

INVESTIGATIVE REPORT

Frequency of Bacteria, *Candida* and *Malassezia* Species in Balanoposthitis

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Balanoposthitis is an inflammatory disorder of the prepuce and glans penis. Microbes involved in balanoposthitis have been investigated, but no single study has covered the growth of both bacteria, *Candida* and *Malassezia*. We report here the prevalence of these microbes in 100 patients with balanoposthitis and in 26 control patients. Among patients with balanoposthitis there was a significantly higher frequency of positive cultures than in the control group (59% and 35%, respectively, $p < 0.05$). In the balanoposthitis group *Staphylococcus aureus* was found in 19%, group B streptococci in 9%, *Candida albicans* in 18% and *Malassezia* in 23% of patients. In the control group *S. aureus* was not found at all, whereas *C. albicans* was found in 7.7% and *Malassezia* in 23% of patients. Different microbes did not correspond with distinct clinical manifestations. In summary, we report increased frequency of microbes, specifically *S. aureus*, in the area of the prepuce and glans penis in balanoposthitis. Key words: balanoposthitis; microbes; *S. aureus*; *candida*; *malassezia*.

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Balanoposthitis is a common form of dermatitis among patients attending dermatological, venereological and genitourinary healthcare facilities (1). Symptoms include erythema of the glans penis (balanitis) and prepuce (balanoposthitis), often accompanied by papules, pustules, scaling, white membranes, transudation or fissures. The causes of balanoposthitis are diverse and include inflammatory dermatological disorders, such as Zoon balanitis, lichen planus, seborrhoeic dermatitis and psoriasis, premalignant conditions including Bowen's disease and penile intraepithelial neoplasia, various infections and contact allergies (2).

There are previous reports of the frequency of microbes in balanoposthitis (3–6). These studies have predominantly found *Candida* species (10–60%) and group B streptococci (4–28%). In 31–41% of cases no specific aetiological factor could be detected. Among these studies, only a few have included a healthy control

group for comparison (4, 6). Abdennader et al. (6) have published data revealing a significantly higher frequency of *Candida* and bacteria (with group B streptococci being the most common) in balanoposthitis.

Mayser et al. (7) reported the frequency and spectrum of *Malassezia* in the area of the prepuce and glans penis in 130 healthy men, most of whom were uncircumcised. *Malassezia* was found in 49% of these men and the authors suggested a role in pathological processes of the penis, such as balanoposthitis, seborrhoeic dermatitis and psoriasis. Aridogan et al. (8) presented *Malassezia* in only 7% of circumcised men without genital dermatoses in an outpatient urology clinic.

In recent years increasing attention has been given to the possible role of *Malassezia* in dermatological disorders other than seborrhoeic dermatitis and pityriasis versicolor. The importance of *Malassezia* in atopic dermatitis has been investigated (for review see 9, 10) and a link between psoriasis and *Malassezia* has been suggested (for review see 11).

To our knowledge, the presence and simultaneous presence of bacteria, *Candida* and *Malassezia* in patients with balanoposthitis have not been described. The aim of this investigation was to examine balanoposthitis from this point of view and compare our findings with that of a control group in order to determine the role of these microbes in the pathogenesis of balanoposthitis. Cases of balanoposthitis where distinct dermatoses, such as Zoon balanitis, lichen planus, genital herpes, or premalignant conditions, could explain lesions have not been included.

In addition to evaluating the presence of microbes in the area of the prepuce and glans penis in balanoposthitis, a further aim was to investigate whether different pathogens corresponded with distinct clinical patterns. In view of the suggested link between *Malassezia*, balanoposthitis and other dermatological disorders we also examined the frequency of seborrhoeic dermatitis and psoriasis in the patients participating in this study.

MATERIALS AND METHODS

Patients

The study was performed in our venereology clinic, where we see patients with sexually transmitted infections (STI) and genital dermatological diseases. A total of 102 patients presenting with balanoposthitis between 2004 and 2006 were

included in the study. Twenty-six patients with no clinical signs or medical history of balanoposthitis served as a control group. The study design was reviewed and approved by the regional ethics committee, Gothenburg, Sweden. Participants received oral and written information about the study before their written consent was obtained.

Ages in the balanoposthitis group ranged from 19 to 71 years (median 28, mean 33 years). Ages in the control group ranged from 18 to 44 years (median 29, mean 29 years).

