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Faruk Konjhodžić
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MALASSEZIA YEASTS AS COMMENSALS ON HUMAN SKIN

Asja Prohić,
M.D., Ph.D.Department of Dermatology Sarajevo University Clinical Center

Summary

Yeasts of the genus Malassezia are known to be members of the skin microflora of humans and other warm-blooded animas. Under the influence of predisposing factors they become pathogenic and are associated with several skin disorders and even systemic infections. The taxonomy of these lipophilic yeasts has recently been modified and includes seven species referred as *Malassezia*. The aim of this study was to analyze the prevalence of *Malassezia* species in normal skin and assess the distribution of the species according to patient sex and age. Forty subjects with clinically healthy skin were studied. The samples were obtained by scraping the skin surface of the scalp of all subjects and then incubated on modified Dixon agar. The yeasts isolated were identified by their morphological and physiological properties according to Guillot *et al* method. *M.sympodialis* was the predominant species on trunk skin and *M.restricta* on scalp skin. We found no differences between isolated species and the sex and age of the individuals.

Key words: *Malassezia*, species, healthy skin

Introduction

Genus *Malassezia* was created by Baillon in 1889 and has never been properly classified (1).

By means of molecular methods the genus was taxonomically revised and enlarged to seven species – in addition to *M.furfur, M.pachydermatis, and M.sympodialis,* four new taxa have been described, namely *M.slooffiae, M.globosa, M.obtusa,* and *M.restricta* (2-4). *M.pachydermatis* is the only non lipid-dependent species. It is considered zoophilic, because it is mainly isolated from animals (5), whereas the remaining six species are obligatory lipophilic and found primarily in humans (6).

The presence of *Malassezia* species in healthy human skin had been detected as early as the second half of 19th century (7). The frequency and density of colonization in healthy individuals is related to the age and to the activity of the sebaceous glands in the area studied (8). It has been demonstrated that *Malassezia* yeasts inhabit various body sites including scalp, forehead, nose, shoulders, abdomen, lower axillae, groin and forearm. Roberts reported that 97% of clinically healthy people carry fungus on the scalp and 92% on their trunk (9). However, under the influence of predisposing factors they become

pathogenic and are associated with several diseases such as pityriasis versicolor, folliculitis, seborrheic dermatitis, confluent and reticulate papillomatosis, and even systemic infections (10).

The aim of this study was to analyze the prevalence of *Malassezia* species from clinically normal skin of the scalp and trunk and to examine if the range of species varies with patients sex and age.

Patients And Methods

Patients

This prospective study was conducted at the Department of Dermatovenerology, University Clinical Centre, Sarajevo, Bosnia and Herzegovina, during the period from April till December 2001. Forty individuals with clinically healthy skin (20 women and 20 men; age range, 13-76 years) were included in the study.

Samples

All samples consisted of scales and scrapings from the upper and middle part of trunk and from scalps in all participants. Collected samples were divided into two portions – one for microscopic examination and the other for culture.

Microscopic examination of the samples was performed after the treatment with lactophenol solution.

The samples for culture were inoculated on modified Dixon agar consisting of 3.6% malt extract, 0.6% mycological peptone, 2.0% desiccated ox bile (Sigma Chemical Co. Ltd, UK), 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 0.05% chloramphenicol, 0.05% cycloheximide, and 1.2% agar pH 6.0. The medium was always used within one week of preparation and the cultures were incubated at 32°C for seven days.

Identification of Malassezia yeasts

Malassezia species were identified according to the scheme established by Guillot *et al* (11), and their macroscopic and microscopic features and physiological properties were recorded. The macroscopic features of the predominant colonies included their shape, size, color consistency, and the characteristics of the medium around them. Microscopic features of the yeast cells in cultures were described after lactophenol staining and included the predominant morphology, size, and budding base of the yeasts. To assess the physiological properties of the yeasts, catalase reaction was used. A drop of

hydrogen peroxide (30% solution) was added to a culture smear on a glass slide. The production of gas bubbles, indicative of release of oxygen, was considered a positive reaction.

