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Increased Likelihood of Bacterial Pathogens in the Coronal Sulcus and Urethra of Uncircumcised Men in a Diverse Group of HIV Infected and Uninfected Patients in India

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Abstract

Background:

The biological mechanism of circumcision as potentiating HIV prevention is poorly understood. Foreskin microbiota has been postulated as having a potential role; however, little is known about the relationship between bacterial pathogens and circumcision in adults.

Materials and Methods:

We sampled the coronal sulcus of a diverse group of circumcised and uncircumcised men (n=315) from a government chest hospital and fertility clinic in Hyderabad, Andhra Pradesh, India. Genital examination was conducted on three groups of men: Group 1 – HIV infected; Group 2 – TB infected; Group 3 – control. Aerobic and anaerobic specimens were cultured according to standard clinical protocols, and results were analyzed following multivariate logistic regression models.

Results:

Three hundred fifteen study participants – 47.6% of Group 1, 36.5% of Group 2, and 15.9% of Group 3 – were enrolled in the study and included in all analyses. Overall 37.1% of the participants were circumcised without variation across groups (P=0.29). Smegma was observed in 18.7% of the participants with no cases observed in Group 3 (P<0.001). Gram-negative pathogens were more prevalent among study participants in Group 1 (22.7%) and Group 2 (30.4%) as compared with those in Group 3 (6.0%) (P=0.003). In multivariate regression analysis, controlling for group, age, and presence of smegma, uncircumcised men were more likely to be colonized with gram positives [Adjusted Odds Ratio (AOR) 1.9; P<0.05)], gram negatives (AOR 2.4; P<0.05), or any pathogen (AOR 2.8; P<0.005).

Conclusions:

Uncircumcised men in this population in South India are more likely to harbor bacterial pathogens in the coronal sulcus than do their circumcised counterparts. Future studies should examine the relationship between foreskin microbiota and HIV transmission.

Keywords: Circumcision, HIV, *Staphylococcus aureus*, Tuberculosis

INTRODUCTION

Even after three randomized controlled trials in Africa demonstrated reduction in HIV acquisition following circumcision,[1–3] the biologic mechanism of protection remains poorly understood. Several hypotheses have been postulated that include differences in penile microbiota, rates of sexually transmitted infections, presence of Langerhans cells, level of keratinization, and penile hygiene between circumcised and uncircumcised persons.[4–7] However, a clear understanding of the relationship between the foreskin and the penile microbiological environment is lacking.

Unlike considerable efforts to investigate vaginal characteristics that affect HIV transmission, such as particular microbiomes in bacterial vaginosis,[8] little attention has been paid to the microbiota of the uncircumcised penis. A single study identified decreased numbers of anaerobic genera and increased numbers of facultative anaerobic genera including *Staphylococcus* spp. after circumcision, which were examined through cultivation-independent methods.[9] Yet other known pathogens such as *Pseudomonas* spp. were also prevalent in both circumcised and uncircumcised individuals.

A few studies have focused on microbiota among adolescents. Gram-negative bacteria such as *Escherichia coli, Pseudomonas* spp., *Proteus* spp., and *Klebsiella* spp.[10,11] are predominantly found in and around the urethra (UR), with circumcision increasing the presence of coagulase-negative *Staphylococcus* spp. and decreasing the number of gram-negative pathogens and enterococci.[12] After circumcision, relatively low levels of *Staphylococcus aureus* and no changes in *S. aureus* prevalence are reported.[12] However, these studies have focused on adolescents, populations with low levels of sexual activity and low HIV risk, those who have not evaluated resource-restricted regions, or those in which circumcision would be difficult to implement.[13]

MATERIALS AND METHODS

Participants and specimen collection

Between April and September 2009, three groups of study participants were recruited from the Andhra Pradesh Government Chest Hospital and an infertility clinic, both in Hyderabad, Andhra Pradesh, India. The firs group included inpatients infected with HIV (Group 1: HIV), the second group included inpatients infected with tuberculosis but not HIV (Group 2: TB), and the third group included patients presenting at a male fertility clinic (Group 3: Control). Exclusion criteria included use of short-term antibiotics (within previous 4 weeks) for an active bacterial or fungal infection other than tuberculosis. In Group 1, those who were on antibiotics other than trimethoprim-sulfamethoxazole were excluded, and in Group 2, those on a category II, III, or DOTS-plus regimen with agents other than rifampicin, ethambutol, isoniazid, and pyrazinamide were excluded. Participants with unretractile foreskin, evidence of an active *sexually transmitted infection* (STI), cellulitis, abscess, or a tight urethral meatus were excluded. All participants provided written informed consent after agreeing to participate in the study. Data were collected through a face-to-face interview and chart abstraction, and all samples were collected by a single male physician trained by a local STI specialist. Ethics review boards at SHARE-India and the University of Chicago approved the protocol and procedures.

