Epidemiology of pityriasis versicolor in Adana, Turkey

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ABSTRACT
Pityriasis versicolor is a common superficial mycoses of the skin. It is now recognized that the causative organisms of this infection are different species of Malassezia. The aim of this study was to determine the distribution of Malassezia species in patients with pityriasis versicolor in Adana, Turkey. In total, 97 patients positive for Malassezia elements, namely, yeast cells and short hyphae in microscopic examination, were included in the study. All samples were inoculated in plates containing modified Dixon’s medium. However, only 44 of the patients (45.4%) showed Malassezia spp. in culture. Malassezia globosa (47.7%) was the most commonly isolated species followed by Malassezia furfur (36.4%) and Malassezia slooffiae (15.9%). Mixed Malassezia species were not isolated. In conclusion, M. globosa was found to be the predominant PV isolate in Adana, Turkey.

Key words: lipophilic yeasts, Malassezia furfur, Malassezia globosa, Malassezia slooffiae, pityriasis versicolor.

INTRODUCTION
Pityriasis versicolor (PV) is a superficial fungal infection of the skin caused by yeasts of genus Malassezia. The causative fungus is a normal inhabitant of the skin flora, seen especially in such seborrheic areas as the scalp, face, trunk and upper back, and it is in yeast form. However, as a result of endogenous (e.g. hyperhidrosis, use of oral contraceptives and systemic corticosteroid) and exogenous (e.g. humid and hot climates) factors, it mutates from yeast form to a mycelial form of organisms. As a result of such mutation, it causes an infection characterized by hypopigmented or hyperpigmented macules which are mildly squamous. The lesions, which can be scattered over the neck, shoulders and arms, cause cosmetic problems and occasionally some slight itching. This dermatosis can arise at any age, with most cases occurring during adolescence and young adulthood.

In 1996, a taxonomic revision divided the genus Malassezia into seven different species according to morphological, ultrastructural and physiological features. While Malassezia furfur, Malassezia globosa, Malassezia obtusa, Malassezia restricta, Malassezia slooffiae, and Malassezia sympodialis are lipophilic, Malassezia pachydermatis is not. In addition to these, five new Malassezia species (Malassezia dermatis, Malassezia equi, Malassezia japonica, Malassezia nana and Malassezia yamatoensis) have recently been reported; yet further biochemical and molecular characterization is required for their identification as distinct species.

The purpose of this study was to: (i) discuss the epidemiological characteristics of patients with PV in Adana, Turkey; (ii) determine the distribution of Malassezia species in such patients by using morphological, biochemical and physiological criteria; and (iii) find out whether there were any associations between the identified Malassezia species and recurrence, anatomical site, excess sweating, hypopigmented or hyperpigmented lesions.
METHODS

Study population
Between August 2004 and October 2005, 97 patients with PV who applied to the outpatients clinic of the Department of Dermatology at the Faculty of Medicine, University of Çukurova, were included in the study. Informed and written consent was provided by the patients with a clear description of the objectives and procedures of the study.

All of the participants filled a questionnaire stating their ages (patients were divided into four age groups: group I, ≤15 years; group II, 16–30 years; group III, 31–45 years; group IV, 46–60 years; and group V, >60 years), sex, clinical form of PV (hypo-pigmented or hyperpigmented), anatomical sites of lesions in sun-protected (trunk) or sun-exposed (face, neck or extremities), primary or recurrent episode of PV, duration of infection (<1 years; 1–5 years; 6–10 years; >10 years) and excess sweating.

Diagnosis
The diagnosis of PV was established by the characteristic “spaghetti-and-meatballs” appearance reflecting the presence of both dense clusters of budding yeast cells, and short hyphae were microscopically observed in 15% potassium hydroxide, and clinical picture when typical hypopigmented or hyperpigmented and scaling lesions were present. In addition, fungal culture was carried out using the composition of modified Dixon agar as follows: 1 L distilled H2O, 3.6% malt extract, 0.6% mycological peptone, 2% ox bile, 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 1.2% agar and 0.5% chloramphenicol.5–7

Clinical sampling
Pityriasis versicolor lesions were sampled with a scalpel blade to scrape the skin surface into sterile paper packets after cleaning with 70% alcohol.5 Due care was given in order to not collect the samples from healthy looking areas.6 Then, they were transported at room temperature to the Mycology Laboratory, Department of Microbiology, at the same institution.