Table I summarizes the reason for visit and/or additional diagnoses in the patients in the balanoposthitis and control groups. A majority of patients in the control group came for routine STI check-up or genital warts. Others were diagnosed with *molluscum contagiosum*, folliculitis, non-specific urethritis, pruritus scrotalis, urethritis caused by *Chlamydia trachomatis* or *Mycoplasma genitalium* and, in one case, varicella zoster virus infection of the right leg. In none of these cases was the area of the prepuce or glans penis affected.

The diagnosis of balanoposthitis was based on the presence of one or more of the following criteria; erythema, papules, pustules, transudate, white membranes or fissures in the area of the prepuce and glans penis. Presence or absence of the predetermined clinical criteria was assessed in each patient. In addition, patients were asked if they had experienced or were currently suffering from seborrhoeic dermatitis or psoriasis, and a clinical examination of the skin including the scalp was performed.

Cultures

One swab sample for bacterial culture and one for semi-quantitative *Candida* culture were obtained from the area of the prepuce and glans penis. Furthermore, one culture for *Malassezia* was obtained using a contact plate with Leeming-Notman agar pressed against the prepuce and glans penis. Bacterial cultures were sent to the hospital department of bacteriology according to standard procedure. *Candida* species were cultured on Sabouraud agar in

our laboratory, differentiated on chrome agar and growth visually quantified as very discrete, discrete, moderate or heavy. Semi-quantitative cultures for *Malassezia* were made on Leeming-Notman agar followed by differentiation of the isolates based on the combined methods of Guillot et al. (12) and Mayser et al. (13). Specifically, the colonies on Leeming-Notman agar were counted and the isolates then purified on Sabouraud dextrose agar in 32°C and modified Dixon agar in 38°C. Further differentiation was based on β -glucosidase activity assayed by using esculin agar tubes, the catalase reaction determined by application of hydrogen peroxide and culture with Cremophor IL.

Statistics

The frequencies of microbes in the balanoposthitis group and in the control group were compared with Fisher's exact test. The same statistical instrument was used to compare prevalence of seborrhoeic dermatitis between groups. The significance level was $p < 0.05$.

RESULTS

A total of 102 patients with clinical signs of balanoposthitis were examined. Eighty-eight of these patients reported having female sexual partners, 2 patients had male sexual partners, one patient had both male and female sexual partners and 11 patients did not report sexual orientation. Two out of the 102 patients were circumcised.

The control group consisted of 26 patients, whereof 25 reported having female sexual partners and one patient did not report sexual orientation. One out of the 26 patients was circumcised.

On clinical suspicion of Zoon balanitis a punch biopsy was taken from the glans penis of a 64-year-old man and this confirmed the diagnosis. Furthermore, a 36-year-old man had a positive PCR for herpes simplex virus type 2. The data of these 2 patients were not included in the statistical analysis or discussion, since Zoon balanitis and viral aetiology were outside the scope of this investigation.

Patients with balanoposthitis had positive cultures in 59% (59/100) of cases, whereas positive cultures were found in only 35% (9/26) of cases in the control group ($p < 0.05$). A positive culture was defined as growth of one or several of the following pathogens; staphylococci, streptococci, *Candida* and *Malassezia*. In the following, the different microbes will be presented separately. In addition, the cultures of 2 patients in the balanoposthitis group, 34 and 26 years old, respectively, showed heavy growth of *Trichosporon* species.

Staphylococcus aureus

In the balanoposthitis group 19 patients (19%) exhibited *S. aureus* in the area of the prepuce and glans penis. In the control group no staphylococci were detected in any of the patients. There was a statistically significant difference between groups ($p < 0.05$) (Table II).

Table I. Additional diagnoses in the balanoposthitis group and reasons for the visit in the control group

	Balanoposthitis group n = 102*	Control group n = 26
Balanoposthitis	102	—
Bacterial STIs		
<i>Chlamydia trachomatis</i> urethritis	3	2
<i>Mycoplasma genitalium</i> urethritis	—	1
Viral STIs		
Herpes simplex type 2	1	—
Varicella zoster (right leg)	—	1
<i>Condylomata acuminata</i>	2	8
<i>Molluscum contagiosum</i>	1	2
Other		
Eczema nummularis (extragenital)	1	—
Folliculitis	1	1
Non-malignant preputial epithelial hyperplasia	1	—
Non-specific urethritis	5	2
Pruritus scrotalis	—	1
STI check-up	—	8
Zoon balanitis	1	—

*The total number of diagnoses in this column exceeds the number of patients, since some patients received diagnoses in addition to balanoposthitis.

STI: sexually transmitted infection.

