Effect of circumcision on genital colonization of Malassezia spp. in a pediatric population

SERDAR İSKIT*, MACIT İLKİT‡, AYGÜL TURAÇ-BİCER‡, HAKAN DEMİRİRİN‡ & MERDAN TÜRKER*

Departments of *Pediatric Surgery, ‡Microbiology, and †Public Health, Faculty of Medicine, University of Çukurova, Adana, Turkey

This study aimed to determine the prevalence of yeast fungi in samples from the glans penis and prepuce of circumcised and uncircumcised children. Impression preparations were made on modified Dixon and Leeming-Notman agar without cycloheximide. The isolates were identified by morphological and physiological characteristics. The yeasts were detected in 32 (28.6%) of 112 children, 12 (18.2%) being among 66 circumcised and 20 (43.5%) among 46 uncircumcised children. The most common species was Malassezia globosa (40.6%) followed by, Malassezia furfur (31.3%), Malassezia slooffiae (15.6%), Candida albicans (6.3%), Candida tropicalis (3.1%) and Candida parapsilosis (3.1%). The colonization with yeasts, and especially lipophilic species was more frequently observed among uncircumcised versus circumcised children. While age was not found to be a factor (P > 0.05), circumcision was responsible for increasing the colonization rate by 4.8 times (95% CI: 1.6–14.5) (P < 0.01). The circumcision status was not found to effect the distribution of Malassezia spp.

Keywords children, circumcision, glans penis, Malassezia, Candida

Introduction

Malassezia species form part of the normal microbial flora of the skin of humans and other warm-blooded animals and most infections are of endogenous origin, such as pityriasis versicolor (PV) [1,2]. In 1996, a taxonomic revision divided the genus Malassezia into seven different species according to morphological, ultrastructural and physiological features. Malassezia furfur, M. sympodialis, M. slooffiae, M. obtusa, M. globosa and M. restricta are lipophilic, while M. pachydermatis is a non-lipophilic species [3]. Yeasts of this genus are most often found in sebum-rich areas of the skin such as scalp, face, trunk and upper back [1,2,4]. The incidence of skin colonization rises from around 25% in children to almost 100% in adolescents and adults. It is thought that colonization with Malassezia species primarily occurs at the time that concentration of lipids on the skin increases [2].

The colonization of the yeast of the genus Malassezia on the glans penis and prepuce has been reported so far only in adults, and mostly uncircumcised men [5]. The first study comprising circumcised adults had recently originated in our laboratory [6]. This current study, not only presents the glans penis and prepuce colonization of Malassezia spp. in a pediatric population for the first time in the literature, but it is also the first study comparing the situation between circumcised and uncircumcised populations.

We aimed to determine the prevalence of yeasts in samples from the glans penis and prepuce in a pediatric population both circumcised and uncircumcised, and to compare the efficacy of modified Dixon (mDixon) medium and Leeming-Notman (LN) agar used in the isolation of Malassezia spp.

Materials and methods

Informed and written consent was obtained from either the children and/or their families according to the age
of the child with clear description of the objectives and procedures of the study.

Study population

Between August 2004 and February 2005, 112 patients who applied to the outpatients clinics of the Department of Pediatric Surgery at the Faculty of Medicine, Cukurova University, were included in the study. The complaints or diagnosis of the patients were healthy in 40 (35.7%), corrosive esophageal stricture in six (5.4%), cryptorchidism in five (4.5%), gastroesophageal reflux in four (3.6%), hidatid cyst of the liver in four (3.6%), leukemia in four (3.6%), anal fissure in four (3.6%), posterior urethral valve in four (3.6%) and various other pathologies in 41 (36.6%). Patients with genital dermatoses, i.e., seborrheic dermatitis, atopic eczema, allergic contact dermatitis and urinary tract infections, were excluded from the study. The age distribution of the children was between eight days and 18 years.

Media and sampling procedure

The diagnosis was made by an impression preparation of the glans penis on mDixon and LN media [1,5/C1]/7]. Both media were prepared with chloramphenicol, but without cycloheximide, to allow isolation of all species of Candida. These two media were always used within one week of preparation. The plates were incubated in air at 32°C in a moist atmosphere. Cultures were examined daily over a period of 14 days. Positive results were then evaluated for the number, color and composition of yeast colonies according to Mayser et al. [5]. Colonies compatible with Malassezia spp. were subcultured on Sabouraud glucose agar (SGA, Acumedia, Baltimore, Maryland, USA) plates to confirm their lipophilic character [3].

