*Malassezia* and *Candida* colonisation on glans penis of circumcised men

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**Summary**

The *Malassezia* yeast are members of the normal human cutaneous flora in adults. They also are reported as part of the microflora of the male genital region in mostly uncircumcised males. It has been reported that *Malassezia sympodialis* and *Malassezia globosa* are the most frequent yeast belonging to the resident microflora of the penis as in other human skin areas. The aim was to evaluate the prevalence of *Malassezia* and *Candida* yeast colonisation on the glans penis of circumcised males. Impression preparations were made on modified Dixon agar. The isolates were identified by morphological and physiological characteristics. A total of 245 circumcised males were included in the study. Of the 245 patients examined, 55 (22.4%) were found to have a mycologically proven yeast fungi on their glans penis. In 17 (30.9%) *Malassezia*, in 36 (65.5%) *Candida*, in one (1.8%) *Malassezia* and *Candida*, and in one (1.8%) *Saccharomyces* strains were detected. *Malassezia furfur* (66.7%) was the most common species among the lipophilic yeast, followed by *Malassezia globosa* (11.1%), *Malassezia obtusa* (11.1%) and *Malassezia slooffiae* (11.1%). *Candida albicans* was the most common non-lipophilic yeast (46.0%), that was isolated among the other yeast, followed by unidentified *Candida* strains (18.9%), *Candida tropicalis* (8.1%), *Candida glabrata* (8.1%), *Candida parapsilosis* (8.1%), *Candida zealandiae* (5.4%), *Candida guilliermondii* (2.7%) and *Saccharomyces cerevisiae* (2.7%). The results of this study showed that *Malassezia* species were also colonised like *Candida* on the glans penis of circumcised males.

**Key words:** *Candida*, circumcision, glans penis, *Malassezia*, yeast.

**Introduction**

The *Malassezia* yeast are not only the members of the normal human cutaneous flora in adults, but also associated with several skin diseases.1, 2 A taxonomic revision has divided the yeast of the genus *Malassezia* into seven different species according to morphological and physiological features. *Malassezia furfur*, *Malassezia sympodialis*, *Malassezia slooffiae*, *Malassezia obtusa*, *Malassezia globosa* and *Malassezia restricta*, which are lipophilic species and *Malassezia pachydermatis* as non-lipophilic.1–4 According to previous reports *M. sympodialis*, *M. globosa* and *M. furfur* were found as the most commonly isolated species from patients with pityriasis versicolor (PV), seborrheic dermatitis, atopic dermatitis and healthy individuals.5–10 The isolated species were reported to differ not only between healthy individuals and patients with various diseases, but also between various countries.1 Gupta et al. [9] stated that there is very likely possibility that, as in other fungal dermatoses, there may be significant differences worldwide in species prevalences, both in aetiological and commensal *Malassezia* colonisation.

*Malassezia sympodialis* dominated among the species recovered from the skin of healthy individuals.10–12 However, Nakabayashi et al. [6] detected *M. globosa* at the highest rate, followed by *M. sympodialis*. On the
contrary, M. obtusa, M. pachydermatis, M. slooffiae and M. restricta were infrequently isolated from healthy individuals.\textsuperscript{5–10} Recently, Mayser et al.\textsuperscript{[13]} reported Malassezia spp. as part of the microflora of the male genital region in 49.2\% of the men, mostly uncircumcised. The authors reported M. sympodialis and M. globosa as the most frequent yeast belonging to the resident microflora of the penis as in other human skin areas.

The dimension of Malassezia and Candida colonisation on the glans penis of male circumcised patients who were admitted with various pathologies to outpatient clinics of the Department of Urology were examined.

\textbf{Patients and methods}

This study was a single-centre randomised study. All patients were fully informed (oral and written) of the objectives and implications of the study and a written informed consent was obtained from each patient before they were admitted to the study.

Between March 2004 and July 2004, 245 patients who were admitted to the outpatient clinics of Department of Urology at the Faculty of Medicine, Çukurova University were included in the study. The complaints or diagnosis of the patients were urolithiasis in 43 (17.5\%), benign prostate hyperplasia (BPH) in 47 (19.2\%), bladder cancer in 24 (9.8\%), prostate cancer in 14 (5.7\%), urinary tract infection (UTI) in eight (3.3\%) and various other pathologies in 109 (44.5\%). Patients with genital dermatoses, i.e. psoriasis, seborrheic dermatitis, atopic eczema, allergic contact dermatitis, balanoposthitis, idiopathic penile oedema or with human papillomavirus, herpes simplex virus, mollusca contagiosa were admitted to the study.

