

## Distribution of *Malassezia* Species in Patients with Pityriasis versicolor Compared with Healthy Individuals in Yazd, Iran

Abbas Ali Jafari<sup>1</sup>, Hossein Zarrinfar<sup>2,3</sup>, Farzaneh Mirzaei<sup>1</sup>, Farzad Katiraei<sup>4,\*</sup>

<sup>1</sup>Medical Parasitology and Mycology Department, Medical School, Shahid Sadoghi University of Yazd Medical Sciences, Yazd, IR Iran

<sup>2</sup>Department of Medical Parasitology and Mycology, Ghaem Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>3</sup>Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>4</sup>Department of Pathobiology, Section of Mycology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, IR Iran

\*Corresponding author: Farzad Katiraei, Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, IR Iran. Tel:+98-9126047311, Fax: +984116378743, E-mail: katiraei\_f@yahoo.com.

Received: Jun 21, 2012; Revised: September 30, 2012; Accepted: October 03, 2012

**Background:** *Malassezia* species as skin microflora of humans and other warm-blooded vertebrates are the lipophilic yeasts associated with various human diseases, especially pityriasis versicolor (PV).

**Objectives:** The objective of this study was to identify the *Malassezia* species of scraped skin of PV patients in Yazd, Iran, using morphological, biochemical and physiological methods. We also compared the obtained results of PV patients with normal healthy volunteers.

**Materials and Methods:** A total of 200 persons, including 100 patients (with skin lesion) referred to Yazd Central Laboratory and 100 healthy volunteers as controls, were evaluated for *Malassezia* infection by morphological and biochemical methods.

**Results:** The most commonly isolated species from PV lesions, were *M. globosa* (38.3%), *M. furfur* (29.4%), *M. sympodialis* (14.9%), *M. pachydermatis* (9.6%) and *M. slooffiae* (5.3%). Also the most commonly isolated species from healthy skins were *M. furfur* (37.2%), *M. globosa* (25.6%), *M. sympodialis* (16.3%), *M. pachydermatis* (13.9%) and *M. slooffiae* (4.6%). Totally *M. globosa* and *M. furfur* were the most frequented isolated.

**Conclusion:** Highest prevalence of PV in our study was observed in the 20 – 39 years old group, suggesting that the peak of the infection is coincided with ages and increasing sebum production in the highest level. *M. globosa* was the most commonly isolated *Malassezia* species of the patients group and *M. furfur* is the most common isolated species obtained from normal individuals skin samples. We couldn't find any significant differences between groups. The rate of isolated *Malassezia* species from patients was higher than normal individuals.

**Keywords:** *Malassezia*; Pityriasis versicolor, *Malassezia globosa*; Iran

### 1. Background

*Malassezia* species are lipophilic yeasts and parts of skin microflora of humans and other warm-blooded vertebrates that associated with various human diseases, especially pityriasis versicolor (PV), a chronic superficial scaling dermatomycosis (1-3). This chronic superficial fungal disease is characterized by the appearance of round-to-oval lesions. This disease is common in young adults and usually presented as variable pigmented scaling macules (3). These lesions vary in color, and can be hypo or hyperpigmented. The most common sites of disease are the upper trunk and the neck (4). Numerous studies have shown the relationship between *Malassezia* species and their role as causative agents and/or triggers of diseases. Also *Malassezia* species, are etiological agents of catheter-associated fungemia, seborrheic dermatitis (5), and folliculitis (3, 6) dacryolitis, blepharitis as well as nosoco-

mial bloodstream infections in pediatric care units (7, 8).

The taxonomy of genus *Malassezia* was controversial and has undergone several taxonomic revisions. In 1996, in the reclassification by Gueho et al. seven distinct species were identified within this genus namely *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta* and *M. slooffiae*. Recently, on the basis of DNA relatedness, six new species have been included in this genus: *M. dermatis*, *M. nana*, *M. japonica*, and *M. yamatoensis*, *M. equine*, *M. caprae* (6, 9-14). Among them, *M. restricta* and *M. globosa* are considered to be as the most important pathogenic organisms in the development of seborrheic dermatitis. However, some reports have also linked *M. furfur*, *M. sympodialis*, *M. obtusa*, and *M. slooffiae* with seborrheic dermatitis. Our data about the epidemiology of PV in Iran is limited in some regions such as Tehran, Kashan, Ahvaz and Mazandaran (6, 15-18). Also there are evidences suggesting that the geographical variations of the spe-

#### Implication for health policy/practice/research/medical education

The results of this study indicating the recovery rate of *Malassezia* species on the pityriasis versicolor lesions and skin of normal persons.

