

Frequency, Distribution and Genotyping of *Malassezia* Species in Patients with *Psoriasis vulgaris*

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Abstract

Background: *Malassezia* species are reported to play a role in the etiology of *Psoriasis vulgaris*. **Objective:** The aim of this study was to determine the presence, frequency, distribution, and genotyping of skin colonization of *Malassezia* species in *Psoriasis vulgaris* and to compare with healthy individuals and to investigate its relationship with the severity of the disease. **Methods:** Skin samples were taken from scalp, arm, body, and leg of 34 psoriasis patients (lesional/ non-lesional skin) and 30 healthy volunteers. Overall, 392 skin scraping samples were taken for the isolation of *Malassezia* species, which were incubated on the modified-Dixon agar. Conventional culture methods were used for *Malassezia* species identification. In isolates, genotyping was carried out by PCR-RFLP method. **Results:** In the samples from psoriatic lesions, most frequently isolated *Malassezia* species were *M.globosa* and *M.furfur*. Similarly, the most frequently isolated species in healthy volunteers was *M. globosa*; followed by *M.restricta* and *M.symphodialis*. The *M.furfur* isolation rate in psoriatic scalp and leg lesions of the patients was significantly higher than in healthy volunteers. There was no relationship between the severity of the disease and the isolated species. **Conclusion:** It was found that there was a difference between patients with psoriasis and healthy controls regarding presence and frequency of *Malassezia* species. Therefore, our study results support the view that *Malassezia* species may be associated with the etiopathogenesis of psoriasis. In addition, we surmise that the treatment applications for the regulation of skin microbiota of psoriasis patients will contribute positively to the treatment of psoriasis.

Keywords: psoriasis vulgaris, malassezia, PCR-RFLP, genotyping, modified-Dixon agar, microbiota

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Introduction

Psoriasis vulgaris with unknown etiology is a chronic, recurrent, immune-mediated, inflammatory skin disease which is common in the society. It is thought that the disease is associated with hereditary polygenic predisposition as well as the irregular interaction between environmental triggers, including keratinocytes, immune cells, and microbiota (1,2). In recent years, it has been reported that changes in skin microbiota (dysbiosis) play a role in the pathogenesis of chronic plaque psoriasis disease. It is reported that some skin microbiota members, such as *Streptococcus*, *Staphylococcus*, and *Cutibacterium*, as well as *Malassezia* and *Candida* may aggravate psoriasis. It is also suggested that the cellular immune response thought to be developing against these agents may be responsible for the pathogenesis (3-6). It has been reported that lesions compatible with psoriasis occur after the application of inactivated *Malassezia* species to the non-lesional skin of patients with psoriasis by patch test method (7). In addition, since the soluble components obtained from *Malassezia* are chemoattractant for polymorphonuclear leukocytes of psoriatic patients, it has been shown that *Malassezia* species play a role in koebnerization of psoriasis (8-9).

Malassezia species are among the fungal members of commensal skin microbiota. These species have been reported to be associated with some skin diseases. Fourteen species of *Malassezia* fungi are known today. Some of them are zoophilic and some of them are located in humans as members of human microbiota including *M.furfur*, *M.sympodialis*, *M.globosa*, *M.obtusa*, *M.restricta*, *M.slooffiae*, *M.dermatis*, *M.japonica*, *M.yamatoensis* (10). *Malassezias* prefer fat-rich body areas in humans, and their density varies in the area according to localization (11). They are especially colonized particularly in the scalp, upper body, and flexural areas.

Malassezia species do not infect hair, nails, and mucous membranes (12).

There are controversial results in the studies on the clinical significance of *Malassezia* species since *in vitro* cultivation is difficult for these organisms and there are challenges in their identification (13). Special media are needed for the growth of *Malassezia* species; thus, limited study has been carried out so far. Modified Dixon agar containing peptone (14) has been reported as the most suitable medium for isolation of *Malassezia* species (15).

The aim of this study was to determine the presence, frequency, distribution, and genotyping (by PCR-RFLP method) of *Malassezia* species in samples taken from patients with *Psoriasis vulgaris* and to compare with healthy individuals. In addition, we also investigated whether there is a relationship between *Malassezia* species and Psoriasis area severity index (PASI).

