Identification of *Malassezia* species isolated from Iranian seborrhoeic dermatitis patients

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**Abstract.** – Background and Objectives: In recent years, the genus *Malassezia* has come to be considered important in the etiology of seborrhoeic dermatitis (SED). The aim of present study was identification of *Malassezia* species on the lesions of Iranian SED patients.

**Methods:** 100 patients with SED were enrolled in the study. The patients were evaluated both clinically for the severity of SED and microscopically for the presence of the yeast *Malassezia*. Diagnosis of *Malassezia* was made after the yeast *Malassezia* was microscopically observed on skin scales stained with methylene blue. All samples were also cultivated on Leeming and Notman and Sabouraud’s dextrose agar culture media. The agar plates were incubated at 32°C for 2 weeks and evaluated for the existence of growth every day for one week. Identification of isolated yeast was based on morphological and physiological characteristics.

**Results:** From 100 patients with SED, 60% were female. The age range was 12-65 years with median 27.3 years. The highest prevalence of SED was seen in 20-29 years age group. 59% and 41% of patients had local and generalized lesions, respectively. 58% of patients showed lesion on scalp. Microscopic examination of skin scales was positive in 100% of SED lesions. 96% of patients showed more than 1-3 yeasts in each microscopic field whereas only 4% patients showed 1-3 yeasts in whole slide. Totally, 77% of the specimens yielded *Malassezia* in culture. *Malassezia globosa* was the most commonly isolated *Malassezia* species (55.8%). *Malassezia globosa* had also most frequencies on scalp and face lesions. *Malassezia furfur* had most frequency on trunk lesions.

**Conclusion:** The results of our study showed high recovery rate of *Malassezia* species on lesions of patients with SED. So it might be playing a causative role in the etiology of this disease.

**Key Words:** Seborrhoeic dermatitis, *Malassezia* species, Tween assimilation.

**Introduction**

Yeasts of the genus *Malassezia*, formerly known as *Pityrosporum*, are ubiquitous skin residents of humans and other warm-blooded animals and also associated with several dermatological disorders. In recent years, the genus *Malassezia* has come to be considered important in the etiology of seborrhoeic dermatitis (SED)\(^1\). The improvement of SED (and the related condition, dandruff) by treatments directed against *Malassezia* supports the role of the yeasts in these conditions, although other factors such as an impaired barrier function are also thought to be important\(^1\). So the epidemiology and the ecology of *Malassezia* species in SED has been considered by different investigators\(^1\)-\(^5\). Epidemiological data suggest geographical variations in the rate of the isolated *Malassezia* species from SED\(^1\)-\(^5\).

The taxonomy of *Malassezia* has undergone extensive revisions in the last 10 years and is still in a state of flux. Guillot and Gueho described and named 7 species of *Malassezia* (M.): *M. furfur*, *M. sympodialis*, *M. obtusa*, *M. globosa*, *M. restricta*, *M. slooffiae* and *M. pachydermatis*. Subsequently, other species have been described, including *M. dermatis*\(^10\), *M. japonica*\(^11\), *M.
Species Identification

We identified Malassezia species according to Guillot et al.\textsuperscript{17}. Isolates from the primary cultures were used for identification. M. pachydermatis is able to grow on Sabouraud’s agar. Tween assimilation and catalase test were applied for identification of other Malassezia species. Briefly, Malassezia yeast suspensions were prepared in sterile distilled water, which were adjusted to McFarland No. 2 turbidity, mixed with Mycobiotic agar with cyclohexamide and chloramphenicol, and poured into the plates. Four holes were made in the agar by means of a 3-mm diameter punch and filled with 30 μl each of tween 20, 40, 60 and 80, respectively. The agar plates, incubated at 32°C for 1 week, were examined each day for the existence of any growth around the wells that contained tween compounds.

Catalase Test

One drop of hydrogen peroxide solution was applied onto a yeast colony, which was on a glass slide. Production of gas bubbles was considered a positive catalase reaction.

Statistical Analyses

Chi-square test was performed using SPSS software (version 13.0) and differences were considered significant at $p<0.05$.

Results

60% of the patients with SED were female. The age range was 12-65 yrs with median and SDF yrs of 27.3 and 10.2, respectively. The highest prevalence of SED was seen in patients with 20-29 yrs old. 59% and 41% of patients had local and generalized lesions, respectively. 58% of patients showed lesion on scalp (Table I).

Materials and Methods

Patients

100 patients with SED from the Department of Dermatology, of Boali Hospitals (Sari City) and from the Department of Dermatology, Hospital Razi, Tehran, were enrolled in the study. The patients filled out the consent form to participate in research which was approved by the Ethical Committee of Mazandaran University of Medical Sciences. The severity of the SED lesions was evaluated using a semiquantitative scale of 0 to 3 for each of the symptoms (erythema, scaling, itching and seborrhoea), where a value of 0 corresponded to the absence of symptoms, 1 to mild symptoms, 2 to moderate symptoms and 3 to severe symptoms. The sum of these values is regarded as score of SED.