Mycological examination
All samples were inoculated in plates containing modified Dixon’s medium. The media were used within 1 week of preparation. The plates were incubated in air at 32°C in a moist atmosphere. Cultures were examined daily during a period of 14 days. Malassezia yeasts were identified as pale ochre-brown waxy or crumbly colonies with a smooth surface.3,8 Presumptive Malassezia colonies were subcultured on Sabouraud’s glucose agar (SGA; Acumedia, Baltimore, MD, USA) to confirm their lipophilic character.3

Identification of Malassezia species
Malassezia yeasts were identified on the basis of microscopic observation of cells and physiological properties such as presence of catalase and the ability to utilize Tween compounds.8 The following reference strains were obtained from Centraalbureau voor Schimmelcultures (CBS; Utrecht, the Netherlands): M. furfur CBS 1878, M. globosa CBS 7966, M. obtusa CBS 7968, M. restricta CBS 7877, M. slooffiae CBS 7861 and M. sympodialis CBS 7822.

Statistical analysis
Variables were compared using the Friedman test, Pearson’s χ2-test, Fisher’s exact test (distribution using the Statistical Packages of Social Sciences ver. 9.0 for Windows), in which P ≤ 0.05 was considered statistically significant.

RESULTS
Of the 97 patients with PV, 65 were (67%) men and 32 (33%) women, all of whose ages ranged 13–60 years (mean age ± standard deviation, 42.2 ± 23.6). No statistically significant differences were found between sex and Malassezia species (P > 0.05). Of the 97 clinical samples, only 44 (45.4%) yielded Malassezia in culture. M. globosa (n = 21; 47.7%) was the most commonly isolated species followed by M. furfur (n = 16; 36.4%) and M. slooffiae (n = 7; 15.9%). Mixed Malassezia species were not isolated.

The age groups mainly affected by PV were those whose ages ranged 16–30 years: 50 patients (51.5%) from group II, 28 patients (28.9%) from group III, 11 patients (11.3%) from group I, eight patients (8.3%) from group IV, and no patients from group V. We found no statistically significant differences in Malassezia species isolated in these five groups (P > 0.05; Fig. 1).
As for duration of infection (<1 year \( n = 72; 74.2\% \); 1–5 years \( n = 17; 17.5\% \); 6–10 years \( n = 6; 6.2\% \); >10 years \( n = 2; 2.1\% \)), the patients stated that lesions had been existing for some time ranging between 1 week and 240 months. There was a statistically significant difference in the distribution of isolated species according to duration of the lesions \( (P < 0.05; \text{Fig. 2}) \). Out of 97 lesions, 61 (62.9%) were located on sun-protected and 36 (37.1%) on sun-exposed regions. A statistically significant difference was observed in the distribution of isolated species in the sun-protected and sun-exposed regions \( (P < 0.05; \text{Fig. 3}) \).

The lesions were hypopigmented in 49 (50.5%) patients, and \textit{M. globosa} was the predominant species. Hyperpigmented lesions detected in 46 (47.4%) patients with \textit{M. furfur} was the predominant species, and both types of lesions were observed in two (2.1%) patients. There was a statistically significant difference in the distribution of isolated species according to clinical picture \( (P < 0.05; \text{Fig. 4}) \). Clinical findings of PV patients such as hyperhidrosis \( (n = 30; 30.9\%) \), hypo- and hyperpigmentation of lesions, sun-protected or sun-exposed region, and recurrence \( (n = 32; 32.9\%) \) are illustrated in Table 1. Additionally, recurrence and hyperhidrosis were found to be statistically significant \( (P < 0.05) \) as well.