Utilization of Tween compounds was done according to the test originally described by Guillot *et al* (11) and later modified by Gupta *et al* (12, 13).

Statistics

Chi-squared test with Yates' correction for a small sample size was carried out to determine the statistical significance of differences in proportions. We used a statistical software package Minitab 13.0. Significance level was set at $p<0.05$.

Results

Direct Microscopy

Direct microscopic examination of scales from trunk showed the presence of yeast cells in 25 patients (62,5%), but short filaments were observed in only one (2,5%). Spherical and large yeast cells with a narrow budding base were found in 9 patients (22,5%). Smaller yeast cells, oval or cylindrical in shape, were seen in remaining 20 cases (50%). In 11 slides (27,5%) neither yeast cells nor hyphae were observed (Table 1).

In the scales from healthy scalp skin, yeast cells were observed in 21 patients (53%), whereas the remaining 19 cases (47%) were negative. Short filaments were seen in only one patient. Oval and cylindrical yeast cells dominated; they were found in 14 cases (35%). Spherical yeasts were recorded in 7 slides (18%).

No significant statistical differences were found in the direct microscopic findings from healthy trunk and scalp skin (Table 1).

	HEALTHY TRUNK SKIN			HEALTHY SCALP SKIN		
	F	M	Σ	F	M	Σ
Filaments	1	0	1	1	0	1
Yeast cells	11	13	24	11	9	20
Negative	8	7	15	8	11	19
Total	20	20	40	20	20	40
F= female; M=male; Σ= total;						

Table 1. Direct microscopic examination of scales from trunk and scalp of normal subjects

Cultures

Malassezia yeasts were found in 29 (72,5 %) samples taken from healthy trunk skin. The most frequently isolated species was *M.sympodialis* found in 12 (30 %) patients, followed by *M.globosa* (22,5 %), *M.furfur* (17,5%) and *M.restricta* (2,5 %). The percentage of negative cultures was 27,5 %.

The results of culture obtained from healthy scalp skin were positive for *Malassezia* yeasts in 26 (65%) cases. The predominant species was *M.restricta,* found in 12 (30%) patients and the prevalence of other species was 17,5% for *M.globosa,* 10% for *M.sympodialis,* 5% for *M.slooffiae,* and 2,5% for *M.furfur.* The remaining 14 (35%) cultures showed no growth of the colonies (Table 2.).

Statistically significant differences were found in the distribution of the species isolated from healthy trunk and scalp skin – *M.sympodialis* was more frequent in the healthy trunk skin cultures (respective ratio 3.0), whereas *M.restricta* was more commonly positive in healthy scalp skin cultures (ratio 12.0) (Table 2).

	HEALTHY TRUNK SKIN			HEALTHY SCALP SKIN		
	F	M	Σ	F	M	Σ
M. globosa	2	7	9	3	4	7
M. sympodialis	7	5	12	1	3	4
M. furfur	4	3	7	1	0	1
M. obtusa	0	0	0	0	0	0
M. slooffiae	0	0	0	0	2	2
M. restricta	1	0	1	8	4	12
M. pachydermatis	0	0	0	0	0	0
Negative	6	5	11	7	7	14
Total	20	20	40	20	20	40
F= female; M=male; Σ= total;						

Table 2. *Malassezia* species obtained from trunk and scalp skin of normal subject

Distribution of Species Isolated from Healthy Trunk Skin According to Relevant Parameters

Sex

The same number were woman and man (50%).

No statistically significant differences were found between sexes in the species isolated (Fig. 1).

