14	32	147	3	***
T. rubrum	56	181	599	46	***
T. tonsurans	26	6	20	4	***
T. violaceum	51	7	26	16	***
Zoophilic					
T. mentagrophytes granulare	62	27	79	11	***
M. canis	478	41	65	6	***
T. verrucosum	14	5	7	1	**
Geophilic					
M. gypseum	26	4	9	0	***
Others	11	0	5	0	NS
Total	776	360	1095	126	***

$^{**}P \leq 0.01$, $^{***}P \leq 0.0001$; NS, not significant.

Table 3 Distribution of dermatophyte species isolated by gender

Dermatophyte species	Gender		*P*-values
	Male	Female	
Antropophilic			
E. floccosum	52	19	***
T. megninii	117	84	*
T. mentagrophytes interd.	102	94	NS
T. rubrum	514	368	***
T. tonsurans	17	39	**
T. violaceum	30	70	***
Zoophilic			
T. mentagrophytes granulare	76	103	*
M. canis	257	333	**
T. verrucosum	16	11	NS
Geophilic			
M. gypseum	14	25	NS

$^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.0001$; NS, not significant.

clinical forms, age-stratified groups, and gender with the occurrence of dermatophytoses ($P < 0.0001$; data not shown). In order to investigate the basis of this association, the data were further analyzed in respect to the variables in the study and the dermatophyte species isolated (Tables 1–3).

Predominance of dermatophytoses in a year-based analysis

Distribution of the most relevant dermatophyte species isolated by periods of 5 years clearly illustrates variations in the predominance of the different dermatophyte species, with

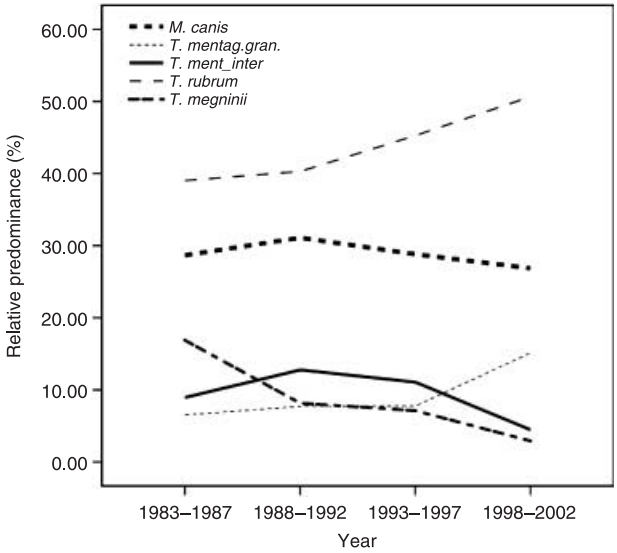

Figure 1 Relative predominance of the five most common dermatophyte species isolated by periods of 5 years, normalized by the number of patients attending the service of dermatology with suspected cutaneous fungal infection in the same period

T. rubrum and *M. canis* being the most frequently isolated species (Fig. 1). However, after 1988–92 the percentage of isolates of *T. rubrum* increased, whereas *M. canis* decreased. In addition, *T. mentagrophytes* var. *granulare* increased in predominance relative to *T. mentagrophytes* var. *interdigitale*. A constant decrease in the isolation of *T. megninii* was observed throughout the studied period, accounting for < 2% in 1998–2002.

Distribution of etiological agents of dermatophytoses per clinical forms

The relationship between clinical forms and the etiologic agents, accordingly to their natural habitat, is summarized in Table 1. The results reveal significant differences in the frequency of dermatophytoses among the clinical forms, with tinea corporis being the most common followed by tinea capitis and tinea pedis (column "Total", Table 1). From an association of the clinical forms and the etiologic agents, significant differences were obtained (Table 1).

Distribution of etiological agents of dermatophytoses per age-stratified groups

Distribution of dermatophytoses in the four age-stratified groups shows statistical differences and reveals a high predominance of infections in middle-aged people and in children (line "Total", Table 2). Specifically, the etiological agents distributed along the age-stratified groups with an associative pattern, unless for those species included in the group termed "Others" (Table 2). One should stress that the results not only show a

high predominance of *M. canis* in the prepubertal stage, but also that this species is mainly associated with this group. Also, *T. rubrum* was the principal etiological agent accounting for all dermatophytoses in the groups 26 > 65 and 15 > 25 with 54.7% and 50.2%, respectively. A low frequency of dermatophytoses was observed in individuals > 65 years (Table 2).