Copyright © 2013, Ahvaz Jundishapur University of Medical Sciences; Licensee Kowsar Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

cies are available.

## 2. Objectives

The objective of this study was to identify the *Malassezia* species on the scalp of patients with PV, in Yazd (a central province in Iran) using morphological, biochemical and physiological modifying methods. We also compared the results of PV patients with normal healthy volunteers.

## 3. Patients and Methods

### 3.1. Subjects and Collecting Samples

A total of 200 samples, including 100 patients (with skin lesion) referred to Yazd Central Laboratory and 100 healthy volunteers as control group were subjected to this study (Table 1). A questioner included, gender, age, duration of disease, lesion type and involved area were filled for each person. Initially, PV on the lesions was clinically diagnosed, after that the final diagnosis was confirmed by direct microscopic examination with Sellotape method and methylene blue staining of the samples collected from scraped skin of patients (19 - 21). In control group, scraped skin was collected as same as patients, from chest, back, arms, face and neck and abdominal area.

**Table 1.** Age and Gender Distribution of Subjects

Age Groups	Pityriasis versicolor			Control Group		
	Male, No. (%)	Female, No. (%)	Total, %	Male, No. (%)	Female, No. (%)	Total, %
< 19, y	14 (25.9)	8 (17.4)	22	11(21.6)	9 (18.3)	20
20 - 39, y	21 (38.9)	19 (41.3)	40	23 (45.1)	22 (44.9)	45
40 - 59, y	15 (27.8)	13 (28.2)	28	12 (23.5)	14 (28.6)	26
> 60, y	4 (7.4)	6 (13.1)	10	5 (9.8)	4 (8.2)	9
<b>Total</b>	54 (100)	46 (100)	100	51 (100)	49 (100)	100

### 3.2. Culture of Samples

The scraped skin was inoculated in plates containing modified Dixon medium (Merck, Germany) (mDixon). This medium consisted of 3.6% malt extract, 2.0% desiccated Ox-bile, 1.0% Tween 40, 0.2% glycerol, 0.2% oleic acid, 0.05% chloramphenicol, 0.5% cycloheximide, and 1.2% agar. Inoculated cultures incubated at 32°C for 1 - 10 days. According to Gueho et al. and Mayer et al. suspected *Malassezia* spp. identified on the basis of the colony morphology, microscopic appearance of the fungal cells obtained in the culture, by examining the fungal ability to grow without lipid supplementation (13, 22).

### 3.3. Tween Assimilation Test

Utilization of different Tween compounds (Tween 20, 40, 60 and 80) (Sigma-Aldrich, USA) as a lipid supplement for *Malassezia* species performed according to the Guillot et al. (20) and Gupta et al. (3). Briefly, the yeast suspension (at least  $10^5$  to  $2 \times 10^5$  CFU/mL) was made in 2 mL sterilized distilled water and poured into plate containing Sabouraud dextrose agar (Merck, Germany) at 45°C. The inoculums were then spread evenly. After solidification of each plate, four wells with 2 mm diameter were made and filled with 10  $\mu$ L Tween 20, 40, 60 and 80, respectively. These plates were incubated for a week at 31°C and the growing degree was assessed by measuring the sedimentation zone of each well after 2, 4 and 7 days (21, 23).

### 3.4. Catalase Reaction

Presence of catalase was determined by using a drop of

hydrogen peroxide (3% solution) and production of gas bubbles that considered as a positive reaction. Lack of catalase activity is a characteristic feature of *M. restricta*, (20, 21) and also *M. pachydermatis* is variable in catalase reaction (21).

### 3.5. Splitting of Esculin

The  $\beta$ -glucosidase activity of different *Malassezia* species was assayed using method described by Mayser et al. (22). Briefly, a loop of fresh yeasts was inoculated deeply in the esculin agar (Merck, Germany) tube and incubated for 5 days at 31°C. The splitting of esculin is revealed by darkening of the medium. This test was used to differentiate *M. furfur*, *M. slooffiae* and *M. sympodialis* from other *Malassezia* species. However *M. pachydermatis* has a variable reaction (1). In order to differentiation of *M. sympodialis* and *M. obtusa*, they were incubated at 40°C for one week (1, 13).