Sampling and Microbiological Evaluation

Sampling was performed from the lesions after diagnosis of SED. Skin scales were scraped off with a sterile blade and transferred to the laboratory. Diagnosis of Malassezia was made after budding yeast cells that were microscopically observed on skin scales stained with methylene blue. To counting of yeast cells, each slide was examined under high power field (hpf) of microscope and it was recorded as number of observed yeast cells per hpf. All samples were also cultivated on Leeming and Notman medium and Sabouraud’s dextrose agar culture media. The agar plates were incubated at 32 °C for 2 weeks and evaluated for the existence of growth every day for one week. Identification of isolated yeast was based on morphologic and physiologic tests, namely tween assimilation profiles and catalase reaction.

Table I. Distribution of SED patients on the basis of age and gender.

<table>
<thead>
<tr>
<th>Total n (%)</th>
<th>Male n (%)</th>
<th>Female n (%)</th>
<th>Age Yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 (24)</td>
<td>11 (28.9)</td>
<td>13 (21)</td>
<td>10-19</td>
</tr>
<tr>
<td>44 (44)</td>
<td>14 (36.8)</td>
<td>30 (48.4)</td>
<td>20-29</td>
</tr>
<tr>
<td>16 (16)</td>
<td>6 (15.8)</td>
<td>10 (16.1)</td>
<td>30-39</td>
</tr>
<tr>
<td>13 (13)</td>
<td>7 (18.4)</td>
<td>6 (9.7)</td>
<td>40-49</td>
</tr>
<tr>
<td>3 (3)</td>
<td>0 (0)</td>
<td>3 (4.8)</td>
<td>50≤</td>
</tr>
<tr>
<td>100 (100)</td>
<td>38 (100)</td>
<td>62 (100)</td>
<td>Total</td>
</tr>
</tbody>
</table>
Direct microscopic examination (DME) of specimens was positive in 100% of SED lesions. Figure 1 shows the observed yeast cells number in DME on lesions of patients with SED. 96% (96/100) of patients showed more than 1-3 yeast cells per hpf whereas only 4% (4/100) patients showed 1-3 yeast cells in whole slide.

Totally, 77% of the specimens yielded *Malassezia* in culture. The most commonly isolated *Malassezia* species was *M. globosa* (55.8%), followed by *M. furfur* (32.5%), *M. restricta* (9.1%), *M. sympodialis* (1.3%) and *M. japonica* (1.3%). *M. globosa* was most common isolated species from face (17/27, 63%) and scalp (25/45, 55.5%). *M. furfur* had most frequency in trunk (4/5, 80%) (Table II). There was no statistically significant difference between cultured *Malassezia* species and body sites (p=0.613).

Table III shows the culture results from different areas of the body in SED patients. There was not significant difference between culture result and body sites (p=0.935).

Table IV shows the frequency of observed yeasts number in DME on lesions of patients with SED based on age groups. 48% and 49% of patients in 20-29 years age group showed 4-7 and >7 yeast cells per hpf, respectively. There was significant difference between observed yeasts number in DME on lesions of patients with SED and age groups (p=0.003).

Out of 100 patients with SED, 9, 74 and 17 patients showed mild, moderate and severe SED, respectively (Table V). With respect to the observed yeasts number in DME on lesions, 22.2%, 56.7% and 41% of patients with mild, moderate and severe SED had a density of >7 yeast cells per hpf, respectively (Table V). There was not significant difference between the observed yeasts number in DME and severity of SED (p=0.176).

### Discussion

In our study the most frequency of SED was observed in women and the age group of 20-29 years. The most common species isolated from patients with SED was *M. globosa* (55.8%). *M. furfur* was the second most common species (32.5%). *M. restricta*, *M. sympodialis* and *M. japonica* were isolated in lower frequencies (9.1%, 1.3% and 1.3%, respectively). There was no significant difference between the frequency of isolated *Malassezia* species and age groups (p=0.613). However, the frequency of isolated *Malassezia* species was significantly different between different body sites (p=0.003). The observed yeasts number in DME on lesions of patients with SED was significantly different between mild, moderate and severe SED (p=0.003).
yrs old. Although it has suggested that SED can appear at any age, the highest prevalence is observed in individuals aged 30 to 60 years and it is more common in males than in females, in contrast to the results of our study.

In DME, 100% of samples yielded a positive result which is the same reported by Crespo et al. in pityriasis versicolor. Moreover, 96% of our patients showed more than 1-3 yeast cells per hpf. This is a further evidence that our patients with SED harbour a higher number of *Malassezia* yeasts. One reason may be the increased amount of lipids in the skin of seborrhoeic patients. Some Authors have also shown a greater number of *Malassezia* cells in lesional skin of patients with SED or a greater detectable incidence in affected subjects. However, others have reported a decrease in the density of recovered yeasts from lesional skin. It has also been suggested that seborrhoeic dermatitis is not caused by an overgrowth of *Malassezia* cells, but by an abnormal host response to the yeasts on the skin.