Distribution of etiological agents of dermatophytoses per gender

No significant differences in the distribution of dermatophytoses between males and females were observed (line 'Total', Table 3). However, significant differences were detected when the etiology of dermatophytoses and the gender of the patients were compared, with the exception of *T. mentagrophytes* var. *interdigitale*, *T. verrucosum*, and *M. gypseum* (Table 3).

Discussion

Dermatophytoses are not usually life-threatening nor pose serious physical injuries; however, they assume significant relevance in clinical practice.[13] The results of the present study revealed that dermatophytes are the main causal agents of skin cutaneous infections in the Braga district for 20 years. These data reiterate the predominance of dermatophytes among cutaneous fungal infection described by others worldwide.[14,15]

Trichophyton rubrum

Our results showed that *T. rubrum* was by far the main causal agent of cutaneous fungal infection over the 20-year study period (Fig. 1), accordingly to descriptions in European countries and the USA.[14,16–18] A historical analysis of *T. rubrum* frequency in Portugal reveals that its predominance has been increasing since 1962.[19] In 1959, this species accounted for only 5% of the clinical isolates, whereas in 1971 it was already the third most common species[20], and since 1983 it has been the leading causative agent of dermatophytoses. In light of the behavior of this species worldwide, in Portugal the increased frequency is mainly associated with increased urbanization. Specifically, the observed increasing frequency of *T. rubrum*, after the period 1988–92 (Fig. 1), may also expose changes in social habits resulting from both demographic expansion of Braga city and transformation from an extensively rural region into an industrialized region, with a more cosmopolitan way of life.

Distribution of *T. rubrum* by age-stratified groups, gender, and the clinical forms revealed statistical differences (Tables 1–3). As described elsewhere,[15,21,22] this species was mainly isolated from cases of tinea corporis followed by tinea pedis, tinea cruris and tinea ungium (Table 1). Chronic infections caused by *T. rubrum* in the glabrous skin, crural region, feet and nails are possibly owing to occlusive footwear and tight underwear.[7] Risk factors are on the basis of the higher frequency of *T. rubrum* among middle-aged persons, and in most clinical forms has a bias towards male patients, excluding the cases of tinea corporis and tinea unguium, where *T. rubrum* is equally distributed with patient gender (Tables 2 and 3).[26] Although *T. rubrum* plays a significant role in tinea pedis in Portugal, it is noticeably less than that observed in Canada and USA.[14,23]

Microsporum canis

Microsporum canis was the second most common etiologic agent isolated from the dermatophytoses cases analyzed in this study (Fig. 1, Table 1). This zoophilic dermatophyte is usually acquired from infected domestic pets, such as dogs and cats, typically associated to an urban life-style, and was found to be the most frequent isolated species in several countries.[16,18,24–28] As shown in Fig. 1, the predominance of *M. canis* showed a slight decrease after the period 1988–92 in favor of *T. rubrum*.

The pattern of *M. canis* distribution by age-stratified group, gender and the seven clinical forms was shown to be highly associative (Tables 1–3). This species was the predominant isolate among tinea capitis and the second in tinea corporis (Table 1). Notably, the results showed that from all *M. canis* isolates, 81.0% were obtained from patients in the prepubertal stage, and within this group *M. canis* was responsible for 61.6% of the dermatophytoses. This result can be associated with the fact that tinea capitis and tinea corporis can spread rapidly among children in day-care settings, together with the high virulence and contagiousness of *M. canis* owing to the tendency of pets at home.[28–30] In addition, the development of early immune responses to this dermatophyte species may reduce the possible later infectious episodes.[30]

Overall, *M. canis* is predominantly distributed in female patients (Table 3), as described by others elsewhere.[31] However, in childhood this clinical form is equally distributed over male and female patients. Taking into consideration the distribution of this species over the clinical forms and subdivided by the age-stratified groups and genders, it is clear that its association with tinea corporis is highly dependent of gender with a male : female ratio of 1 : 2.6 in childhood.

Trichophyton megninii

Further results of this study found that *T. megninii* was the third etiologic agent of dermatophytoses (Table 1). As described elsewhere,[20,32] the distribution of *T. megninii* by the clinical forms, age-stratified groups and gender, revealed a statistically different pattern (Tables 1–3). Most of the isolates of this species were associated with tinea corporis, tinea barbae and tinea manuum. In addition, 70.1% of the isolates were from patients > 25 years old (Table 2), and in all age-stratified groups a higher predominance was found among males, with a higher male : female ratio of 2.3 : 1 in prepuberty.