Materials and Methods

The study was approved by the Ethics Committee of Hatay Mustafa Kemal University, Faculty of Medicine (2009-47/ 05-20). Overall, 64 subjects were included in the study: 34 patients diagnosed with psoriasis and 30 healthy individuals who presented to the Dermatology Outpatient Clinic of Hatay Mustafa Kemal University Faculty of Medicine between 2010 and 2011.

All subjects signed and informed consent before participation. In the study, demographic data (age, gender, and disease duration of the patients) of the individuals included were collected by face-to-face interview method. In addition, PASI values were calculated and recorded in all patients. In the study, exclusion criteria comprised: the use of systemic steroid and other immunosuppressive therapy, and systemic or topical antifungal therapy within the prior month, topical steroid use within the prior week, and moisturizer use within the prior 24 hours, bathing within

the prior 3 days and the presence of immunosuppressive disease, disability or pregnancy.

Mycological evaluation

In the study, both psoriatic lesional skin samples (136 pieces) and non-lesional skin samples (136 pieces) were taken from the patients. Samples from four different body regions (scalp, arm, body, and leg) were taken from healthy volunteers. Overall, 120 skin scraping samples were taken from healthy volunteers. Before taking the samples, the skin area was cleaned using 70% ethyl alcohol and the samples were taken with sterile scalpel after waiting for a while. Overall, 392 specimens taken from the skin as scrapings were inoculated to modified Dixon agar (mDIX; Oxoid, UK). Each sample was inoculated on two petri plates. The inoculated plates were incubated for 10 days at 32°C under aerobic conditions. In all plates, growth was controlled daily. At the end of incubation, microorganisms with yeast-style growth were tested with conventional techniques to determine whether they were *Malassezia* (16).

Isolation of Malassezia Species

Clinical samples were taken under sterile conditions. The isolation of the samples was carried out according to the method described by Bayraktar et al. (17). After the samples were inoculated into Dixon agar plates, sterile olive oil was added to cover the surface of the medium. Plates were incubated at aerobic conditions for 10 days at 32°C.

Polymerase chain reaction (PCR)

Genomic DNA extraction was performed using commercially available glass bead isolation kits (Roche, ZR Fungal DNA Kit, Germany). DNA isolation was performed in accordance with the test procedure. The following primary sequence based on the 26S rDNA sequence of *Malassezia* species was used for PCR amplification

(forward: 5'-TAACAAGGATTCCCCTAGTA 3', reverse: 5'ATTACGCCAGCATCCTAAG 3'). PCR amplification was prepared as follows: with the final volume of 50 µL. 1 µL template DNA, 0.5 µM from each primer, 0.20 mM from each deoxynucleotide triphosphate (dNTPs; AB gene, UK), 5 µL 10x PCR buffer (MgCl₂ Chlorine-free; Promega corp.) and 1.25 U Taq DNA polymerase (Fermentas, USA).

PCR amplification was performed in the Thermal cycler (Bioder/ Thermal Blocks xp cycler, Tokyo Japan) in the following steps. The first denaturation; 5 minutes at 94°C, denaturation; 45 seconds at 94°C, annealing; 45 seconds at 55°C, 1 minute at elongation at 72°C, 7 minutes at last at 72°C. Denaturation, annealing, and elongation periods were applied for 30 cycles.

The amplified PCR products were digested with CfoI (Roche Diagnostics, Mannheim Germany) and BstF51 (Sib Enzyme, Russia) restriction endonuclease enzymes. Genotyping of *Malassezia* species (*Malassezia* (*M*).*globosa*, *M.furfur*, *M.restricta*, *M.sympodialis*, *M.slooffiae*, *M.obtusa* and *Malassezia spp.*) was performed according to species-specific band patterns. The final volume was adjusted to 25ml for the restriction procedure. For digestion, PCR-amplified DNA and the restriction enzymes were incubated for 3 hours at 37°C. Thereafter, the presence of specific amplification products for *Malassezia* species was visualized by running on a 1.5% agarose gel (Wealtec, Dolphin- View, USA).

Statistical Analysis

Categorical measurements are summarized as number and percentage, while numerical measurements are summarized as average and standard deviation. Chi-Square test was used to analyze non-parametric data in the patient and the healthy control group. Mc Nemar test was used to compare patients regarding *Malassezia* species that were determined from lesional and non-lesional skins. Mann-Whitney U test was

used to determine the relation between *Malassezia* species and PASI. In addition, Spearman correlation test was performed to compare the disease duration of psoriatic patients with presence of *Malassezia* species. Statistical significance level was taken as $p < 0.05$ in all tests. SPSS 16.0 package software was used in the statistical analysis of data.

