Identification of *Malassezia* species from immunocompetent and immunocompromised patients with seborrheic dermatitis

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**Abstract. — Background and Objectives:** Differences in prevalence, clinical and histological manifestations between seborrheic dermatitis (SD) in immunocompetent and immunocompromised patients suggest that these two populations might also differ in a spectrum of isolated *Malassezia* species. The purpose of our study was to analyse the prevalence of *Malassezia* species in immunocompromised and non-immunocompromised patients with SD and to examine if the range of isolated yeasts varies between these two study groups.

**Patients and Methods:** Specimens were taken from 50 patients with SD: 30 without any underlying disease and 20 with confirmed immunosuppression. The samples were obtained by scraping the skin surface of the scalp and trunk lesions 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.

**Results:** In both groups, the most commonly isolated species from the scalp lesions were *Malassezia restricta* and *Malassezia globosa*, the later being the most common species isolated from lesional trunk skin. No significant differences were found between immunocompromised and immunocompetent patients from both sampled sites.

**Conclusions:** There is no difference in the distribution of *Malassezia* species isolated from SD lesions between immunocompetent and immunocompromised patients. However, the much higher percentage of positive cultures in immunocompromised patients confirms that impaired cellular immunity may facilitate fungal survival on the skin.

**Key Words:** *Malassezia*, Identification, Immunocompetent, Immunocompromised.

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**Introduction**

The genus *Malassezia* (former *Pityrosporum*) comprises lipophilic yeasts found in normal cutaneous microflora of humans and other warm-blooded animals. However, these yeasts have been identified as a causative organism in pityriasis versicolor and have also been involved in the pathogenesis of a variety of dermatological disorders, such as seborrheic dermatitis (SD).

In 1996, the genus *Malassezia* (M) was revised to include seven species: *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. slooffiae*, *M. globosa*, *M. obtusa* and *M. restricta*.

*M. pachydermatis* is the only species confirmed to be associated with animals, while remaining species are found primarily in humans. Since 2002, six new species have been isolated from human (*M. dermatis*, *M. japonica* and *M. yamotensis*) and animal skin (*M. nana*, *M. caprae* and *M. equine*).

Among many endogenous and exogenous factors that contribute to the development of SD, a prominent role of *Malassezia* is supported by numerous treatment studies, demonstrating parallel decreases in the number of yeasts and the severity of the disease.

The incidence of SD is much higher in immunosuppressed patients with rates up to 83% as against 1-3% in general population, suggesting that immunological alterations may play a role in the pathogenesis of the disease. Some Authors reported that SD in immunosuppressive patients, especially in HIV positive and AIDS patients, should be regarded as a distinctive entity. This hypothesis is supported by the fact that lesions become more prevalent and severe with advances of immunosuppression. Addi-
tionally, there are also histological and molecular differences between lesions in the two populations\textsuperscript{14,16}.
Considering these facts, the present study aimed to analyse the prevalence of \textit{Malassezia} species in the lesional skin of immunocompromised and non-immunocompromised patients with SD and to examine if the range of isolated yeasts varies between these two study groups.

**Patients and Methods**

**Patients**
Fifty adult patients with SD with active disease and with scalp and trunk involvement were enrolled into the study. They included 30 patients (9 females and 21 males, mean age 43.0 years; range, 21-56) otherwise healthy individuals without any known underlying disease and 20 patients (6 females and 14 males, mean age 52.0 years; range, 30-69) on immunosuppressive therapy for a variety of disorders. The immunosuppression in later patients group was confirmed by low CD4 counts. Although not serologically tested, none of the participants was known to be HIV-positive or from a high risk group. None had taken topical or oral antifungal agents in the previous two months. All the subjects gave their written informed consent with the requirements of the Institutional Ethics Committee.

**Samples**
All samples consisted of skin scales and scrapings taken from the lesions from the scalp and from upper part of the trunk. Collected samples were inoculated into Sabouraud dextrose agar and into modified Dixon agar consisting of 3.6% malt extract, 0.6% mycological peptone, 2.0% desiccated ox bile (Sigma Chemical Co. Ltd, Dorset, 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 inoculated at 32°C for seven days.