Trichophyton megninii, an anthropophilic dermatophyte with a generally low incidence but with a broad geographic

distribution, assumed particular importance in Portugal. A longitudinal study carried out in Portugal has revealed that this species increased from 4% (1959–63) to 16.0% (1983–85), probably owing to the increased movement of people from provinces in Africa to Portugal.[33] In particular, the changes of *T. megninii* frequency of isolation over these 20 years may indicate that this species tends to disappear in Portugal, at least in this region, even though it is also found in the neighboring Spanish province of Galicia with northern Portugal, of which Braga is a district.[32]

Trichophyton mentagrophytes

Trichophyton mentagrophytes was the fourth most commonly isolated species in this study, taking into account the two variants *T. mentagrophytes* var. *interdigitale* and *T. mentagrophytes* var. *granulare* (Table 1). Distribution of this species among the clinical forms and age-stratified groups revealed statistical significances (Tables 1 and 2), whereas only *T. mentagrophytes* var. *granulare* was found to be associated with female patients (Table 3). As described by others,[34] the antropophilic *T. mentagrophytes* var. *interdigitale* was mainly isolated with tinea pedis (Table 1) and corresponded to 35% of all cases of this clinical form. Moreover, it seems that this etiologic agent is predominantly associated with dermatophytoses amongst patients aged $25 \geq 65$ years old; equally distributed over male and female patients (Tables 2 and 3). It is likely that exposure to *T. mentagrophytes* var. *interdigitale* is a common occurrence owing to chronicity and permanence of spores in the host and in the environment. On the other hand, the zoophilic form *T. mentagrophytes* var. *granulare* was shown to be associated with tinea corporis in childhood female patients and in the $25 \geq 65$-year group. Moreover, a year-based analysis over the last 20 years showed an overall decrease of dermatophytoses caused by *T. mentagrophytes*, mainly owing to the high reduction of *T. mentagrophytes* var. *interdigitale* related cases.

Species with low predominance

If one considers all the results obtained for the species with low predominance, the decrease in the isolates of all the species from the beginning of the study (1983–87) until the last period (1998–2002) is evident (data not shown). This result could be interpreted as indicative of species that have, nowadays, a low clinical relevance and could tend to disappear from our community. *Trichophyton violaceum* distribution among the clinical forms, gender and age-stratified groups showed significant differences (Tables 1–3). Most of the isolates of this species were obtained from cases of tinea capitis and corporis, with half of the cases being from patients in childhood (Tables 1 and 2). The ratio male : female cases revealed a higher predominance among female patients (Table 3), moreover differences were observed with this female bias when age-stratified groups were taken into account. The *E. floccosum* was found to be associated with male dermatophytoses (Table 3), being the second etiologic agent causative of tinea cruris (Table 1). In addition, this species was almost non-existent among patients over 65 years old (Table 2). *Trichophyton tonsurans* was mainly found in cases of tinea corporis and tinea capitis (Table 1) in female patients (Table 3) either in prepuberty or in the group $25 \geq 65$ years (Table 2). In contrast to the epidemiologic surveys in the USA,[14,28] *T. tonsurans* is not a predominant species in Portugal, and the present longitudinal study evidenced an accentuated decrease in its predominance (data not shown). *Microsporum gypseum* and *T. verrucosum*, were mainly found in cases of tinea corporis (Table 1) of prepuberty patients (Table 2) without gender bias (Table 3). Overall, the decrease of occurrence of these agents could once more be associated with Braga's urbanization. The dermatophytoses associated with the etiologic agents included in the group called "Others", such as *M. ferrugineum*, *M. nanum*, *M. audouinii* and *T. schoenleinii*, were shown to be linked to tinea capitis cases (Table 1) in female patients (Table 3), being distributed equally in all age-stratified groups (Table 2).

Conclusion

In conclusion, gender, clinical forms and age appear to be highly associated with the occurrence of dermatophytoses. Moreover, this study shows that *T. rubrum* remains the prevalent species responsible for dermatophytoses, especially in tinea corporis in adults. While anthropophilic dermatophytes infected mainly adults, geophilic and zoophilic species preferentially affected prepubertal individuals. In addition, the high predominance of tinea corporis may be partly explained by the dispersal of fungi from other lesions into the trunk as a result of the sharing facilities. Changes in dermatophyte predominance patterns during the 20 years studied were noted, and could be associated with the radical changes in Braga's demography, from a rural to a cosmopolitan lifestyle. An overall analysis showed no significant change in the relative predominance of each of clinical form of dermatophytoses over the 20-year study (data not shown), revealing the changes of epidemiological profile in the etiology of the agents causing disease, as described above.

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