The diagnosis is made by an impression preparation of the glans penis on a modified Dixon medium. The complaints or diagnosis of the patients were urolithiasis in 43 (17.5\%), benign prostate hyperplasia (BPH) in 47 (19.2\%), bladder cancer in 24 (9.8\%), prostate cancer in 14 (5.7\%), urinary tract infection (UTI) in eight (3.3\%) and various other pathologies in 109 (44.5\%). Patients with genital dermatoses, i.e. psoriasis, seborrheic dermatitis, atopic eczema, allergic contact dermatitis, balanoposthitis, idiopathic penile oedema or with human papillomavirus, herpes simplex virus, mollusca contagiosa were excluded from the study. All patients were between 18 and 80 years of age and heterosexual male as reported.

The diagnosis was made by an impression preparation of the glans penis on a modified Dixon medium. The composition of modified Dixon agar is as follows: 1 l distilled H\textsubscript{2}O, 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. Agar was prepared by adding distilled water to the dry ingredients, heating to dissolve and then adding the Tween 40 and glycerol. Agar was autoclaved at 121°C for 15 min. After cooling for approximately 15 min, 18 ml of agar was dispensed into 90-mm plastic Petri dishes. The medium was always used within 1 week of preparation. The plates were incubated in air at 32°C in a moist environment. Cultures were examined daily over a period of 14 days.\textsuperscript{3} Positive results were then evaluated for the number, colour and composition of yeast colonies according to Mayser et al.\textsuperscript{[13]} Malassezia yeast were identified as pale ochre-brown colonies with a smooth or rough surface, however Candida spp. as typical cream-coloured, smooth surfaces waxy colonies.\textsuperscript{2–1}\textsuperscript{3}\textsuperscript{13} Semiquantitative evaluation of all positive cultures after 10-day incubation (graded as +, if one macroscopic colony was formed after 10 days; ++, if 2–5 macroscopic colonies; and +++, if more than five macroscopic colonies).\textsuperscript{13}

Colonies compatible with Malassezia spp. were subcultured on Sabouraud glucose agar (SGA; Acumedia, Baltimore, MD, USA) plates to confirm their lipophilic character. Malassezia species were identified on the basis of microscopic observation of cells and physiological properties such as the presence of catalase and the ability to utilise Tween compounds. Presence of catalase was determined by application of a drop of 3% hydrogen peroxide onto the culture smear on a glass slide. Production of gas bubbles, indicative of release of oxygen, was considered positive. Utilisation of Tween compounds was carried out according to Gupta et al.\textsuperscript{[8, 9]} modified from Guillot et al.\textsuperscript{[4]}. Yeast suspensions (at least 10\textsuperscript{7} CFU ml\textsuperscript{−1}) were formed in sterilised normal saline (0.85\%) and inoculated on SGA. The inoculum was evenly spread over the surface of each plate. Each plate was divided into four sections and a well was made in the centre of each section. Four drop of a Tween compound, i.e. Tween 20, 40, 60 and 80, were applied in each separate well. These plates were incubated at 32°C for 7 days and the resulting growth in each section was recorded.

Non-lipophilic yeast fungi were identified by germ-tube formation in human serum at 37°C for 2 h, by micromorphology on cornmeal agar–Tween 80 according to the Dalmau’s method and by using the commercial API 20C AUX (Bio-Merieux, Marcy-l’Etoile, France) system.\textsuperscript{14} Mycological data were analysed by using a chi-squared test to compare the results of yeast colonisation according to age groups. Statistical significance was set at P < 0.05.

\textbf{Results}

The mean age of the patients was 53.8 ± 14.6 (minimum = 18, maximum = 80). Yeast fungi were isolated in 55 (22.4\%) of the 245 circumcised male patients with species isolated as Malassezia in 17 (30.9\%), Candida in 36 (65.5\%), Malassezia and Candida in one (1.8\%) and Saccharomyces cerevisiae in one (1.8\%). More than two colonies of Malassezia species in 94.4\%, more
than five colonies in 55.5% were isolated. This was 77.8% and 63.9% for *Candida* species, respectively (Table 1).

*Malassezia furfur* (66.7%) was the most common species among the lipophilic yeast, followed by *M. globosa* (11.1%), *M. obtusa* (11.1%) and *M. slooffiae* (11.1%). In one patient *M. furfur* was isolated in addition to *C. albicans*. *Candida albicans* was the most common non-lipophilic yeast (46.0%), that was isolated among the other yeast, followed by unidentified *Candida* strains (18.9%), *Candida tropicalis* (8.1%), *Candida glabrata* (8.1%), *Candida parapsilosis* (8.1%), *Candida zeylanoides* (5.4%), *Candida guilliermondii* (2.7%) and *Saccharomyces cerevisiae* (2.7%). However, growing mould fungi are a serious problem, as no cycloheximide has been added to allow the detection of *Candida* spp. to the Dixon medium. Therefore, the seven (18.9%) contaminated *Candida* species could not be identified.

