Original paper

Distribution of *Malassezia* species in patients with atopic dermatitis – quality assessment

Elżbieta Rup[1], Magdalena Skóra[2], Paweł Krzyściak[2], Anna B. Macura[2]

[1]Department of Dermatology, Jagiellonian University, *Collegium Medicum*, Krakow, Poland
Head: Prof. Anna Wojas-Pelc MD, PhD
[2]Department of Mycology, Chair of Microbiology, Jagiellonian University, *Collegium Medicum*, Krakow, Poland
Head: Prof. Anna B. Macura MD, PhD

Post Dermatol Alergol 2011; XXVIII, 3: 187–190

Abstract

Introduction: The fungi *Malassezia* are a constituent of the skin microbiota in humans and some animals. The fungi may cause skin diseases or even organ and/or generalized infections in the presence of appropriate predisposing factors.

Aim: To evaluate the occurrence of *Malassezia* fungi on the skin in patients with psoriasis and search for a relationship between the occurrence of *Malassezia* and the severity of skin lesions, age and sex of the patients.

Material and methods: The materials comprised smears sampled from four sites: scalp, face, chest and back. *Malassezia* spp. were isolated in cultures on modified Dixon medium and identified on the basis of morphological and biochemical features.

Results: *Malassezia* spp. were isolated in 55.1% of the patients. The fungi were most prevalent on the back (33.3%) and least on the scalp (17.5%) and face (19%). The prevalence on the chest was 30.2%. In the group of patients with AD localized to the head and neck *Malassezia* spp. were cultured in 71.4% of patients.

Conclusions: No statistically significant differences were found between *Malassezia* prevalence in males vs. females. No relationship with the patients' age was found. Higher values of the atopic dermatitis severity index (SCORAD) were found in the patients in whom *Malassezia* spp. were isolated. *Malassezia* spp. were particularly common in the group of patients with AD localized to the head and neck.

Key words: *Malassezia* spp., atopic dermatitis, skin diseases.

Introduction

The fungi *Malassezia* are opportunists of significant clinical importance in humans and in animals. *Malassezia* spp. may be isolated from healthy human and animal skin. These fungi are involved in certain dermatological diseases and, in the case of considerable immunological insufficiency, they can cause systemic infections [1-6]. Currently, by techniques of molecular biology, 13 *Malassezia* species were identified, with 12 lipid-dependent species (*M. furfur*, *M. globosa*, *M. obtusa*, *M. sympodialis*, *M. slooffiae*, *M. nana*, *M. dermatis*, *M. restricta*, *M. equina*, *M. japonica*, *M. yamatoensis* and *M. caprae*) and one lipid-independent species (*M. pachydermatis*) among them [1, 6-9]. Frequency of the superficial fungal infections caused by species of the *Malassezia* genus depends on many different factors, such as socio-economic, climatic or geographical factors [10].

Atopic dermatitis (AD) is a chronic recurrent pruritic dermatosis, affecting mainly children. Atopic dermatitis may occur as an isolated disease or as a manifestation of a systemic allergic disorder, together with hay fever, bronchial asthma or food allergies. Atopic dermatitis is also observed in 1-3% of adults. The disease may occur for the first time in adulthood, persist from childhood or recur after a long period of remission [11-13]. *Malassezia* spp. seem to be an important factor in the pathogenesis of AD in adults, particularly when the skin lesions are localized to the head and neck. Since the 1980s it has been observed that the skin lesions in patients with AD disappear after the administration of local and systemic antifungal agents [14]. The prevalence of *Malassezia* spp. on the skin of adult

Address for correspondence: Prof. Anna B. Macura MD, PhD, Department of Mycology, Chair of Microbiology, Jagiellonian University, *Collegium Medicum*, 18 Czysta, 31-121 Krakow, Poland, tel. +48 12 633 08 77, ext. 231, e-mail: mbmacura@cyf-kr.edu.pl

Tab. 1. Characteristics of patients included in the study

Sex	Number	Age, $x \pm SD$	Number of *Malassezia* spp. isolates from particular skin regions				SCORAD, $x \pm SD$ (Me)
			Scalp	Face	Chest	Back	
F	29	34.3 ±13.8	7	8	11	11	42.1 ±16.7 (42.5)
M	20	31.9 ±12.2	4	4	7	10	38.8 ±16.5 (36.5)
Σ	49	33.3 ±13.1	11	12	18	21	40.8 ±16.5 (38.7)

F – female, M – male, x ± SD – mean ± standard deviation, Me – median

patients with AD compared to the healthy population is similar and is between 50% and 80%, depending on the testing method. However, it was noticed that in patients with AD, positive results of skin tests with *Malassezia* spp. allergens and high plasma levels of specific IgE antibodies against *Malassezia* spp. are found significantly more often than in the control groups [15-17]. Therefore, currently, *Malassezia* spp. are considered to be an allergenic rather than an infectious agent, in the case of patients with AD. Exacerbation of the skin lesions in patients with AD may occur also as a result of activation of inflammatory reactions by the release of hydrolytic enzymes by *Malassezia* spp. [18, 19].