**Identification of Malassezia Yeasts**
\textit{Malassezia} species were identified according to their macroscopic and microscopic features and physiological characteristics.
The macroscopic features of the predominant colonies included their shape, size, color, consistency, and the characteristics of medium around them. Microscopic features of the yeast cells in culture 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 determined by using a drop of hydrogen peroxide (30%) onto 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\textsuperscript{17} and later modified by Gupta et al\textsuperscript{18}. Yeast suspension, obtained by inoculating 5 mL of sterile water with a loopful of actively growing yeasts, was inoculated on Sabouraud glucose agar. The inoculum was then spread evenly. Each plate was divided into four sections and 5 mL of Tween 20, 40, 60 and 80 were added into a hole made in center of each section and incubated for a week at 32°C. Utilization of Tweens was assessed by the degree of growth and/or reaction of the lipophilic yeasts around individual holes.

**Measure of Immunity**
Immunity was measured by lymphocyte CD4 counts, expressed as a percentage of the total number of lymphocytes in the peripheral blood. Two colour direct immunofluorescence flow cytometry (FCM) was performed using a Simultest\textsuperscript{TM} IMK-Lymphocyte kit (Becton Dickinson, Franklin Lakers, NJ, USA).

**Statistics**
Statistical analysis was carried out with software package Minitab 13.0. Chi-squared test with Yates’ correction for a small sample size was performed for evaluation of the differences in proportions. Significance level was set at $p < 0.05$.

**Results**
Seven \textit{Malassezia} species (\textit{M. furfur}, \textit{M. pachydermatis}, \textit{M. sympodialis}, \textit{M. slooffiae}, \textit{M. globosa}, \textit{M. obtusa} and \textit{M. restricta}) were identified from both study groups. Mixed cultures were not observed.
The most frequently isolated species from scalp lesions of immunocompetent patients were \textit{M. restricta} (26.7%), \textit{M. globosa} (20.0%) and \textit{M. sympodialis} (13.3%). Both \textit{M. slooffiae} and \textit{M. furfur} were cultured in 10.0%. \textit{M. pachydermatis}
was isolated in one case (3.3%). The percentage of negative cultures was 16.7%. The results of culture obtained from immunocompromised subjects were positive for Malassezia yeasts in 95% cases. M. restricta was also the dominant species found in 30.0% patients and the prevalence of other species was 25.0% for M. globosa, 15.0% for M. sympodialis and M. slooffiae each and 10.0% for M. furfur. Two species, M. obtusa and M. pachydermatis were not isolated.

There was no significant difference in the distribution of the species cultured from scalp lesions between two groups (p<0.05).

Malassezia yeasts were found in 73.3% samples taken from lesional trunk skin of non-immunocompromised patients. Only three species were identified: M. globosa (33.3%), M. furfur (23.3%) and M. sympodialis (16.7%). All cultures from the same body site in immunosuppressed patients were positive. Isolation pattern was: M. globosa 45.0%, M. furfur and M. sympodialis 25.0% each and M. obtusa 5.0%. M. slooffiae and M. pachydermatis were not isolated. The results showed no significant difference in the distribution of the species isolated from trunk lesions between immunocompetent and immunosuppressed patients (p<0.05).

Table I shows the distribution of Malassezia species isolated from the scalp and trunk lesions from both study groups.

Discussion

In recent years, the genus Malassezia has come to be considered important in the etiology of various skin and systemic diseases. Both immunocompetent and immunosuppressed patients may be affected by this type of infection. In immunologically competent hosts, Malassezia species are implicated in the pathogenesis of variety of skin infections such as pityriasis versicolor, Malassezia folliculitis, SD, and, rarely, in a range of other dermatological disorders. In contrast, in immunocompromised patients, including patients with AIDS, immune-haematological, oncological, and solid organ and bone marrow transplant recipients, these yeasts have been associated with catheter-related fungemia, sepsis and a variety of deeply invasive infections.

SD is perhaps the most common disease associated with Malassezia species. It is believed that its increased incidence in immunosuppressed patients may be due to immunological alterations which lead to more severe skin involvement than in immunocompetent hosts. This hypothesis is further supported by the observation that degree and duration of exposure to immunosuppression is important in the aetiology of lesions in immunosuppressive patients.

