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The Effect of Medical Male Circumcision on Urogenital *Mycoplasma genitalium* among Men in Kisumu, Kenya

Supriya D. Mehta, MHS, PhD[1], **Charlotte Gaydos, DrPH**[2], **Ian Maclean, PhD**[3], **Elijah Odoyo-June, MBChB, MSc**[4], **Stephen Moses, MD, MPH**[3], **Lawrence Agunda**[4], **Nicole Quinn**[2], and **Robert C. Bailey, PhD, MPH**[1]

[1]Division of Epidemiology and Biostatistics, University of Illinois Chicago School of Public Health, Illinois, USA

[2]Department of Medicine, The Johns Hopkins University School of Medicine, Maryland, USA

[3]Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada

[4]UNIM Project, Kisumu, Kenya

Abstract

Background—We determined the prevalence of urethral *Mycoplasma genitalium* (MG) infection and whether infection was associated with circumcision status, among men enrolled in the randomized trial of medical male circumcision to prevent HIV acquisition in Kisumu, Kenya.

Methods—*M. genitalium* and *Trichomonas vaginalis* (TV) were detected in first void urine (FVU) by APTIMA transcription mediated amplification assay. FVU and urethral swabs were assessed for *N. gonorrhoeae* (NG) and *C. trachomatis* (CT) by polymerase chain reaction assay. HSV-2 antibodies were detected by IgG ELISA. Multivariable logistic regression identified factors associated with MG infection.

Results—Specimens were collected between July and September 2010, and 52 [9.9%; 95% CI: 7.3–12.4%] MG infections were detected among 526 men. NG and TV were not associated with MG. CT co-infection was 5.8% in MG-infected men, and 0.8% among MG-uninfected men (p=0.02). MG infection was predominantly asymptomatic (98%). The prevalence of MG was 13.4% in uncircumcised men vs. 8.2% in circumcised men (p=0.06). Being circumcised nearly halved the odds of MG [adjusted OR=0.54; 95% CI: 0.29–0.99], adjusted for other variables significant at the p<0.05 level: HSV-2 infection [aOR=2.05; 95% CI: 1.05–4.00], CT infection [aOR=2.69; 95% CI: 1.44–5.02], and washing the penis <=1 hour after sex [aOR=0.47; 95% CI: 0.24–0.95].

Conclusions—MG infection was reduced among men who were circumcised, adding to the benefits of male circumcision in preventing several sexually transmitted infections.

Keywords

circumcision; *Mycoplasma genitalium*; Kenya; HSV-2; *Chlamydia trachomatis*

Corresponding Author: Dr. Supriya Mehta, 958 SPHPI M/C 923; 1603 W. Taylor St.; Chicago, IL 60622; phone: (312) 413-7485; fax (312) 996-0064; supriyad@uic.edu.

Introduction

Mycoplasma genitalium is a causative pathogen in urethritis, cervicitis, pelvic inflammatory disease (PID), and tubal factor infertility [1–3]. In a meta-analysis of 19 studies, *M. genitalium* doubled the odds of HIV infection, and the association increased to 2.6 among studies conducted in sub-Saharan Africa [4]. In South Africa, the prevalence of *M. genitalium* ranges from 2–17%, with highest prevalence among men and women with genital discharge or genital ulcers [5–8]. Studies in western Africa found prevalences of 2% in the general population [9]; 12–18% in HIV infected or symptomatic persons [10–11]; and 26% in female sex workers [12]. In eastern Africa the prevalence of *M. genitalium* has been reported to be 3–5% in the general population in Tanzania [13–14], 16% in female sex works in Nairobi, Kenya [15], and 17% in HIV-positive women in Mombasa, Kenya [16]. No published studies have examined the association of male circumcision status with *M. genitalium* infection.

We determined the prevalence of *M. genitalium* and its association with circumcision status, socio-demographic characteristics, and behavioral factors among young men enrolled in a randomized trial of male circumcision to reduce HIV incidence in Kisumu, Kenya.

Methods

Study Design and Participants

From 2002–2005, our trial in Kisumu enrolled 2,784 men aged 18–24 years. Trial recruitment, enrollment, reasons for refusing enrollment, and follow-up have been previously described [17]. For inclusion men had to be: uncircumcised, HIV-negative, sexually active in the last 12 months, and aged 18–24 years; have a hemoglobin > 9.0 mmol/L; and reside in Kisumu District. Exclusion criteria included: foreskin covering less than half of the glans, a bleeding disorder, keloid formation, other conditions that might increase the risks of elective surgery, or a medical indication for circumcision. Following written informed consent, participants were randomized 1:1 to either immediate circumcision or delayed circumcision after a two-year follow-up period (the control group). Both groups underwent risk reduction counseling for STIs and HIV and were provided unlimited supplies of free condoms. Detailed evaluations were conducted at baseline, and at 1 month, 3 months, and every 6 months from randomization for both the circumcision and the control groups. The trial ended in December 2006, but follow-up continued until September 30, 2010. This study examines a cross-sectional sample of enrolled men, with specimens collected between July 16 and September 30, 2010.

The current study was approved by the Institutional Review Boards of the University of Illinois at Chicago, the Kenyatta National Hospital, RTI International, the Johns Hopkins University, and the University of Manitoba.