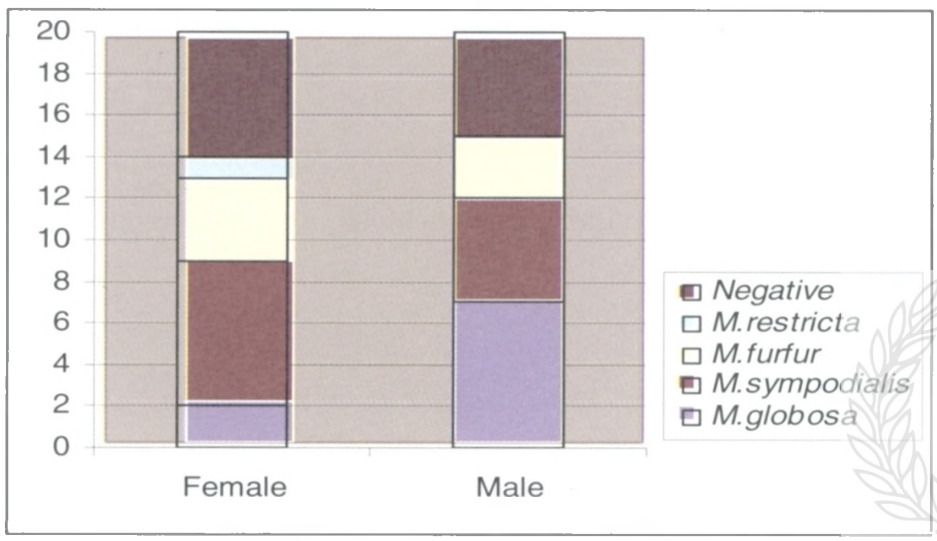

Figure 1. *Malassezia* species distribution from healthy trunk skin according to patient sex.

Age

According to age, patients were divided into five groups, as follows: ≤15 (n=3; 7,5%), 16-30 (n=10; 25%), 31-45 (n=9; 22,5%), 46-60 (n=8; 20%), and ≥61 years of age (n=10; 25%).

No statistically significant differences were found in the species isolated in these five groups (Fig. 2).

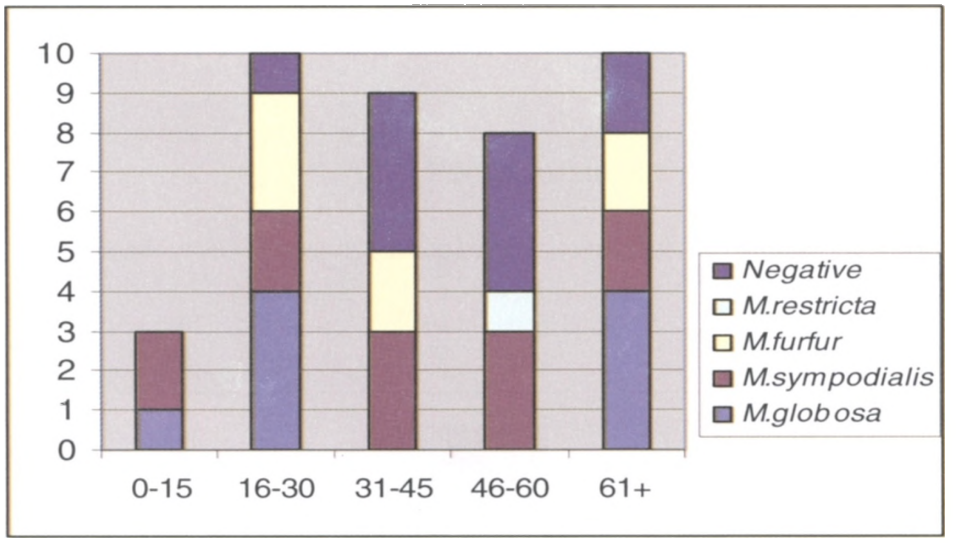

Figure 2. *Malassezia* species distribution from healthy trunk skin according to patient age.

Discussion

A number of investigators have conducted studies of *Malassezia* colonization of healthy skin (9, 10, 14, 15). Such studies have demonstrated that these yeasts are unique among the fungal kingdom as the only species to form part of normal human cutaneous commensal flora. Since the description of the new species some studies have focused on their distribution on normal skin.