Aim

The objective of the study was to evaluate the occurrence of *Malassezia* fungi on the skin in patients with psoriasis and search for a relationship between the occurrence of *Malassezia* and the severity of skin lesions, age and sex of the patients.

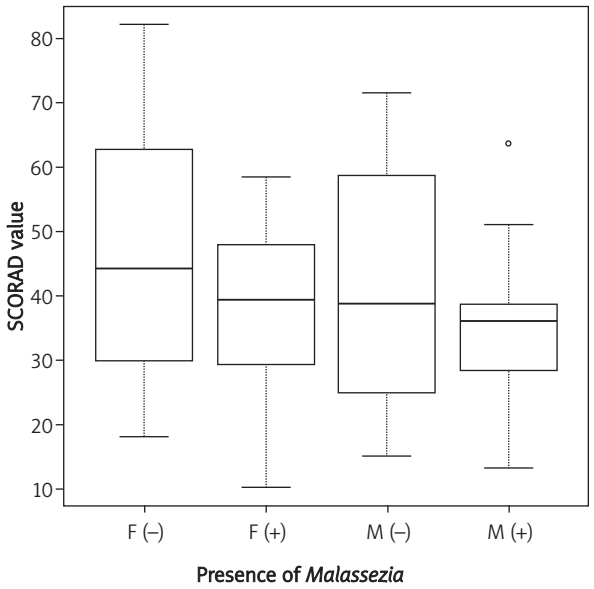

Fig. 1. Comparison of the SCORAD index values in patients with and without *Malassezia* skin colonization. M(–)/F(–) – male/female without *Malassezia* skin colonization, M(+)/F(+) – male/female with *Malassezia* skin colonization. The box represents 1-3 quartiles, the median is marked by a horizontal line, the range value by whiskers, the outliers by a circle

Material and methods

Experimental group

The experimental group consisted of adult patients of both sexes, who were diagnosed with atopic dermatitis according to the criteria of Hanifin and Rajka. Clinical condition of the patients was assessed using SCORAD (Severity Scoring of Atopic Dermatitis). Isolation and identification of *Malassezia* spp.

Material for the mycological examination was obtained from each patient using sterile swabs moistened with saline. Smears were sampled from four sites of the body: 1) scalp, 2) face (nasolabial grooves and forehead), 3) chest (sternal region), 4) back (interscapular region). The material was inoculated on modified Dixon medium, which was composed of: malt extract 36 g, peptone 6 g, ox bile 20 g, Tween 40 10 ml, glycerol 2 ml, oleic acid 2 ml, chloramphenicol 250 mg, agar 12 g, distilled water 1000 ml. Cultures were incubated at 30°C for 10 days. Fungi were identified to the level of species on the basis of the colony morphology, microscopic appearance of the fungal cells obtained in the culture, by examining the ability of the fungi to grow without addition of lipids and by performing the urease test on Christensen's solid medium (with Tween 40 and Tween 80).

Results

The obtained data were analysed using the programming language and software environment for statistical computing R (version 2.9.1, 2009).

The study included 49 patients (29 women and 20 men) with atopic dermatitis, aged between 18 and 69. The results are presented in the Table 1 and in the Figure 1.

Malassezia spp. were isolated from 27 patients (55.1%), including 16 women (55.2%) and 11 men (55%). In total, 63 isolates of *Malassezia* spp. were obtained from different body locations (37 from women and 26 from men). No statistically significant difference was observed in the frequency of isolation of *Malassezia* spp. between women and men (χ^2 test, $p = 0.9905$).