**DISCUSSION**

A great number of epidemiological studies using culture or molecular-based (polymerase chain reaction restriction fragment length polymorphism [PCR-RFLP] or nested PCR) have been performed in different geographical regions in order to investigate \textit{Malassezia} species in patients with PV.\(^9\)\(^{-20}\) In some
M. Karakaş et al.

culture-based studies carried out in Spain,6,11,20 Japan,12 Bosnia and Herzegovina,18 Tunisia,10 Iran13 and India,14 using morphological and physiological characteristics – particularly in tropical areas – M. globosa either alone or associated with other Malassezia spp. (e.g. M. sympodialis, M. slooffiae) was the most commonly isolated Malassezia species (Table 2). This ratio that indicated the predominance of M. globosa was between 53.3% and 63.3%.6,10–14,18,20 In contrast to these studies, a study carried out by Gupta et al.,16 M. sympodialis was the predominant PV isolate (59.5%), followed by M. globosa in 25.2%, M. furfur in 10.8%, M. slooffiae in 2.7%, M. restricta in 0.9% and M. obtusa in 0.9% in Canada.

Additionally, in molecular-based non-culture methods utilizing either PCR-RFLP15 or nested PCR,9 M. globosa were predominantly detected from the lesions of PV. In a recent study, Gaitanis et al.15 noted that M. globosa was isolated from 90% of PV cases, alone in 77% and in combination with M. sympodialis, M. furfur or M. slooffiae in 13%, while the other species were isolated only in 10% of the patients. These authors also reported that PCR single-strand conformation polymorphism (SSCP) differentiated five subgroups of M. globosa with one being associated with extensive clinical disease whereas all M. sympodialis isolates displayed a homogenous ITS 1 PCR-SSCP profile. However, in a similar molecular-based study by Makimura et al.21 in Japan, while M. sympodialis and M. furfur were recovered, M. globosa failed to be detected.

In the present study, the most frequent species observed were M. globosa (47.7%) followed by M. sympodialis (15.9%). Nevertheless, M. sympodialis was observed to not isolate from PV lesions.

Our findings for M. globosa were most comparable to that of other similar studies.6,11–14,18,20 However, M. furfur and M. slooffiae seemed to occur more frequently than in other studies carried out in Canada,5 Spain,6,11,20 Japan,12 Iran,13 Tunisia10 and Bosnia,18 in which a frequency between 0% and 25.3%, and 0% and 5.3%, respectively, is reported. In this study, significant differences were found in species isolated according to the clinical type or anatomical sites of lesions (sun-protected or sun-exposed). On the contrary, in Bosnia, Prohic et al.18 and in Spain, Erchiga et al.11 reported that no significant differences were found in the species isolated according to the clinical form or anatomical sites of lesions.

In this study, the recovery rate of Malassezia spp. from the PV lesions was 45.4%. In similar studies, this ratio tends to vary between 43.8% and 91.3%.5,8,8–19 To the best of our knowledge, this

### Table 1. Characteristics of patients with pityriasis versicolor

<table>
<thead>
<tr>
<th>Sampling region</th>
<th>No</th>
<th>%</th>
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<tbody>
<tr>
<td>Sun-exposed</td>
<td>36</td>
<td>37.1</td>
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<tr>
<td>Sun protected</td>
<td>61</td>
<td>62.9</td>
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<table>
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<tr>
<th>Clinical presentation</th>
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<th>%</th>
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<tr>
<td>Hypopigmented</td>
<td>49</td>
<td>50.5</td>
</tr>
<tr>
<td>Hyperpigmented</td>
<td>46</td>
<td>47.4</td>
</tr>
<tr>
<td>Hypo- and hyperpigmented</td>
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<td>2.1</td>
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<table>
<thead>
<tr>
<th>Recurrence</th>
<th>No</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>No</td>
<td>65</td>
<td>67</td>
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<table>
<thead>
<tr>
<th>Hyperhidrosis</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>30</td>
<td>30.9</td>
</tr>
<tr>
<td>No</td>
<td>67</td>
<td>69.1</td>
</tr>
</tbody>
</table>