Table II. Frequency of bacteria and yeasts in the balanoposthitis and control groups

	Balanoposthitis group n=100		Control group n=26	
<i>S. aureus</i>	19	(19%)*	0	—
Group B streptococci	9	(9%)	1	(3.8%)
Group C streptococci	3	(3%)	0	—
<i>C. albicans</i>	18	(18%)	2	(7.7%)
<i>Malassezia spp</i>	23	(23%)	6	(23%)

* $p < 0.05$.

Group B streptococci

Group B streptococci were present in 9 (9.0%) patients in the balanoposthitis group. In the control group one patient (3.8%) had group B streptococci in cultures ($p = 0.69$).

β -haemolytic group C streptococci

β -haemolytic group C streptococci were found in 3 patients (3.0%) with balanoposthitis and not at all in the control group ($p = 1.0$).

Candida

In fungal cultures the only *Candida* species detected was *C. albicans*. Only cultures with growth that was moderate to heavy were included in our analysis of data.

In total, moderate to heavy growth of *C. albicans* was found in 18 patients (18%) in the balanoposthitis group. The corresponding number for the control group was 2 patients (7.7%) ($p = 0.24$). All positive *Candida* cultures, including those with very discrete or discrete growth, are presented in Table III.

Malassezia

Several different *Malassezia* species were detected and quantified in both the balanoposthitis group and the control group.

Table III. Quantification of growth of *Candida albicans* in the balanoposthitis and control groups

	Balanoposthitis group n=100		Control group n=26	
<i>C. albicans</i>				
Heavy growth	10	(10%)	0	
Moderate growth	8	(8%)	2	(7.7%)

In the balanoposthitis group very discrete or discrete growth of *C. albicans* was found in 13 additional patients. In the control group very discrete or discrete growth of *C. albicans* was found in 2 additional patients.

No statistically significant differences.

Malassezia was detected in 23 patients (23%) in the balanoposthitis group. The frequency of *Malassezia* in the control group was similar to that in the balanoposthitis group, with positive cultures in 6 patients (23%).

Malassezia isolates were quantified and further differentiated as described in the Materials and Methods section. Five of the 7 known species were found; *M. globosa*, *M. obtusa*, *M. sympodialis*, *M. restricta* and *M. slooffiae*. Combinations of 2 different species were observed in 7 individual patients. The frequency and distribution of *Malassezia* is presented in detail in Table IV.

Simultaneous presence of bacteria and yeasts

It was noted that *C. albicans* or *Malassezia* coexisted with bacteria in a subgroup of patients with balanoposthitis. The combination of *C. albicans* and *Malassezia*, with or without bacteria, was not found in any patient in the study. Table V illustrates the distribution of all positive cultures in the balanoposthitis group and in the control group. For patients with *S. aureus*, 3 out of 19 had simultaneous growth of *C. albicans* and 6 out of 19 had simultaneous growth of *Malassezia*. Similarly, patients with group B streptococci had positive cultures for *C. albicans* in 3 out of 9 cases and for *Malassezia* in one out of 9 cases. In one of the 3 patients with β -haemolytic group C streptococci there was a simultaneous

Table IV. Frequency, distribution and quantification of *Malassezia* in the balanoposthitis and control groups. For each *Malassezia* species, quantification and number of patients are shown. When more than one *Malassezia* species was detected the combined number of colonies is presented

	Balanoposthitis group n=100			Control group n=26		
	>5 colonies	2–5 colonies	1 colony	>5 colonies	2–5 colonies	1 colony
<i>M. globosa</i>	—	5	3	—	—	1
<i>M. obtusa</i>	1	4	2	—	—	1
<i>M. sympodialis</i>	—	—	3	—	—	2
<i>M. obtusa</i> + <i>M. globosa</i>	1	1	—	—	—	—
<i>M. obtusa</i> + <i>M. sympodialis</i>	—	1	—	—	—	—
<i>M. obtusa</i> + <i>M. restricta</i>	1	—	—	—	—	—
<i>M. sympodialis</i> + <i>M. restricta</i>	—	1	—	1	—	—
<i>M. globosa</i> + <i>M. slooffiae</i>	—	—	—	1	—	—

Table V. Simultaneous presence of bacteria and yeasts in the balanoposthitis and control groups