Predominantly, *Malassezia* spp. were isolated from the interscapular region. Overall, 21 isolates were obtained from this site (33.3% of all isolates of *Malassezia* spp.): 11 isolates from women and 10 isolates from men. Least frequently, *Malassezia* spp. were isolated from the scalp (11 isolates – 17.5%) and only slightly more often from the seborrhoeic regions of the face (12 isolates – 19.0%). This tendency was seen in both women and men. However, in patients with particularly severe skin lesions on the skin of the head and neck, in the course of AD, *Malassezia* spp. were isolated from these sites in 71.4% of cases. From the skin of the chest, *Malassezia* spp. were isolated in 30.2% of the patients (11 isolates from women and 8 isolates from men). In 74.1% of the included patients in whom *Malassezia* spp. were found (68.7% women and 81.8% men), these fungi could be isolated from at least 2 different sites of the body. In 18.5% of the patients (25% women and 9% men), *Malassezia* spp. were found in all examined locations, at the same time.

In the study, no difference in the frequency of occurrence of *Malassezia* spp. depending on the age of the patients could be seen (Student's *t*-test, $p = 0.3656$).

Severity of skin lesions and subjective symptoms (pruritus, sleep disorder) in the course of AD expressed by the SCORAD index was between 10.2 and 80 (mean 40.8). Higher values of the SCORAD index were seen in the group in which *Malassezia* spp. could not be found (Mann-Whitney test, $p = 0.004347$). In patients with severe AD (SCORAD index above 40) *Malassezia* spp. were isolated in 43.5% of the patients, whereas in the group with the SCORAD index below 40, the fungi were isolated from 65.4% of the patients.

Discussion

The role of *Malassezia* spp. in AD has been studied since the beginning of the 1980s, when it was observed that the skin lesions in this dermatosis disappear after the administration of antifungal agents [14]. It was found that fungi of the *Malassezia* genus, on the one hand, can be a component of the normal flora in healthy people, and on the other hand can act as an allergen and be a direct infectious factor causing superficial and systemic fungal infections [20, 21]. Clemmensen *et al.*, examining patients with skin lesions on the face and neck and with concomitant pruritus, observed positive reactions in the skin prick tests with *M. furfur* antigen [14]. Many clinical trials confirm the presence of specific IgE antibodies against *Malassezia* spp. antigens in the plasma in patients with AD [15-17]. The frequency of occurrence of *Malassezia* spp. on the skin in patients with AD and in the healthy population is similar and varies between 50% and 80%. These differences are due to the testing method. In our study *Malassezia* spp. were isolated from 55.1% of the patients with atopic dermatitis, most frequently from the interscapular region (33.3%). Similar results were obtained by Nakabayashi *et al.* and Yim *et al.* [22, 23]. In the first of the cited studies, positive culture results were obtained in 54% of the samples from patients with AD and in 50% of the samples from healthy people [22]. In the latter one, Yim *et al.* obtained positive culture results in the case of 51.7% of the patients with AD (the 26S rDNA PCR-RFLP method was used) [23]. The species most frequently isolated was *M. sympodialis* (16.3%). There were significant differences in the occurrence of particular species, depending on the site of sampling. In samples obtained from the scalp and the face, *M. restricta* was isolated most often (30% and 16.7% respectively), whereas in samples from the skin of the chest, *M. sympodialis* was found most frequently (28.3%). In the study conducted by Nakabayashi, predominantly isolated species were *M. globosa* (22%) and *M. furfur* (17%). *Malassezia furfur* was isolated more often from the sites where skin lesions were observed (21%) than from the unchanged skin (11%) [22]. In our study, in the group with particularly severe skin lesions on the head and neck, positive culture results for *Malassezia* spp. were obtained in samples from the mentioned sites in the case of 71.4% of the patients.

In the study, positive culture results for *Malassezia* spp. were more frequent in the case of patients with less severe AD (SCORAD index below 40) compared to the patients with severe AD. A possible reason for that could be a significantly decreased level of skin lipids in the latter group of patients. In the quantitative studies, it was found that in patients with severe AD, the number of colonies of *Malassezia* spp. was lower in the case of isolates from skin lesions compared to isolates from the unchanged skin [24]. Another explanation for this phenomenon could be intensified migration of the inflammatory cells to the skin and high concentrations of released mediators having antifungal properties in patients with severe AD [23].

The results of the study suggest that *Malassezia* is an allergenic rather than an infectious agent in patients with AD. It is worth stressing the role of particular species of the *Malassezia* genus in patients with skin lesions in different locations. Studies on the pathogenicity of *Malassezia* spp. have been conducted in the Department of Mycology since the 1980s [25-30]. In order to specify the exact role of *Malassezia* spp. in patients with AD, application of molecular biology methods is necessary. The characteristics of particular strains will be a subject of further studies in our department.

The study was done at the Department of Mycology, Chair of Microbiology, Jagiellonian University, *Collegium Medicum*, Krakow.

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