Colony morphology

Malassezia yeasts were identified as pale ochre-brown waxy or crumbly colonies with a smooth or rough surface, however Candida spp. as typical cream-colored, smooth or rough surfaced, soft colonies [1,3,5–7]. All positive cultures were semiquantitatively evaluated after 10 days of incubation as (+) if one macroscopic colony was formed, (+ +) for 2–5 macroscopic colonies, and (+ + +) for more than 5 macroscopic colonies [5].

Identification of Malassezia species

Malassezia species 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.

Physiological tests

Catalase reaction. 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.

Utilization of Tween compounds. This test was done according to Gupta et al. [8,9] modified from Guillot et al. [10]. Yeast suspensions – (of at least 10^7 cfu ml^-1) were prepared in sterilized 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 center of each section. Four drops of a Tween compound, i.e., Tween 20, 40, 60, 80, were applied in each separate well. These plates were incubated at 32°C for seven days and the resulting growth in each section was recorded.

Organisms

The following reference strains were obtained from the Centraalbureau voor Schimmelcultures (CBS), Baarn, 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 7222.

Identification of Candida species

Candida species 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 method, and by using the commercial API 20C AUX (bio-Merieux, Marcy-l’Etoile) system [11].

Statistical analysis

The data were analysed by using chi-square tests, Kendall’s tau-b correlation and binary logistic regression.

Results

The mean age of the children was 6.4 years (with one standard deviation of 4.5). The yeasts were detected in 32 (28.6%) of 112 children, 12 (18.2%) being among 66 circumcised and 20 (43.5%) among 46 uncircumcised children, with a significant difference (x^2 = 8.50, df = 1, P < 0.01). Among the 32 isolates, 28 belong to the genus Malassezia and four to the genus Candida.
Thirty (93.8%) strains were isolated on both of the media, while two (6.2%) strains of *M. globosa* were solely isolated on mDixon medium. The results of both media were compared and a very good and significant correlation was found ($r=0.909$, $P<0.001$).

The most common species was *M. globosa* (40.6%) followed by, *M. furfur* (31.3%), *M. slooffiae* (15.6%), *C. albicans* (6.3%), *C. tropicalis* (3.1%) and *C. parapsilosis* (3.1%). Mixed colonization was not observed in any of the cases. Among circumcised cases, *M. globosa* was isolated in 5 (41.7%), *M. furfur* in 4 (33.3%), *M. slooffiae* in 2 (16.7%) and *C. tropicalis* in 1 (8.3%); while among uncircumcised cases, *M. globosa* was detected in 8 (40%), *M. furfur* in 6 (30%), *M. slooffiae* in 3 (15%), *C. albicans* in 2 (10%) and *C. parapsilosis* in 1 (5%) (see Table 1).

When compared to uncolonized cases, the portion of colonized cases were found to be lower in all age groups between 2 and 14 years, with values varying from 12.8–30.4%. In the group older than 14 years of age and infants (younger than 2 years of age), colonization prevailed at 50% and 60%, respectively. As age was found to affect the circumcision status of the children, with older children being more frequently circumcised, the effect of age and circumcision on the colonization was tested by multivariate analyses. While age was not found to be effective ($P=0.329$), the circumcision was calculated to increase the colonization rate by 4.8 times (with a 95% confidence interval between 1.6 and 14.5) ($P<0.01$). The diagnoses of the children were found not to affect the colonization status ($P=0.306$).

### Discussion

Circumcision is the most commonly performed operation in the world. Recently, Gatrad *et al*. [12] estimated one-third of the global male population to be circumcised. The circumcision, known to be performed for religious and cultural reasons as accustomed, is now being more frequently indicated by medical reasons. The neonatal period is suggested as the most appropriate time for circumcision, as uropathogens have been reported to have a high affinity to the prepuce and 10 to 12 fold increase of urinary tract infections has been reported among uncircumcised compared to circumcised children [13,14]. Pathogenic microorganisms have been reported to decrease in the periurethral flora and bacteriuria to be impeded following circumcision, which was also reported to decrease the frequency of invasive penile cancer and sexually transmitted disease [14–16].