### 3.6. Demonstration of *M. pachydermatis*

As *M. pachydermatis* is the only non-obligatory lipid dependent species of *Malassezia* spp. (21, 24), the yeast isolated on mDixon agar was smeared with a sterile swab on Sabouraud glucose agar (Merck, Germany) plus 0.05% chloramphenicol and 0.05% cycloheximide devoid of lipids. Incubation was performed at 31°C for one week.

### 3.7. Statistical Analysis

Quantitative data were analyzed by t-test. The data of the patient and healthy controls were analyzed using chi-square test and Fisher exact test. P-values of < 0.05 were

considered significant.

#### 4. Results

The study included 100 patients (46 women and 54 men) with PV, aged 18 - 72 and 100 healthy individuals aged 19 - 70 years. No statistically significant difference was observed in the frequency of isolated *Malassezia* spp. between women and men within group or between groups ( $P: 0.34$ ). The highest prevalence of PV was observed in patients with 20 - 39 years old. Significant differences in the rate of *Malassezia* species isolation between age groups were observed in both groups ( $P < 0.04$ ). 94% of the specimens in patients with PV and 43% of healthy individuals yielded *Malassezia* in culture. Besides, culture positive cases were higher in patient group than healthy controls and this difference was statistically significant ( $P < 0.05$ ).

In PV lesions, the most commonly isolated species was *M. globosa* (38.3%), *M. furfur* (29.4%), *M. sympodialis* (14.9%), *M. pachydermatis* (9.6%) and *M. slooffiae* (5.3%), respectively. the most commonly isolated species were *M. furfur* (37.2%), *M. globosa* (25.6%), *M. sympodialis* (16.3%), *M. pachydermatis* (13.9%) and *M. slooffiae* (4.6%), respectively. Totally *M. globosa* and *M. furfur* were the frequent isolations. Overall, no differences in distribution of *Malassezia* isolated species were noted in 2 groups ( $P = 0.1$ ). However

in patient's group isolation of *M. globosa* was higher than *M. furfur*. Predominantly, *Malassezia* species were isolated from the chest. Overall in patients group, 26 isolates were obtained from this organ. On the other hand, in healthy individuals *Malassezia* species predominantly were obtained from back and chest area (13 and 12 cases respectively). Also the lowest isolation of *Malassezia* species was from arm in both groups (Figure 1). Tables 1 and 2; show the distribution of subjects and *Malassezia* species, based on the sites of sample collection.

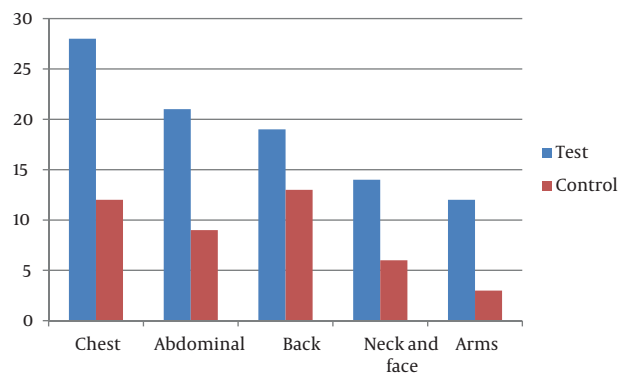


Figure 1. Distribution of *Malassezia* Isolated Based on Anatomical Sites

Table 2. Distribution of Different *Malassezia* Species Isolated in Patients and Healthy Individual

Species	Patients			Normal			Total, No. (%)
	Male, %	Female, %	Total, No. (%)	Male, %	Female, %	Total, No. (%)	
<i>M. globosa</i>	22	14	36 (38.3)	6	5	11 (25.6)	47 (34.3)
<i>M. furfur</i>	15	12	27 (29.4)	9	7	16 (37.2)	42 (30.6)
<i>M. sympodialis</i>	4	10	14 (14.9)	3	4	7 (16.3)	21 (15.3)
<i>M. pachydermatis</i>	6	3	9 (9.6)	4	2	6 (13.9)	15 (10.9)
<i>M. slooffiae</i>	2	3	5 (5.3)	2	0	2 (4.6)	7 (5.2)
<i>Malassezia</i> . Species	1	2	3 (3.2)	1	0	1 (2.3)	4 (2.9)
<b>Total</b>	50	44	94 (100)	25	18	43 (100)	137 (100)