We found that the predominant species on normal trunk skin was *M.sympodialis* isolated in 30% of cases. This species emerges as the predominant species on healthy skin, especially on the trunk, where it can be recovered in great numbers in more than 60% of individuals (16, 17). *M.globosa* is less common species found in 22,5% of healthy individuals. This species is reported to be a causative agent of pityriasis versicolor, found in filamentous form in the scales from this skin disorder (13, 16). Also in Russia, Arzumanian found *M.sympodialis* to be the most common species on the skin of 32 individuals, whereas *M.globosa* encountered much less frequently (18). *M.furfur* was isolated in 17,5% and this species is found to be less common inhabitant of healthy skin (10, 13, 15).

In contrast to healthy trunk skin, *M.sympodialis* was recovered less frequently from the scalp skin of same subjects, whereas *M.restricta* was the commonest

species. This species is isolated regularly from the scalp and face of patients with seborrheic dermatitis and normal individuals (10, 12). Aspiroz *et al.* in Zaragoza also found *M.restricta* to be particularly associated with scalp and *M.sympodialis* with the back, whereas *M.globosa* was evenly distributed on the scalp, forehead and trunk (19). The investigators in Canada also found that *M.sympodialis* was the commonest species in 20 healthy control subjects. *M.globosa* was equally likely to be recovered from the scalp and forehead and trunk, but less so from the arms and legs, and *M. restricta* and *M.slooffiae* were recovered more freq gently from the scalp and forehead than from the lower body (12). In our study *M.slooffiae* was found on scalp skin in only two subjects.

M.obtusa and *M.pachydermatis* were not recovered from any of our samples either from trunk or from healthy scalp skin. However, *M.obtusa* is considered to be very rare and only infrequently isolated from the cases of pityriasis versicolor, atopic dermatitis, and seborrheic dermatitis (12). *M.pachydermatis* is confirmed to be clearly adapted to animals, although it has been involved in some systemic human infection (8). The presence of this species on human skin is rare and transient, occurring possibly by transmission from animal pets and environmental sources (20, 21).

A correlation between the prevalence of *Malassezia* species and the age and sex of the subjects has been observed with a low recovery in infants and the moreover occurring at puberty but without differences among the woman and man (15). In our study all *Malassezia* species were identified equally in both sexes and according to the age.

Conclusion

Our results suggest that *M.sympodialis* is the most frequent isolated species from healthy trunk skin, whereas from scalp skin it is determined to be *M.restricta*. *M.globosa* and *M.furfur* are found to be less frequently isolated species. We found no differences between isolated species and the sex and age of the individuals.

Apstrakt: Kvasnice iz roda *Malassezia* čine dio normalne mikroflore kože ljudi i toplokrvnih životinja. Pod utjecajem predisponirajućih faktora postaju patogene i udružene sa nekim kožnim oboljenjima, pa čak i sistemnim infekcijama. Taksonomija ovih lipofilnih kvasnica nedavno je modifikovana, tako da obuhvata sedam specijesa, označenih kao *Malassezia*. Cilj ovog istraživanja jeste utvrditi prevalencu *Malassezia* specijesa na normalnoj koži i utvrditi njihovu distribuciju u odnosu na spol i dob pacijenata. Ispitivano je četrdeset pacijenata sa klinički zdravom kožom. Uzorci kod svih ispitanika su dobiveni struganjem površnog dijela kože sa gornjeg i središnjeg dijela trupa i sa vlasišta, a potom inkubirani na modificirani Dixon agar. Izolirane kvasnice identifikovane su na osnovu njihovih morfoloških i fizioloških osobenosti prema metodi Guillota i suradnika. Nađeno je da je *M.sympodialis* najdominantnija vrsta izolirana sa kože trupa, a *M.restricta* sa kože vlasišta. Nije nađena razlika u izoliranim specijesima u odnosu na spol i dob pacijenata.

Ključne riječi: *Malassezia*, specijesi, zdrava koža

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