Results

The study included 34 patients diagnosed with *Psoriasis vulgaris* (18 men, 16 women) and 30 healthy volunteers (14 men, 16 women). The mean ages of the patients and healthy volunteers were 42.0 ± 13.3 and 41.7 ± 11.9 years, respectively. The average disease duration was 12.1 ± 8.6 years. Mean PASI values were determined as 12.7 ± 8.9 in the patients.

The culture positivity rate was determined as 55.9% (76/136) in the samples taken from psoriatic lesions and 48.5% (66/136) in the samples taken from non-lesional areas in the patient group whereas 32.5% (39/120) in healthy volunteers. There was no statistically significant difference in culture positivity between the lesional and non-lesional skin areas of patients with psoriasis ($p = 0.203$). Statistically significant difference was found in culture positivity between the samples from the lesional areas of patients with psoriasis and the samples from healthy volunteers ($p < 0.001$). Similarly, a statistically significant difference was found between non-lesional skin samples of patients and samples taken from volunteers ($p = 0.011$).

PCR results

The presence of *Malassezia* species, i.e. PCR positivity, isolated and confirmed by PCR was compared with patients with psoriasis and healthy volunteers. The PCR positivity rate of *Malassezia* species was found to be significantly higher in the patients from a statistical point of view.

PCR positivity was found in 59.6% (81/136) of the samples taken from the lesions of the patients with psoriasis and 51.5% (70/136) of the samples taken from the non-lesional skin. In healthy volunteers, it was found as 34.2% (41/120). In samples taken from psoriatic-lesional and non-lesional areas of patients with psoriasis, there was no statistically significant difference in terms of PCR positivity ($p = 0.127$). However, PCR positivity in samples taken from psoriatic lesions was found to be significantly higher than healthy volunteers ($p < 0.001$). Similarly, a statistically significant difference was found between the samples taken from the lesions-free areas of the patients and the volunteers ($p = 0.008$).

PCR positivity of *Malassezia* species in psoriatic-lesional and non-lesional samples according to anatomic regions in psoriasis patients were as follows: 61.8% and 50.0% on the scalp, 61.8% and 44.1% on the arm, 58.8% and 55.9% on the body, 55.9% and 55.9% on the leg, respectively. No statistically significant difference was detected in PCR positivity between lesional and non-lesional skin areas ($p > 0.05$). PCR positivity was found as 56.7%, 26.7%, 40.0%, and 13.3% on the scalp, arm, body, and leg samples of healthy volunteers, respectively.

The most common *Malassezia* species isolated in the psoriatic lesional and non-lesional skin samples of psoriasis patients were *M.globosa* and *M.furfur*. The rate of *M.globosa* was determined as 30.1% (41/136) in psoriatic lesional skin and 24.3% (33/136) in non-lesional skin. The rate of *M.furfur* was detected as 23.5% (32/136) in psoriatic lesional skin and 18.4% (25/136) in non-lesional skin. *Malassezia* species isolated from the skin samples of patients and healthy volunteers were compared. The frequency of *M.furfur* was found to be significantly higher in the lesional scalp ($p = 0.015$) and leg ($p = 0.029$) samples of the patients.

The most commonly isolated species in healthy volunteers were found to be *M.globosa* 21.7%

(26/120), *M.restricta* 13.3% (16/120), and *M.sympodialis* 11.7% (14/120). In the scalp samples, *M.restricta* (43.3%) and *M.globosa* (23.3%) were the two most frequently isolated species. In the body, *M.globosa* (36.7%), *M.sympodialis* (20.0%), and *M.slooffiae* (13.3%) were the three most frequently isolated species. Nevertheless, *Malassezia* colonization in the arms and legs was found to be less than other areas. *M.globosa* was the most frequently isolated species in the arm (16.7%) and leg samples (10.0%), followed by *M.sympodialis* (13.3%- 6.7%). Statistically, when the lesional/non-lesional skin samples of psoriasis patients and healthy volunteers were compared, isolation of *M.restricta*, *M.sympodialis*, *M.slooffiae* (4.2%) species was significantly higher than the healthy group.