	<i>C. albicans</i> n (%)	<i>Malassezia</i> spp n (%)	No fungi n (%)	Total n (%)
<i>Balanoposthitis group, n = 100</i>				
<i>S. aureus</i>	3 (3)	6 (6)	10 (10)	19 (19)
Group B streptococci	3 (3)	1 (1)	5 (5)	9 (9)
Group C streptococci	1 (1)	0	2 (2)	3 (3)
Genital skin flora*	11 (11)	16 (16)	41 (41)	68 (68)
Total	18 (18)	23 (23)	58 (58)	
<i>Control group, n = 26</i>				
<i>S. aureus</i>	0	0	0	0
Group B streptococci	0	0	1 (3.8)	1 (3.8)
Group C streptococci	0	0	0	0
Genital skin flora*	2 (7.7)	6 (23)	17 (65)	25 (96)
Total	2 (7.7)	6 (23)	18 (69)	

*Genital skin flora defined as growth of one or several of the following bacteria/groups of bacteria: alpha streptococci, coagulase-negative staphylococci, *Corynebacterium* spp, enterococci, *Escherichia coli*, Gram-negative mixed flora or Gram-positive mixed flora.

finding of *C. albicans*. In the control group there was no simultaneous growth of bacteria and yeasts.

Clinical assessment

Clinical findings were similar among patients in the balanoposthitis group, with erythema of the prepuce and/or glans penis being most common (83 patients (83%)). Papules were found in 48 patients (48%), transudation in 30 patients (30%) and white membranes in 27 patients (27%). No correlation between specific clinical criteria and the different microbes were found. Also, the presence of a particular microbe did not correlate with the number of clinical criteria noted in an individual patient (data not shown).

Correlation with seborrhoeic dermatitis and psoriasis

Patients with balanoposthitis reported a similar frequency of seborrhoeic dermatitis compared with controls, 29/100 patients (29%) and 9/26 patients (35%), respectively. Clinical findings to support the diagnosis were present in 13/29 (45%) in the balanoposthitis group and in 3/9 (33%) in the control group. One patient in the balanoposthitis group reported psoriasis but had no clinical signs thereof. There were no reports of psoriasis in the control group.

When patients were subdivided according to results of the bacterial and fungal cultures it was noted that patients with *S. aureus* reported seborrhoeic dermatitis in 26% and had clinical signs in 11% of cases. The corresponding number for patients with *C. albicans* was 5.6% (one patient). In the subgroup with *Malassezia* species 44% of patients reported seborrhoeic dermatitis and had clinical signs in 16% of cases. There was no statistically significant difference in the frequency of seborrhoeic dermatitis between subgroups.

DISCUSSION

We report here the frequency and distribution of microbes, including *Malassezia* species, in a group of patients with balanoposthitis. It is shown that patients with clinical signs of balanoposthitis are a diverse group colonized and/or infected with microbes to a larger extent than patients in a corresponding control group. *S. aureus* was significantly more prevalent in the balanoposthitis group. There was no statistically significant difference in the prevalence of group B streptococci or *C. albicans* when they were compared separately. The frequency of *Malassezia* was similar in the balanoposthitis and control groups. The spectrum of *Malassezia* species in balanoposthitis was described.

Previous studies on microbes in balanoposthitis have demonstrated mainly *Candida* species and group B streptococci (3–6, 14, 15). In our patients the frequencies of *C. albicans* and group B streptococci are within the range of those presented by these investigators.

In contrast, *S. aureus* is rarely reported as a possible pathogen in balanoposthitis in adults (2, 6). The patients with balanoposthitis in our study did, however, present with *S. aureus* in 19% of cases. In comparison, the skin of patients with another inflammatory disorder, atopic dermatitis, is colonized by *S. aureus* in up to 90% of cases (16). Although *S. aureus* is not considered the causative agent of atopic dermatitis, it is believed to increase and maintain inflammatory response in the skin (9). A similar mechanism can be proposed for *S. aureus*, and indeed other microbes, in balanoposthitis. The reason that we found a high prevalence of *S. aureus* compared with previous investigators is not apparent. It might reflect a difference in the general bacterial flora in our population, as compared with the others examined. This difference could be geographical, or may represent a progressing change over time, since most of the comparable studies were performed a decade or more ago.

Our findings and the results of other investigators suggest that patients with balanoposthitis are vulnerable to colonization with microbes in the area of the prepuce and glans penis. This vulnerability to microbe colonization is further supported by Jackson et al. (17), who detected high rates of carriage of group B streptococci (38%) among men attending a clinic for sexually transmitted diseases. The subpreputial sac was an important site for carriage and there was a strong association between streptococcal isolation and balanitis.