In the present study, 77% of the specimens yielded *Malassezia* in culture which is nearly concordant to other studies. The most commonly isolated species in SED patients were *M. sympodialis*, *M. globosa*, *M. restricta* and *M. furfur*. Conversely, Swedish SED patients’ skin lesions are more frequently colonized with *M. obtusa*, a species that was only sporadically identified in previous reports. Regarding to the most commonly isolated species, there were differences between patients with various skin diseases and also the reported results from different countries. This difference may be attributable to the sampling and culture techniques as well as to geographical differences and it could be elucidated by worldwide epidemiological studies.

In the present research, there was not a significant difference between the positive culture results and the various body sites. However, *M. globosa* was the most common isolated species on scalp and face lesions and *M. furfur* had most frequency on trunk lesions. This result in part agrees with Gupta et al. that used the contact plates method to isolate and identify *Malassezia* species from the different body sites of individuals with or without dermatoses including SED patients. It has also been suggested that different *Malassezia* species tend to be found on different body sites in both normal and diseased skin.

We have isolated single separated colony from each sample, as suggested by Tarazooie et al. However, in many studies, more than one species have been recovered from each sample. It should be underlined that providing a pure culture and the separation of a species from a mixed sample is too difficult. This might be due to this point that fast growing species usually cover other species in the culture. Besides, because of hydrophobic characteristic of *Malassezia* yeast, preparing ho-

<table>
<thead>
<tr>
<th>Body site culture result</th>
<th>Scalp n (%)</th>
<th>Face n (%)</th>
<th>Trunk n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>45 (77.6)</td>
<td>27 (77.1)</td>
<td>5 (71.4)</td>
<td>77 (77)</td>
</tr>
<tr>
<td>Negative</td>
<td>13 (22.4)</td>
<td>8 (22.9)</td>
<td>2 (28.6)</td>
<td>23 (23)</td>
</tr>
<tr>
<td>Total</td>
<td>58 (100)</td>
<td>35 (100)</td>
<td>7 (100)</td>
<td>100 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age groups</th>
<th>10-19 n (%)</th>
<th>20-29 n (%)</th>
<th>30-39 n (%)</th>
<th>40-49 n (%)</th>
<th>≥50 n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 yeasts in whole slide</td>
<td>1 (25)</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (50)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>1-3 yeast cells per hpf</td>
<td>4 (20)</td>
<td>6 (30)</td>
<td>6 (30)</td>
<td>4 (20)</td>
<td>0 (0)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>4-7 yeast cells per hpf</td>
<td>5 (20)</td>
<td>12 (48)</td>
<td>4 (16)</td>
<td>3 (12)</td>
<td>1 (4)</td>
<td>25 (100)</td>
</tr>
<tr>
<td>&gt;7 yeast cells per hpf</td>
<td>14 (27.4)</td>
<td>25 (49)</td>
<td>6 (11.8)</td>
<td>6 (11.8)</td>
<td>0 (0)</td>
<td>51 (100)</td>
</tr>
</tbody>
</table>
mogenous suspension is very difficult to separate them by culture. Moreover, some \textit{Malassezia} species may lose their viability after several subcultures.

Our data showed the density of \textit{Malassezia} yeast cells on the lesions of patients in the age group of 20-29 yrs is significantly higher in comparison with other age groups. However, the relationship between colonization patterns and age group is less clear. Some studies indicate that there is a difference in the colonization pattern of \textit{Malassezia} species in different age groups. Bergbrant et al. also suggested that the colony counts generally are higher in adults than in children and it decreases again in elderly individuals. This condition may due to high sebum production in above mentioned aged groups.

In the present study there was not a significant difference between the observed yeasts number in DME and severity of SED. The \textit{Malassezia} species density in DME was higher in patients with moderate and severe form in comparison with patients with mild SED. The existing literature generally disagrees whether or not the SED lesions are associated with an excess numbers of \textit{Malassezia} cells, perhaps due to a lack of standardization in the techniques used. Zaidi et al. using direct microscopy, found that \textit{Malassezia} increases in proportion with the severity of SED. These findings are also similar to those of other groups, who used different sampling and culture methods to enumerate yeast cells present in SED or dandruff.

In conclusion, we found an high recovery rate of \textit{Malassezia} species on the lesions of patients with SED. \textit{M. globosa} was the most commonly isolated \textit{Malassezia} species on the SED lesions. In addition, the density of \textit{Malassezia} species on lesions was higher in patients with moderate and severe SED in comparison with patients with mild SED. Therefore, we think that \textit{Malassezia} might be playing a causative role in the etiology of this disease.

### References


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Acknowledgements

This work was supported by grants from the Pharmaceutical Sciences Research Center of Mazandaran University of Medical Sciences, Sari, Iran. We thank the participating patients for their kind cooperation, which has been essential for the completion of the study.