Unidentified *Malassezia* species, namely *Malassezia spp.*, were found in the lesional skin of patients with psoriasis by 8.8% (12/136) and in non-lesional skin by 11.0% (15/136). The incidence of *Malassezia spp.* in healthy volunteers was found to be 5.8% (7/120).

In addition, mixed *Malassezia* colonization was detected in some samples. Psoriasis patients often had *M.globosa* plus *M.furfur*, while healthy individuals had *M.globosa* plus *M.sympodialis*. The rate of detection of mixed *Malassezia* colonization in PCR was 11.0% (15/136) in lesions of patients with psoriasis, 6.6% (9/136) in non-lesional skin and 16.7% (20/120) in healthy volunteers.

The skin colonization frequency of the *Malassezia* species was compared with the PASI value showing the severity of the disease, and no statistically significant difference was found for any *Malassezia* species ($p > 0.05$). No significant relation was found between age, gender, disease duration, and presence of *Malassezia* species ($p > 0.05$).

Figure 1 (A-C) presents distribution of *Malassezia* species isolated from samples taken from different body regions of healthy volunteers,

lesional, and non-lesional patient skin. Figure 2 presents comparison of the *Malassezia* species distributions isolated from psoriatic-lesional and non-lesional samples. Figure 3 presents comparison of the *Malassezia* species distributions isolated from patients psoriatic lesions and healthy volunteers.

Discussion

The mechanism of *Malassezia* in the pathogenesis of psoriasis disease has not been fully elucidated yet. It is suggested that the lipid-rich capsules of *Malassezia* species mask the recognition of the host defense cells and the reduction of this layer reveals the inflammatory response (18). It is reported that the lipid layer of *Malassezia* species regulates cytokines produced in keratinocytes. However, *Malassezia* strains with lipid capsules removed have been reported to significantly increase IL-6, IL-8, IL-1, and TNF- α production and reduce intracellular IL-10 levels (18,19). *Malassezia* species play an immunomodulatory role with a lipid-rich structure and this feature is important in understanding the ability to cause disease (11). *Malassezia* species can activate the complement system from the alternative route and the classic route (20). It has been reported that a specific humoral immune response develops against the yeast form of *M.furfur* (formerly known as *Pityrosporum ovale*) in patients with psoriasis (21). It has been reported that the downregulation effect of *Malassezia* species on anti-inflammatory cytokines may be related to the appearance of guttate psoriasis (22). In *in vitro* experiments, it has been reported that the psoriasis patients with *M.furfur* positivity increase the transforming growth factor-beta 1, integrin and heat shock protein-70 which lead to psoriasis-like phenotype with keratinocyte migration, hyperproliferation (23). *Malassezia* species have been shown to play a role in triggering psoriasis by increasing the expression