Genital examination and specimen collection consisted of a penile swab of the CS and a second swab 1 cm into the proximal UR. Samples from uncircumcised men were taken after foreskin retraction, and no external cleansing was performed before sampling. The specimen was inserted into a liquid thioglycollate medium and transported under ambient temperature to the laboratory within 1 h, where processing was completed upon arrival. Aerobic culture was performed by inoculating swabs on 5% sheep blood agar (COS, bioMeriuex, France) and on a chrome agar (CPS ID, bioMeriuex, France). The culture plates were incubated at 37°C for 24 h. Anaerobic culture was conducted by inoculating the swabs on 5% sheep blood agar and incubating at 37°C for 48 h under anaerobic conditions. The isolation of fungi was attempted by inoculating the swabs on two tubes of Saboraud's dextrose agar and incubating each at 28 and 37°C. The culture tubes were examined for yeast growth after 48 h. A single microbiologist reviewed all plates and isolates. All culture isolates were further identified by using the API or the VITEK 2 system (bioMerieux, France) by the Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, Andhra Pradesh, India.

Data analysis

The chi square or Fisher's exact test for categorical variables and the Mann–Whitney or Kruskal–Wallis test for continuous variables were conducted for bivariate analyses, and multivariate logistic regression models were created for the primary analysis that included variables achieving $P<0.10$ from bivariate analyses – age, circumcision status, and participant group. Interactions between participant group and circumcision status were tested. Multivariate logistic regression models for two sets of subgroup analyses were also developed by using variables that achieved $P<0.10$ from bivariate analyses. In the first subgroup analysis, Groups 1 and 2 were evaluated to assess for the potential effect of clinical (diabetes, history of antibiotic use in past 6 months, previous STI, and presence of warts or other lesions) and behavioral (time spent bathing, time since last genital cleaning, wearing undergarments, and number of sex partners) characteristics, achieving $P<0.10$ from bivariate analyses associated with pathogen prevalence in the CS or the UR. Uncircumcised participants were included in the second subgroup analysis to assess the potential effect of subpreputial wetness, difficulty retracting the foreskin, foreskin length, and the presence of smegma that might be associated with pathogen prevalence in the CS or the UR. The software SPSS version 16.0 (SPSS Inc., Chicago, IL) was used for all analyses.

RESULTS

Three hundred fifteen study participants were enrolled: 150 in Group 1, 115 in Group 2, and 50 in Group 3. Overall, 37.1% of the participants were circumcised without variation across groups ($P=0.29$). Smegma was observed in 18.7% of the uncircumcised participants with no cases observed in Group 3 ($P<0.001$). Among Group 1, CD4 counts of <200, 200-350, and >350 were 40, 37.9, and 22.1%, respectively, and 92.7% of the participants were on highly active antiretroviral therapy. There were no differences between colonization of specific organisms based on the CD4 count. The culture of organisms from the CS yielded 41.6% colonized with *S. aureus*, 3.5% with *Enterococcus* spp., 22.9% with a gram-negative bacterium (*E. coli, Klebsiella* spp., or *Pseudomonas aeruginosa*), 1.6% with an anaerobe (*Clostridium*), 0.3% with a yeast (*Candida albicans*), and 60.0% with any pathogen. A coagulase-negative *Staphylococcus* sp. was present in 97.4% of all study participants. Gram-negative pathogens were more prevalent among study participants in Group 1 (22.7%) and Group 2 (30.4%) as compared with those in Group 3 (6.0%) ($P=0.003$). Urethral colonization demonstrated similar results and patterns.

The association of circumcision status with gram-positive pathogens, gram-negative pathogens, and all pathogens combined, controlling for age and participant group, are presented in Table 1. Interaction terms between the participant group and the circumcision status were not found to be statistically significant and were not included in the final logistic regression models.

Multivariable regression analyses including Groups 1 and 2 participants demonstrated the following significant associations: diabetes was positively associated with gram-positive pathogens in the CS and the UR; increasing frequency of changing underwear was associated with gram-positive pathogens in the CS; having one or more sex partners was associated with any pathogen of the CS and the UR; and not having a genital wart, lesion, or sore was associated with the presence of any pathogen of the UR (Odd ratio (ORs) 1.9–6.8; $P=0.02–0.05$). Not being circumcised remained a significant predictor of the pathogen presence in these analyses (ORs 1.9–3.1; all $P<0.05$). In multivariate analyses of uncircumcised participants, the relationship between smegma, foreskin length, subpreputial wetness, and pathogens was not found to be statistically significant except for the positive association between smegma and gram-positive pathogens of the CS (OR 2.6; $P=0.01$).

DISCUSSION

We found that bacterial pathogens were independently associated with uncircumcised status in a diverse population after controlling for age and clinical population. Findings of colonization in the uncircumcised participants were also robust, with consistent findings across two distinct penile anatomical sites. Several additional clinical and hygiene factors such as diabetes, absence of abnormal genital findings, decreased frequency of changing underwear, and number of sex partners were associated with colonization of the CS and the UR.