#### 5. Discussion

Different studies showed that the frequency and occurrence of *Malassezia* species and their diseases is depending on some factors such as occupational and economical conditions as well as climate and geographical area (6, 18, 25). In many studies *M. globosa* is the predominant species in lesional and normal skin. In our study similar to other investigations, in PV patients *M. globosa* was the predominant species, but in normal individual *M. furfur* was the most isolated species. On the other hand, similar to other investigations the highest prevalence of PV in our study was observed in 20 - 39 years old group, suggesting that the high infection is associated with the age and increasing sebum production at the highest level (6,

17, 26). Previous reports showed that PV is uncommon among children (6) and we didn't found any case of PV in children. However it is only rarely found in the elderly, we had more cases of PV in over 60 years old individuals.

In addition, *M. sympodialis* was isolated more frequently than what has been reported by many Iranian investigators expressing that this species is known as the third or fourth frequented species (6, 15, 17, 21). We couldn't isolate any *M. obtusa* and *M. restricta* in this study, although in other studies in Iran these species were isolated in at a very low frequency. *M. slooffiae* is less common in other studies and we isolated this species in 5.3% of patients and 4.6% in normal individuals. Interestingly, *M. pachydermatis* was isolated in 9.6% and 13.0% of patients and healthy individuals respectively, however this species is

associated with animals.

In other reports from Iran, *M. pachydermatis* was not isolated or low frequency isolated (21). These differences between our study and others may be due to geographical variation and some laboratory techniques such as sampling and differentiation methods. We have isolated a single colony from each sample collected from patients with PV, as suggested by some investigators. However, in many studies, more than one species has been recovered from each sample group. It should be underlined that providing a pure culture and the isolation of a species from a mixed culture is too difficult. This might be due to this point that fast growing species usually cover other species in the culture.

All pathogenic species in patients group were detected in normal individual that showed PV is caused as a secondary complication for patients that is not usually pathogenic. In conclusion, we compared the recovery rate of *Malassezia* species on the lesions of patients with PV and skin of normal persons. *M. globosa* was the most commonly isolated species in the patients group and *M. furfur* in normal individuals. However we couldn't find any significant differences of *Malassezia* species distribution between groups. In addition, the rate of isolation of *Malassezia* species in patients was higher than normal individuals.

## Acknowledgements

This study was financially supported by Shahid Saadoughi University of Yazd Medical Sciences. We express our appreciation to Dr. Fallah Zadeh for his help in the statistical analysis.

## Authors' Contribution

None declared.

## Financial Disclosure

None declared.

## Funding/Support

None declared.