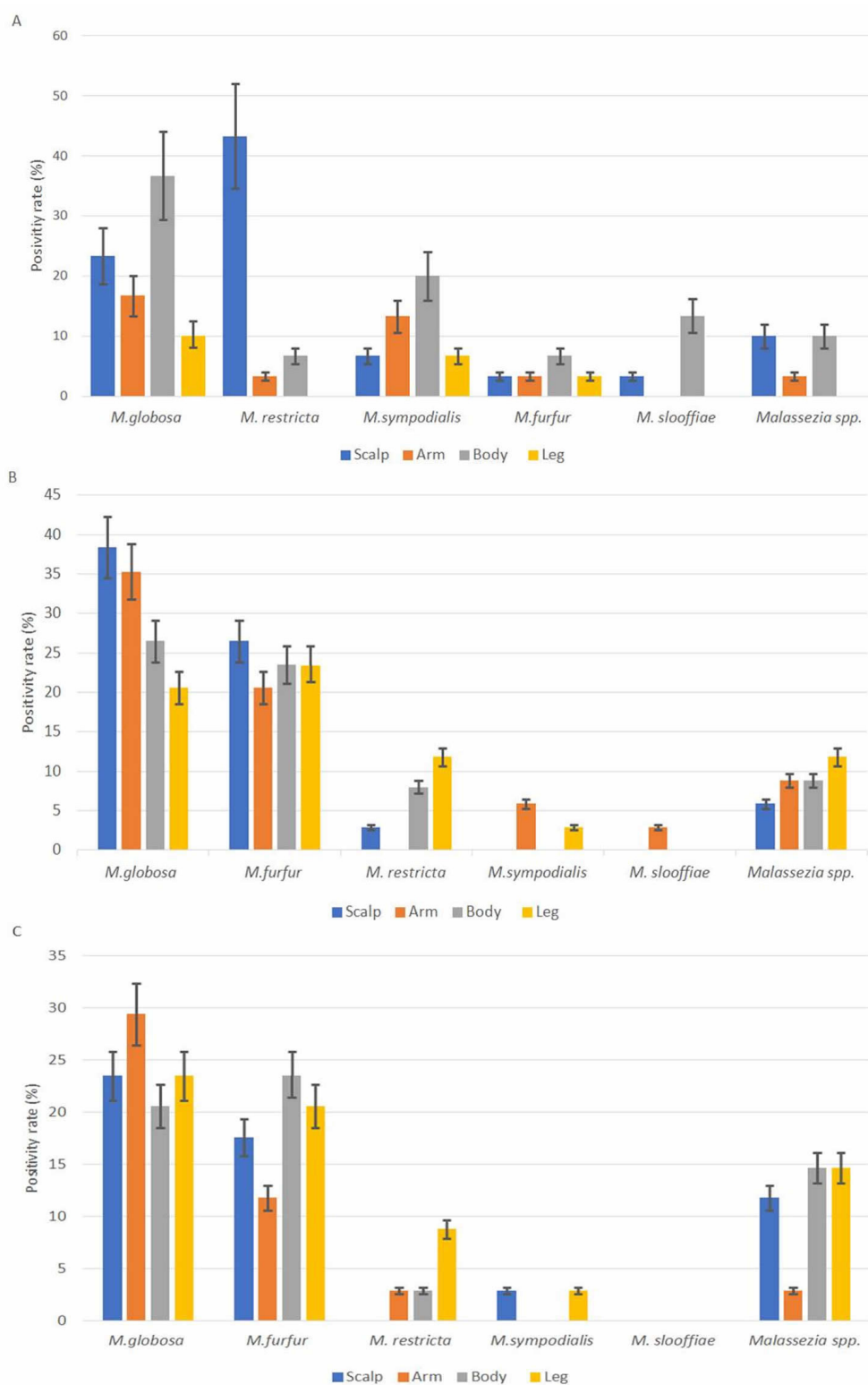


Fig. 1(A-C). Distribution of *Malassezia* species isolated from samples taken from different body regions of healthy volunteers (A), and psoriatic-lesional (B), non-lesional (C) body regions of psoriasis patients.

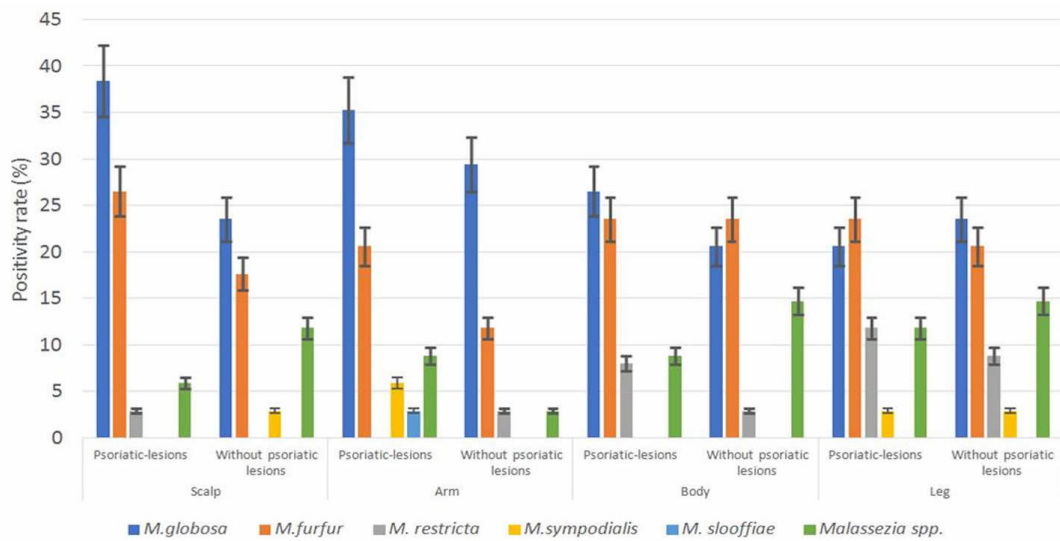


Fig. 2. Comparison of the *Malassezia* species distribution isolated from psoriatic-lesional and non-lesional skin samples.