The colonization of yeast of the genus *Malassezia* on the glans penis and prepuce was reported, to date, by

### Table 1

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Circumcision</th>
<th>Malassezia globosa</th>
<th>Malassezia furfur</th>
<th>Malassezia slooffiae</th>
<th>Candida albicans</th>
<th>Candida parapsilosis</th>
<th>Candida tropicalis</th>
<th>Colonized total</th>
<th>Uncolonized</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>No</td>
<td>6 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>5 (83.3)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>2-4</td>
<td>Yes</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>2 (33.3)</td>
<td>6 (100)</td>
<td>7 (117)</td>
</tr>
<tr>
<td>5-9</td>
<td>No</td>
<td>3 (100.0)</td>
<td>2 (100.0)</td>
<td>2 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>4 (66.7)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>10-14</td>
<td>Yes</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>1 (100.0)</td>
<td>6 (100)</td>
<td>7 (117)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>17 (85.0)</td>
<td>3 (15.0)</td>
<td>12 (50.0)</td>
<td>12 (50.0)</td>
<td>14 (35.9)</td>
<td>25 (64.1)</td>
<td>3 (13.0)</td>
<td>26 (32.5)</td>
<td>66 (89.9)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.
two studies, both observed in adult age [5,6]. First, Mayser et al. [5] reported Malassezia colonization in 64 (49.2%) of 130 mostly uncircumcised men, isolating Candida spp. in 27 (20.8%), Candida and Malassezia spp. in 14 (10.8%). Among all of the six lipophilic species that they had isolated, M. sympodialis and M. globosa were the most common species, like in other human skin areas. More recently, Aridogan et al. [6] observed yeasts isolation in 55 (22.4%) of 245 circumcised males, with species isolated as Malassezia in 17 (30.9%), Candida in 36 (65.5%), Malassezia and Candida in 1 (1.8%) and Saccharomyces cerevisiae in 1 (1.8%). While these authors detected only four of the lipophilic species of this genus, M. furfur was the predominant one. In our study, the colonization with yeasts, and especially lipophilic species, was more frequently observed among uncircumcised versus circumcised children, with the most common species as M. globosa, followed by M. furfur, M. slooffiae and Candida spp.

The determination of different lipophilic fungi as the most common species in each of the three studies mentioned above could be related to the selection of different target populations, which should be verified by further investigations. Gupta et al. [4] stated some questions regarding which species are more commonly found on human skin, whether there is variation in the distribution of the yeasts on different body sites, and whether there is geographic variation in species prevalence. Our finding of a frequency of 12.5% for Candida spp. among children, mostly uncircumcised, seemed relatively low, when compared to the finding of 65.5% in another study performed among adult circumcised men with the same geographical location and evaluated by our laboratory [6]. The higher frequency (65.5%) might be explained by a glans penis colonization due to sexual activity, hence by vaginal Candida resource in the older age group. Further studies may reveal another benefit of circumcision as the impediment or decrease in the colonization of Malassezia spp.

Malassezia species show different colonization patterns according to different age groups. The colonization has been shown to increase with the skin lipid production augmenting at puberty [17–19]. Silva et al. [17] reported a prevalence of 17.8% for yeast of the genus Malassezia on the skin of 107 healthy children, aged 0–15 years. While they found no difference between sexes, the prevalence was highest among children aged 0–18 months (23.3%), and 11–15 years (26.7%). Gupta and Kohli [18] reported Malassezia spp. on scalp, forehead, chest and back samples with a higher frequency among children younger than 14 years of age, when compared to the older ones (≥15 years). Also, Faergemann and Fredriksson [19] found no Malassezia species in children younger than five years of age.

Although Malassezia species are part of the normal microflora, under certain conditions they can cause superficial skin infections [4]. Pityriasis versicolor (PV) can arise at any age, with most cases occurring during adolescence and young adulthood [1,2,4]. Isa- Isa et al. [20] reported that among 5160 cases of PV, 797 (15.4%) were children, with 92 (11.5%) cases under one year of age. Szepietowski et al. [21] reported that 5.2% of patients with PV were children, aged 15 years or younger. Also other surveys reported that 1.1–3.7% of patients with PV were children between the ages of 0–10 years [22,23]. Taking into account the pediatric colonization revealed in our study, PV of the penis reported to be very rare even in adolescence, should be now considered among pediatric superficial fungal infections [24–27].

In our study, mDixon and LN agars, used for the isolation of lipophilic yeasts, i.e., Malassezia spp., have been verified for their efficacy, both by our study and similar studies, like the one performed by Mayser et al. [5], suggesting the selection of either medium.

As a conclusion; the colonization with yeasts, and especially lipophilic species, was more frequently observed among uncircumcised versus circumcised children, with M. globosa as the most frequent species. The study media, i.e., mDixon and LN agars, were found to be equally effective.

References


18 Gupta AK, Kohli Y. Prevalence of *Malassezia* species in various body sites in clinically healthy subjects representing different age groups. *Med Mycol* 2004; **42**:35–42.