Among all the other age groups (14.8–28.3%), the colonisation rate was found highest in the 30–39 years age group (55.6%) (Table 2). This difference was statistically significant ($\chi^2 = 15.32$, df = 5, $P = 0.009$). The clinical diagnoses of 55 patients with yeast fungi were urolithiasis in 10 (18.2%), BPH in 11 (20.0%), bladder cancer in five (9.1%), prostate cancer in two (3.6%), UTI in one (1.8%) and various other pathologies in 26 (47.3%). There was no statistically significant correlation between clinical diagnosis and the rate of colonisation ($\chi^2 = 3.14$, df = 5, $P > 0.05$).

### Discussion

Circumcision is most frequently performed for religious or tribal reasons and it is thought that approximately one-sixth of the world’s male population is circumcised. In Jewish and Muslim community circumcision is a religious ritual and usually performed on the 8th day of life, and between the age of 4 and 13 years respectively.\(^1\) According to Mallon et al. [16], cutaneous penile infections, including human papillomavirus, herpes simplex virus, mollusca and candidal balanitis were more prevalent in uncircumcised male adults. The authors also suggested that absence of the foreskin prevents or protects against common infective

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Malassezia</th>
<th>Candida</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>1</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>++</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>+++</td>
<td>10(^1)</td>
<td>24(^2)</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>37</td>
<td>55</td>
</tr>
</tbody>
</table>

\(^1\)One of these patients colonised with *Candida* sp. (+).

\(^2\)One of these species were *Saccharomyces cerevisiae*.

### Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>18–29 (n = 25)</th>
<th>30–39 (n = 18)</th>
<th>40–49 (n = 46)</th>
<th>50–59 (n = 64)</th>
<th>60–69 (n = 65)</th>
<th>&gt;70 (n = 27)</th>
<th>Total (n = 245)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Malassezia furfur</em></td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>–</td>
<td>12</td>
</tr>
<tr>
<td><em>Malassezia globosa</em></td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td><em>Malassezia obtusa</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td><em>Malassezia slooffiae</em></td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Subtotal</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td><em>Candida zeylanoides</em></td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td><em>Candida guilliermondii</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unidentified <em>Candida</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Subtotal</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>37</td>
</tr>
<tr>
<td>Total colonised</td>
<td>6</td>
<td>10</td>
<td>13</td>
<td>11</td>
<td>11</td>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>Colonised (%)</td>
<td>24.0</td>
<td>55.6</td>
<td>28.3</td>
<td>17.2</td>
<td>16.9</td>
<td>14.8</td>
<td>22.4</td>
</tr>
</tbody>
</table>
penile dermatoses. Fakjian et al. [17] reported higher prevalence of balanitis in the uncircumcised population, especially in patients with diabetes mellitus.

Davidson [18] investigated 66 (48.9%) circumcised and 69 (51.1%) uncircumcised, both heterosexual and homosexual 135 men and isolated yeast at similar rates, 14 and 17%, from coronal sulcus and meatus of the penis respectively. David et al. [19] reported penile Candida colonisation in 74 (16%) of the 462 men who attended a sexually transmitted diseases clinic in Coventry, UK. Of the 74 patients with penile colonisation, 26 (37%) were symptomatic and 20 (27%) had balanitis. Penile carriage and symptomatic balanitis are mostly associated with sexual partners of women who have vaginal candidosis.20 Kwon-Chung and Bennett [21] stated that yeast were infrequently isolated from the penis, except in patients whose sexual partners had vaginal candidosis. Also Schaller [22] isolated Candida spp. in 26.6% of 1000 young men by using impression method.

Maysers et al. [13] reported Malassezia colonisation in 64 (49.2%) of 130 men, while Candida spp. were isolated in 27 (20.8%); colonisation with Candida and Malassezia spp. was found in 14 (10.8%). They also attracted attention to Malassezia infection in eight and Candida in one of 13 circumcised men. The present study also highlighted their results, because all of the patients were circumcised. Yeast fungi were detected in 55 (22.4%) of 245 circumcised males who did not have any dermatological lesion (Table 1). The different colonisation rates of Malassezia and Candida between these two studies may also reflect the differences in the uncircumcised and circumcised male population. In this study, only four of the six recognised lipophilic Malassezia species were identified. Malassezia furfur was the most common isolated species and interestingly M. sympodialis has not been isolated at all (Table 2). Although the incidence of Candida balanitis is a growing problem, PV at the penile sheath and glans penis were reported in a limited number.20, 23–25 This may be due to the lower pH (4.5) of vagina and lack of lipid sources, which is not optimum for the growth of Malassezia.13

The results of this study confirmed that lipophilic yeast fungi were the member of the flora of the glans and coronary sulcus of the penis as Maysers et al. [13] brought up. The rate of colonisation with the yeast of the genus Malassezia between circumcised and uncircumcised children is also under investigation. The authors believe that in the future, the colonisation of Malassezia will be better described by age-related studies reflecting the community and the rate of studies on colonisation of Malassezia will increase.

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References