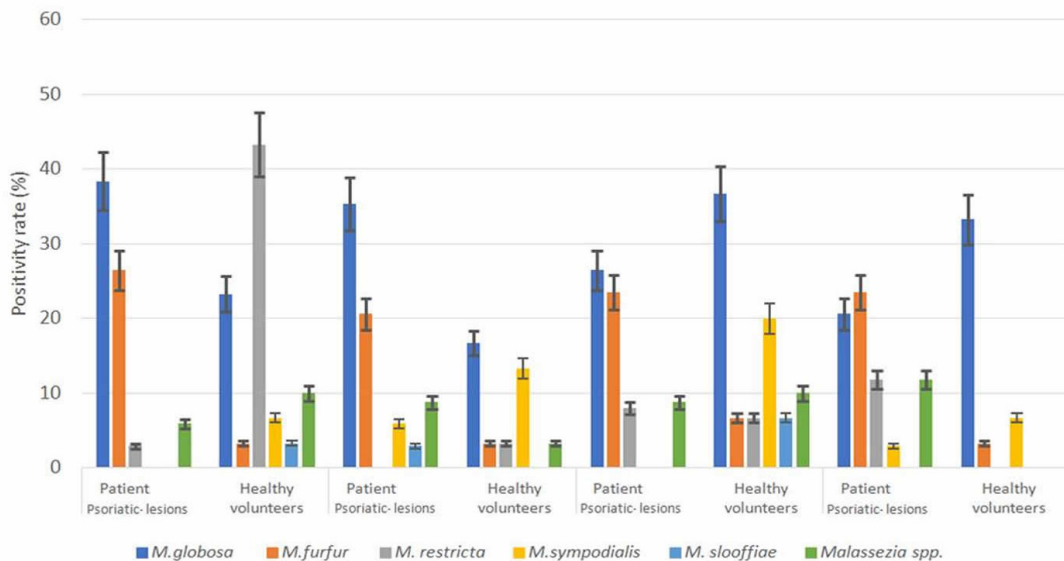


Fig. 3. Comparison of the *Malassezia* species distribution isolated from patients with psoriatic-lesions and healthy volunteers.

of Toll-like receptor 2, human β 2 defensin, and IL-8 (24). In some studies, it has been reported that *M. furfur* can provoke an exacerbation of psoriatic symptoms. It has been detected that arachidonic acid and metabolites, known as skin inflammation mediators, involved in the hyper-

proliferative process of psoriasis are released from epithelial cell membranes by secreting lipase from *M. furfur* (5,25,26).

The PCR-RFLP technique is successfully used for the identification of *Malassezia* species in psoriatic skin lesions. It has been reported that

PCR-RFLP is a more reliable and accurate method in the detection of *Malassezia* species than Nested-PCR method (27). Therefore, in our study, PCR-RFLP method was used for genotyping of *Malassezia* species. Zomorodian et al. (5) compared psoriasis patients with healthy individuals in terms of the presence of *Malassezia*. Skin samples were cultured in modified Dixon agar and identified by PCR-RFLP method. They found that 87% of healthy individuals and 62% of psoriasis patients had *Malassezia* culture positivity. They reported that the isolation rate was higher in the head and extremities in the patient group, and on the head and trunk in healthy individuals. In our study, *Malassezia* culture positivity was 55.9% in lesional skin of the psoriasis patient group, 48.5% in non-lesional skin samples and 32.5% in healthy volunteers. A statistically significant difference was found between patients with psoriasis and healthy volunteers. In accordance with the literature (5), the positivity of *Malassezia* was high in lesional scalp and arm in patients with psoriasis in our study. Similarly, positivity was found to be high in the scalp and body in the healthy control group. In our study, there was no statistically significant difference in *Malassezia* positivity when the skin samples with psoriatic lesions were compared with the non-lesional skin samples in the patient with psoriasis ($p > 0.05$).