Some studies have been performed to determine whether the amount and/or species of Malassezia found on skin of SD patients is different from the normal controls. The obtained results are conflicting although a correlation between yeast density and severity of SD has been reported. It has been suggested that an overgrowth of Malassezia organisms is only important for the individuals who are immunologically predisposed to develop SD. The possibility of an abnormal host response to the yeasts could be another explanation for the development of clinical condition. However, Parry

<table>
<thead>
<tr>
<th>Malassezia species</th>
<th>Scalp IC n (%)</th>
<th>Scalp IS n (%)</th>
<th>Trunk IC n (%)</th>
<th>Trunk IS n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. restricta</td>
<td>8 (26.7)</td>
<td>6 (30.0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. globosa</td>
<td>6 (20.0)</td>
<td>5 (25.0)</td>
<td>10 (33.3)</td>
<td>9 (45.0)</td>
</tr>
<tr>
<td>M. sympodialis</td>
<td>4 (13.3)</td>
<td>3 (15.0)</td>
<td>5 (16.7)</td>
<td>5 (25.0)</td>
</tr>
<tr>
<td>M. obtusa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>M. slooffiae</td>
<td>3 (10.0)</td>
<td>3 (15.0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. furfur</td>
<td>3 (10.0)</td>
<td>2 (10.0)</td>
<td>7 (23.3)</td>
<td>5 (25.0)</td>
</tr>
<tr>
<td>M. pachydermatis</td>
<td>1 (3.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>5 (16.7)</td>
<td>1 (5.0)</td>
<td>8 (26.7)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>20</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

IC = immunocompetent; IS = immunosuppressed.
and Sharpe suggest that the lesions of SD are not caused by an altered immune response to *Malassezia* yeasts and propose the production of toxic metabolites, lipases, and reactive oxygen species by *Malassezia* species\(^2\). Briefly, it is most likely that *Malassezia* yeasts have an indirect effect, mediated via immunological mechanisms on the development of SD.

With regard to the isolated species, some Authors have stated that *M. globosa* predominate\(^18,24\) whilst others have found *M. restricta*\(^28\) or *M. sympodialis*\(^31\) to be the most common species in lesions of SD. Similar to other investigations, we identified *M. restricta* as a predominant species from scalps in SD patients, while *M. globosa* was the most frequently isolated species from lesional skin of the trunk. *M. restricta*, isolated regularly from the scalp and face of patients with seborrhoeic dermatitis and normal individuals, was not recovered from any samples taken from the trunk indicating that this species is strongly restricted on head region. There is a suggestion that measurement variability based on different sampling techniques and inadequate determination of the relative proportion of species on the skin may account for the discrepancies observed among different studies\(^1\).

Considering the role of *Malassezia* species in immunosuppressive patients, some Authors report that there was no difference between HIV-related and non-HIV related SD\(^32\) while others strongly suggest an association for lipophilic yeasts in HIV-related SD, further strengthened by the marked clinical response to antifungals\(^33\). A quantitative correlation between the number of yeast cells adherent to keratinocytes and the clinical severity of SD in immunosuppressed patients seems to lend credence to a causative role for *Malassezia* in SD. Perhaps, other modifications such as sebum and lipid composition on the skin during the HIV infection permit the proliferation of certain strains of *Malassezia* without clinical symptoms. On the contrary, some researches report that this population group demonstrate a decrease in the amount of yeasts that can be cultured from their lesions\(^34\).

No studies to date have specifically investigated the distribution of different *Malassezia* species on SD lesions amongst immunocompromised patients. We found that SD of the scalp and of trunk is associated with different species, as there is already evidence that different species tend to be found on different body sites in both normal and diseased skin\(^18,24\).

Nearly the same range of isolated species is obtained from both sampling sites as in healthy patients with SD. Statistically we could not see any significant difference in the detection rates between immunosuppressed and immunocompetent patients. However, the percentage of positive cultures was much higher in immunocompromised patients confirming that impaired cellular immunity may facilitate fungal survival on the skin.

In conclusion, future studies are necessary to establish whether the range of isolated species correlates with the degree of immunosuppression. Use of new molecular methods applied to more patients is needed to clarify the involvement of each species in the development of SD in both immunocompetent and immunosuppressed patients.

**References**

Malassezia species in seborrheic dermatitis