While increases in both pathogenic and nonpathogenic *Staphylococcus* spp. were found in one study, [14] ecological analyses of microbiota that suggested increases in *Staphylococcus* spp. after

circumcision did not differentiate between pathogenic organisms (e.g., *S. aureus*) and typically nonpathogenic organisms. Such analyses could potentially miss important shifts in species at the family or genus level.[9] Our finding of increased *S. aureus* in uncircumcised men was surprising and warrants further investigation and validation in other settings and contexts. The potential for misclassification of *S. aureus* for other species such as coagulase-negative *Staphylococcus* spp. exists; however, we would not have expected the misclassification to be differentially distributed to uncircumcised individuals. Our results also suggest no differences of colonization between any organism across age strata. This is consistent with a previous work in which variations in colonization of gram-negative organisms in the prepuce and *S. aureus* in the nares are evident in adolescent males[15] and the elderly,[16] respectively. Neither of these age categories were included in this study. Our finding that foreskin length and subpreputial wetness were not associated with the presence of pathogens suggests that if these two qualities are in fact associated with HIV infection,[5,17,18] the mechanism may not be mediated by bacterial pathogens. The presence of smegma, however, was independently associated with gram-positive pathogens in this study. While the secretions related to smegma may have antimicrobial properties, smegma is generally a marker of poor hygiene and further research into the properties and effects of smegma on microbial communities is warranted.

CONCLUSION

Uncircumcised men in this population in South India are more likely to harbor bacterial pathogens in the CS than do their circumcised counterparts. The finding that both gram-positive and gram-negative pathogens are more likely to be found in uncircumcised participants differs from that of a previous work that suggests shifts in microbiota following circumcision with decreases in gram-negative pathogens and increases in gram-positive pathogens.[9,14] An additional research to better understand host and microbiome interactions and their potential relationship with circumcision's efficacy in reducing the risk of HIV and other sexually transmitted infections is required. In many settings or subpopulations, circumcision is not an option given its lack of acceptability.[13] Similar to considerable efforts examining important microbial vaginal interactions and the relation to HIV transmission, further study of these potential foreskin microbial interactions would be required to develop nonsurgical methods that could achieve at least similar risk reduction rates as circumcision.

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Conflict of Interest: None declared.

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Figures and Tables

Table 1

Variable	*N* (% of Total)	Gram positive	Gram negative	Any pathogen
		Adjusted odds of the presence of gram-positive pathogens with multiple logistic regression (95% CI) (CS/UR)	Adjusted odds of the presence of gram-negative pathogens with multiple logistic regression (95% CI) (CS/UR)	Adjusted odds of the presence of any pathogen with multiple logistic regression (95% CI) (CS/UR)
Age (years)				
≤29	60 (19.1%)	Ref	Ref	Ref
30-35	115 (36.6%)	CS: 0.8 (0.4-1.5) UR: 0.9 (0.5-1.8)	CS: 1.0 (0.5-2.3) UR: 1.7 (0.6-4.2)	CS: 1.0 (0.5-1.9) UR: 1.3 (0.7-2.5)
36-45	78 (24.8%)	CS: 1.2 (0.6-2.5) UR: 1.5 (0.7-3.0)	CS: 1.3 (0.5-3.0) UR: 1.5 (0.6-4.2)	CS: 1.6 (0.8-3.4) UR: 1.6 (0.8-3.4)
≥45	61 (19.4%)	CS: 1.6 (0.8-3.4) UR: 1.9 (0.9-3.9)	CS: 1.2 (0.5-2.8) UR: 1.6 (0.6-4.2)	CS: 1.5 (0.7-3.3) UR: 1.5 (0.7-3.2)
Circumcision				
Yes	117 (37.1%)	Ref	Ref	Ref
No	198 (62.9%)	CS: 1.9 (1.2-3.1)* UR: 1.8 (1.1-2.9)*	CS: 2.4 (1.3-4.3)* UR: 2.8 (1.4-5.6)*	CS: 2.8 (1.7-4.6)‡ UR: 2.0 (1.2-3.3)‡
Group				
Control	50 (15.9%)	Ref	Ref	Ref
HIV	150 (47.6%)	CS: 0.4 (0.2-0.7) UR: 0.7 (0.4-1.4)	CS: 4.9 (1.4-16.9)* UR: 2.8 (0.8-9.8)	CS: 0.7 (0.3-1.3) UR: 1.8 (0.9-3.5)
TB	115 (36.5%)	CS: 0.8 (0.4-1.7) UR: 0.9 (0.4-1.8)	CS: 7.7 (2.1-27.9)‡ UR: 6.9 (1.9-25.4)‡	CS: 1.5 (0.7-3.2) UR: 1.7 (0.8-3.6)

*Gram-positive pathogens include *S. aureus* and *Enterococcus* spp.; gram-negative bacteria include *E. coli*, *Klebsiella* spp., and *P. aeruginosa*; any pathogens that include, in addition to gram-positive and gram-negative pathogens, *Clostridium* spp. and *C. albicans*, *$P<0.05$, ‡$P<0.005$.

Presence of pathogens in the coronal sulcus (CS) and the urethra (UR) by circumcision status among a diverse group of circumcised and uncircumcised Indian patients (n=315) in 2009*

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