Malassezia have been suggested as possible pathogens in balanoposthitis, but, to our knowledge, there are no reports of their prevalence in this condition (7). Previously, conflicting data on the frequency of *Malassezia* in other dermatological disorders, such as seborrhoeic dermatitis and atopic dermatitis, have been presented. In patients with seborrhoeic dermatitis increased, de-

creased or unchanged levels of yeasts have been found in lesional skin compared with normal skin (18, 19). The pathogenicity of *Malassezia* in seborrhoeic dermatitis is probably related to the immune response to the yeast rather than to the amount of yeasts on lesional skin (20). Suggested ways for *Malassezia* to induce symptoms are through precipitating antibodies against the yeast, production of toxin, lipase activity and activation of complement (21, 22). Treatment to reduce levels of *Malassezia* has been shown to yield a parallel reduction of symptoms in seborrhoeic dermatitis (23).

In this study we found no difference in the frequency of *Malassezia* in the balanoposthitis and control groups. Seborrhoeic dermatitis was equally distributed in the 2 groups. There was a tendency towards a higher incidence of seborrhoeic dermatitis in those patients who had balanoposthitis and *Malassezia*, but the difference was not statistically significant. It is possible that the inflammatory lesions on the prepuce and glans penis of the patients who presented with *Malassezia* and reported seborrhoeic dermatitis were a genital manifestation of seborrhoeic dermatitis itself.

As a part of this study the *Malassezia* isolates were differentiated. Observations were too few to establish if the distribution of species differed between the balanoposthitis group and the control group. For the differentiation of *M. obtusa* and *M. sympodialis*, growth at 38°C in a Dixon medium is used as one of the criteria. During the study, this step proved to be unexpectedly sensitive to the general state of the medium (date of preparation, viscosity). Therefore, some of the isolates being identified as *M. obtusa* might instead be *M. sympodialis*.

Two patients in the balanoposthitis group exhibited *Trichosporon* species in cultures. *Trichosporon* species are known to cause white piedra; white to light-brown nodules loosely attached to the hairshaft, and are most prevalent in temperate and semitropical climates (24). White piedra most usually affects pubic hair and axillary hair. The patients in question exhibited no clinical signs of white piedra, they attended the clinic on the same day and there was no known connection between them. These facts, in addition to the lack of *Trichosporon* species in the cultures of any of the other patients in the study, suggest that the cultures were contaminated.

Obvious microbes and distinct inflammatory dermatological diseases are often absent in patients with balanoposthitis. The condition could be caused by excessive hygiene, frequent sexual intercourse or other dehydrating or abrasive trauma to the prepuce and glans penis. Birley et al. (25) demonstrated that restrictions in frequency of genital washing abolished symptoms in a large fraction of patients with recurrent balanitis. These investigators also presented an increased lifetime incidence of atopic illness in the 31/43 patients (72%) who had balanitis on the basis of irritant dermatitis.

The diagnosis irritant dermatitis was based on negative bacterial, fungal and viral cultures as well as a 2-mm pinch biopsy showing non-specific dermatitis. In our study it is noteworthy that 41 patients (41%) in the balanoposthitis group exhibited normal genital skin flora in bacterial cultures and no growth of *Candida* or *Malassezia*. This suggests an increased sensitivity to irritants in the genital skin and mucous membranes of these patients.

We attempted to describe distinct clinical patterns related to the different microbes found in balanoposthitis. However, there were no clinical findings that were predominating or unique to any of the microbes investigated. Also, it was not apparent that any microbe was associated with a more severe clinical picture as measured by the number of clinical criteria present. A similar result was reached by Abdennader et al. (6), with the exception that these authors showed that the presence of pustules were highly suggestive of *Candida*. Pustules were noted in only 5 of our patients, 2 of whom presented with *C. albicans*.

In summary, we describe a significantly higher frequency of microbes in balanoposthitis than in a control group. There was a statistically significant difference in the frequency of *S. aureus* when microbes were compared separately. It is proposed that this reflects an increased susceptibility to irritants causing dermatitis and subsequent colonization or infection with microbes in the area of the prepuce and glans penis of these patients. Thus, due to the presence of dermatitis the number of colonizing microbes is indeed expected to be higher. The presence of microbes could increase and maintain pre-existing inflammation. We suggest that the primary therapeutic target in mild to moderate cases of balanoposthitis should be to decrease inflammation with a mild corticosteroid cream. The addition of antimicrobial agents, such as an imidazole, to the cream can reduce microbial load and further help to decrease inflammation. In severe cases of balanoposthitis systemic antibiotics are sometimes needed to resolve symptoms.

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