### Table 2. Distribution of Malassezia species in pityriasis versicolor from different geographical regions

<table>
<thead>
<tr>
<th>Mycological data</th>
<th>Canada (5)</th>
<th>Spain (6)</th>
<th>Spain (11)</th>
<th>Spain (20)</th>
<th>Japan (12)</th>
<th>India (14)</th>
<th>Iran (13)</th>
<th>Tunisia (10)</th>
<th>Bosnia and Herzegovina (18)</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients with</td>
<td>84</td>
<td>79</td>
<td>100</td>
<td>96</td>
<td>19</td>
<td>250</td>
<td>94</td>
<td>87</td>
<td>90</td>
<td>44</td>
</tr>
<tr>
<td>positive cultures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. globosa (%)</td>
<td>25.2</td>
<td>58.2</td>
<td>55</td>
<td>60.4</td>
<td>63.1</td>
<td>54.4</td>
<td>53.3</td>
<td>54</td>
<td>63.3</td>
<td>47.7</td>
</tr>
<tr>
<td>M. globosa mixed (%)</td>
<td>–</td>
<td>31.7</td>
<td>32</td>
<td>36.5</td>
<td>9.2</td>
<td>20.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M. furfur (%)</td>
<td>10.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.3</td>
<td>29.6</td>
<td>25.3</td>
<td>11.5</td>
<td>10</td>
<td>36.4</td>
</tr>
<tr>
<td>M. slooffiae (%)</td>
<td>2.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.3</td>
<td>–</td>
<td>4</td>
<td>1.1</td>
<td>4.4</td>
<td>15.9</td>
</tr>
<tr>
<td>M. sympodialis (%)</td>
<td>59.5</td>
<td>10.1</td>
<td>–</td>
<td>3.1</td>
<td>10.5</td>
<td>–</td>
<td>9.3</td>
<td>4.6</td>
<td>14.4</td>
<td>–</td>
</tr>
<tr>
<td>M. restricta (%)</td>
<td>0.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.4</td>
<td>–</td>
<td>–</td>
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</table>
study seems to be one of the first of its kind to investigate the distribution of the etiological agents of PV in Turkey.

It was stated that detection of different ratios of Malassezia species in similar studies carried out in patients with PV in different parts of the world could be due to different culture media (Leeming–Notman and modified Dixon agar),

climatic differences (tropical region or not),

different sampling methods (swabbing vs scraping),

and characteristics of patients.

In our study, PV was common in group II patients, aged 16–30 years, which indicates similarities with the findings of related studies. In the present study, we found no statistically significant difference between recurrence and Malassezia species, whereas it was reported by Gaitanis et al.

that M. globosa and M. sympodialis have statistically significant levels in recurrent PV patients. Thus, we suggest that further research be carried out in order to shed more light on this issue. In addition, we found statistically significant differences between recurrence and hyperhidrosis, which confirms that hyperhidrosis is prone to PV.

The pathogenesis of hypo- or hyperpigmented lesions of PV is still disputable. It is suggested that hyperpigmentation is a result of both inflammation and increased melanin production. In hypopigmented lesions, the decrease in melanosomes in the stratum spinosum and the blocking of ultraviolet light (tropical region or not),

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However, rare occurrences of the disease in infants not exposed to the sun seems to be a contradiction. In the present study, the predominant Malassezia species in patients with hyperpigmented lesions was M. furfur. The theory of blocking of ultraviolet light by M. furfur, therefore, is rendered invalid. On the other hand, in our patients, lesions were more commonly observed on sun-protected areas, and this observation is in line with published work.

In conclusion, M. globosa was found in hypopigmented lesions and sun-protected areas (P < 0.05). However, M. furfur was found in hyperpigmented lesions, sun-exposed areas and PV of long duration (6–10 years) (P < 0.05). Recurrence of the PV lesions was not found related to Malassezia spp. (P > 0.05), however, it was with hyperhidrosis (P < 0.05). Finally, in the Adana region M. globosa, M. furfur and M. slooffiae were closely linked to PV.

REFERENCES


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