It has been found that *M.restricta* and *M.globosa* species are common in healthy individuals in the most of the studies conducted with various methods such as culture, nested-PCR, real time PCR or PCR-RFLP to identify *Malassezia* species (3, 28-31). In some studies, it has been reported that the most frequently isolated species were *M.globosa*, but the secondary species *M.furfur* (5) and *M.sympodialis* in healthy volunteers (32). On the contrary, some researchers reported that the most isolated species was *M.sympodialis* (33,34). In our study, in accordance with the literature, *M.globosa* was isolated most frequently in healthy

individuals; followed by *M.restricta*, *M.sympodialis*, *Malassezia* spp, *M.furfur*, and *M.slooffiae* species. In addition, *M.restricta* on the scalp of healthy individuals and *M.globosa* on the body were the most frequently isolated species. The frequency of isolation of *Malassezia* species on the arm and leg was low. In two regions, *M.sympodialis* and *M.globosa* were the most frequently isolated species. However, mixed *Malassezia* colonization was higher in healthy individuals than in the patient group.

It has been reported in the literature that the most frequently isolated species in the skin samples of patients with psoriasis are *M.globosa* (5,22,28,31,33,35) and *M.restricta* (3,29,30,36,37). In some studies, it has been reported that *M.furfur* is frequently isolated (34,38). It was emphasized that the isolation of different *Malassezia* species compositions in the current studies in the literature may be related to the difference of the countries in which the studies are conducted (34,39).

A study reported that *M.globosa* and *M.furfur* were major species in the lesional skin of the patients with psoriasis (5). Tett et al. (35) emphasized the diversity of skin microbiome in psoriasis patients. They detected that *M.globosa* and *M.restricta* were dominant type of fungus. However, they also pointed out the availability of many yet-not-defined genomes named *Malassezia* spp. In our study, *M.globosa*, *M.furfur*, *Malassezia* spp. (yet-not-defined genomes), *M.restricta*, *M.sympodialis*, *M.slooffiae* were among the isolated *Malassezia* species. It was found that the species diversity isolated was quite high in accordance with the literature.

Prohic (28) and Gomez-Moyano et al. (31) reported that *M.globosa* was the most common species in the scalp of patients with psoriasis. Also, they reported that *M.restricta* was the most common species in the scalp of healthy persons. A study conducted using real-time PCR method by Paulino et al. (30) found that the most fre-

quently isolated species in both psoriasis patients and healthy individuals were *M.restricta* and *M.globosa*. It was determined that *M.sympodialis* was found at a higher rate in the back region of patients with psoriasis than healthy individuals. In another study, the presence of *M.restricta* in the back region of the patients with psoriasis and *M.sympodialis* from the elbow area was detected. In healthy individuals, it has been reported that *Malassezia spp.* and *M.slooffiae* are highly isolated (37). Zomorodian et al. (5) reported in their study that *M.globosa* was the dominant species and the second frequent species was *M.furfur* on all areas (head, trunk, and extremity) psoriatic skin. In our study, *M.globosa* and *M.furfur* were found to have highest frequency in lesional and non-lesional skin in the patient group. A significant difference was detected statistically on scalp and leg in terms of lesional area upon comparing the presence of *M.furfur* in lesional skin with the control group. This confirms the role of *M.furfur* in psoriasis etiopathogenesis (5,25,26).

In various studies in the literature (36), it was reported that there was no relationship between *Malassezia* species isolated from patients with psoriasis and PASI score. However, it was reported that there was no relationship between isolated species from patients with psoriasis and the age and gender of the patients. In our study, in accordance with the literature, no relation was found between the PASI score and the *Malassezia* species isolated from the patients. Similarly, there was no significant relationship between isolated species and age and gender.

The limitation of this study is that a small number of psoriasis patients was included. The strengths of our study include the evaluation of fungal distribution and frequency by taking samples from four different anatomical regions, statistical comparison of the data of the patient group with the data of healthy individuals, the cultivation of *Malassezia* species in modified Dixon agar, and genotyping using the PCR-RFLP method.

In conclusion, the presence and frequency of *Malassezia* species in psoriatic patients was found to be higher than healthy volunteers in our study. In addition, the distribution of species isolated from patients with psoriasis has been found to differ from healthy individuals. The fact that *Malassezia* species isolated from patients with psoriasis are different from healthy individuals and higher isolation rate supports the view that *Malassezia* species found commensally in the skin microbiome may be related to the etiopathogenesis of psoriasis. In this context, we believe that there is a need to develop prebiotic/ probiotic applications or new treatment strategies such as skin microbiome transfer to correct the current dysbiosis in *Psoriasis vulgaris*. For this purpose, large-scale studies are needed to develop microflora compatible with skin microbiome of healthy individuals.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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