## References

- Midgley G. The diversity of Pityrosporum (*Malassezia*) yeasts in vivo and in vitro. *Mycopathologia*. 1989;106(3):143-53.
- Leeming JP, Notman FH, Holland KT. The distribution and ecology of *Malassezia furfur* and cutaneous bacteria on human skin. *J Appl Bacteriol*. 1989;67(1):47-52.
- Gupta AK, Bluhm R, Summerbell R. Pityriasis versicolor. *J Eur Acad Dermatol Venereol*. 2002;16(1):19-33.
- Crespo Erchiga V, Delgado Florencio V. *Malassezia* species in skin diseases. *Curr Opin Infect Dis*. 2002;15(2):133-42.
- DeAngelis YM, Gemmer CM, Kaczvinsky JR, Kenneally DC, Schwartz JR, Dawson TL, Jr. Three etiologic facets of dandruff and seborrheic dermatitis: *Malassezia* fungi, sebaceous lipids, and individual sensitivity. *J Invest Dermatol Symp Proc*. 2005;10(3):295-7.
- Tarazooie B, Kordbacheh P, Zaini F, Zomorodian K, Saadat F, Zeraati H, et al. Study of the distribution of *Malassezia* species in patients with pityriasis versicolor and healthy individuals in Tehran, Iran. *BMC Dermatol*. 2004;4:5.
- Chryssanthou E, Broberger U, Petrini B. *Malassezia pachydermatis* fungaemia in a neonatal intensive care unit. *Acta Paediatr*. 2001;90(3):323-7.
- Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, Velegraki A. The *Malassezia* genus in skin and systemic diseases. *Clin Microbiol Rev*. 2012;25(1):106-41.
- Sugita T, Takashima M, Shinoda T, Suto H, Unno T, Tsuboi R, et al. New yeast species, *Malassezia dermatis*, isolated from patients with atopic dermatitis. *J Clin Microbiol*. 2002;40(4):1363-7.
- Sugita T, Kodama M, Saito M, Ito T, Kato Y, Tsuboi R, et al. Sequence diversity of the intergenic spacer region of the rRNA gene of *Malassezia globosa* colonizing the skin of patients with atopic dermatitis and healthy individuals. *J Clin Microbiol*. 2003;41(7):3022-7.
- Sugita T, Tajima M, Takashima M, Amaya M, Saito M, Tsuboi R, et al. A new yeast, *Malassezia yamatoensis*, isolated from a patient with seborrheic dermatitis, and its distribution in patients and healthy subjects. *Microbiol Immunol*. 2004;48(8):579-83.
- Hirai A, Kano R, Makimura K, Duarte ER, Hamdan JS, Lachance MA, et al. *Malassezia nana* sp. nov., a novel lipid-dependent yeast species isolated from animals. *Int J Syst Evol Microbiol*. 2004;54(Pt 2):623-7.
- Gueho E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek*. 1996;69(4):337-55.
- Sei Y. [*Malassezia* infections]. *Med Mycol J*. 2012;53(1):7-11.
- Zomorodian K, Mirhendi H, Tarazooie B, Zeraati H, Hallaji Z, Balighi K. Distribution of *Malassezia* species in patients with psoriasis and healthy individuals in Tehran, Iran. *J Cutan Pathol*. 2008;35(11):1027-31.
- Shokohi T, Afshar P, Barzgar A. Distribution of *Malassezia* species in patients with pityriasis versicolor in Northern Iran. *Indian J Med Microbiol*. 2009;27(4):321-4.
- Nazeri M, Amiri S, Moniri R, Moayeri MR, Morveji AR, Asghari B, et al. Isolation and identification of *Malassezia* spp. in pityriasis versicolor patients in Kashan. *Kowsar Med J*. 2009;13(4):293-296.
- Mahmoudabadi AZ, Zarrin M. Pityriasis versicolor in Ahvaz, Iran. *Jundishapur J Microbiol*. 2009;2(3):92-96.
- Hedayati MT, Hajheydari Z, Hajjar F, Ehsani A, Shokohi T, Mohammadpour R. Identification of *Malassezia* species isolated from Iranian seborrheic dermatitis patients. *Eur Rev Med Pharmacol Sci*. 2010;14(1):63-8.
- Guillot J, Guého E, Lesourd M, Midgley G, Chévrier G, Dupont B. Identification of *Malassezia* species: a practical approach. *J de mycologie médicale*. 1996;6(3):103-110.
- Khosravi AR, Eidi S, Katiraei F, Ziglari T, Bayat M, Nissiani M. Identification of different *Malassezia* species isolated from patients with *Malassezia* infections. *World J Zoology*. 2009;4(2):85-89.
- Mayser P, Wille G, Imkamp A, Thoma W, Arnold N, Monsees T. Synthesis of fluorochromes and pigments in *Malassezia furfur* by use of tryptophan as the single nitrogen source. *Mycoses*. 1998;41(7-8):265-71.
- Guillot J, Hadina S, Gueho E. The genus *Malassezia*: old facts and new concepts. *Parasitologia*. 2008;50(1-2):77-9.
- Raabe P, Mayser P, Weiss R. Demonstration of *Malassezia furfur* and *M. sympodialis* together with *M. pachydermatis* in veterinary specimens. *Mycoses*. 1998;41(11-12):493-500.
- Gaitanis G, Velegraki A, Alexopoulos EC, Chasapi V, Tsigonias A, Katsambas A. Distribution of *Malassezia* species in pityriasis versicolor and seborrheic dermatitis in Greece. Typing of the major pityriasis versicolor isolate *M. globosa*. *Br J Dermatol*. 2006;154(5):854-9.
- Crespo-Erchiga V, Gomez-Moyano E, Crespo M. [Pityriasis versicolor and the yeasts of genus *Malassezia*]. *Actas Dermosifiliogr*. 2008;99(10):764-71.