Sexually Transmitted Infection and HIV Testing

For detection of *Mycoplasma genitalium*, an aliquot of first void urine was placed in the appropriate GEN-PROBE Specimen Transport Media for the APTIMA transcription mediated amplification (TMA)-based assay (GEN-PROBE, Inc, San Diego, CA). Aliquotted urine specimens were immediately frozen at −80°C. Specimens were shipped on dry ice to The Johns Hopkins University International STD Reference Laboratory for testing with our previously published methods using the TMA MG research use only assay [1–2]. First void urine specimens were also tested for *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) by polymerase chain reaction (PCR) assay (AMPLICOR® CT/NG Test, Roche Diagnostics, Montreal, Canada), for *Trichomonas vaginalis* (TV) by TMA, and for TV by culture (InPouch™ TV test, Biomed Diagnostics, Oregon, United States). Men with

urethral discharge had a urethral swab taken for detection of NG and CT by PCR, and for detection of NG and TV by culture. Serum specimens were tested for syphilis antibody (rapid plasma reagin with TPHA confirmation) and herpes simplex virus type 2 (HSV-2) antibody (Kalon HSV-2 IgG ELISA, Kalon Biological Limited, Aldershot, United Kingdom). Urine and urethral swab specimens were sent to the University of Nairobi, Department of Medical Microbiology for NG and CT PCR. HSV-2 and syphilis testing and culture for TV and NG were conducted at the UNIM (Universities of Nairobi, Illinois, and Manitoba) lab in Kisumu. All tests were conducted according to manufacturers' instructions.

Testing for HIV infection was conducted using a parallel double rapid test protocol, using Determine® HIV 1/2 (Abbott Diagnostic Division, Hoofddorp, The Netherlands), and the Uni-Gold Recombigen™ HIV Test (Trinity Biotech, Wicklow, Ireland). Men with concordant positive results were informed of their HIV status and followed-up at the study clinic or at the New Nyanza Provincial Hospital. Men who were concordant negative were eligible for the trial.

Data Collection

All consenting participants underwent standardized medical examination and history, and personal interview to obtain socio-demographic and health information and to assess behavioral risk factors. Interviews were conducted by trained, experienced, local language-speaking counselors in the participant's language of choice, English, Dholuo, or Kiswahili.

Data Analysis

The outcome for this analysis was infection with *M. genitalium*. Explanatory variables included demographic characteristics, sexual behaviors, post-coital cleaning, penile trauma, circumcision status, HSV-2 serostatus, and current and prior non-ulcerative STI infection. We used circumcision status (as treated analysis) rather than treatment assignment (intention to treat) because randomization ended in 2006, and since then men have been free to choose to become circumcised if they wish. A cross-sectional sample of men was tested based on resource availability. To assess potential selection bias, we compared demographics, sexual behaviors, and STIs between men who were tested for *M. genitalium* and those who were not.

Differences between categorical explanatory variables and the outcome were assessed by the chi-square test or Fisher's exact test when cell size was < 5. Variables significant at the $p<0.10$ level by likelihood ratio testing from univariate logistic regression were entered into multivariable logistic regression using a stepwise procedure with forwards selection for entry, and likelihood ratio testing for backwards elimination. Those variables with a likelihood ratio p-value <0.05 were maintained in the multivariable model. Wald test p-values are presented for the final multivariable model. Data were analyzed using STATA/SE 11.0 for Windows (Stata Corp., College Station, TX).

Results

Study Sample

From June 13 to September 30, 2010, 526 men were tested for *M. genitalium*. Compared to men being followed but who were not included in the current protocol, men who underwent *M. genitalium* testing were more likely to be aged 26–31 than aged 23–25 (42% vs. 34%, $p=0.006$) and married/cohabiting (60% vs. 46%, $p<0.001$), but did not differ with regards to education, circumcision status, sexual behaviors, HIV prevalence, or baseline or cumulative ulcerative and non-ulcerative STI incidence [results not shown; available from authors].

Participants were 67.3% circumcised, 59.8% married or cohabiting, 5.9% HIV positive (Table 1).

Mycoplasma genitalium Infection

M. genitalium was detected in 52 men [9.9%; 95% CI: 7.3–12.4%]. We traced and treated 46 (88%) of 52 men with oral azithromycin 1g. The majority (98.1%) of men with MG were asymptomatic (Table 1). Dysuria was reported by 1 (1.9%) man with *M. genitalium* and 8 (1.7%) men who were uninfected. There were no examination findings or complaints of genital ulcers (past 6 months, current symptoms) or testicular pain or swelling in any of the men.

Prevalence of Other STIs and Co-Infections

Date matched results to assess co-infection with CT and NG were available for 85% (n=445) of men. Infection with CT was 1.3% [95% CI: 0.3–2.3%], and was more common among men infected with *M. genitalium* than those who were not infected (5.8% vs. 0.8%, Fisher's exact p-value = 0.024; Table 2). The prevalence of NG was 1.4% [95% CI: 0.3 – 2.4%] and did not differ by *M. genitalium* infection status. There was 1 (0.2%) culture detected TV infection, out of 14 TV infections detected by urine TMA [2.67%; 95% CI: 1.3–4.0%]. TV infection did not differ by *M. genitalium* infection status. Infection with HSV-2 was 56.7% [95% CI: 52.4–60.9%] and was more common among men infected with *M. genitalium* (71.2% vs. 55.1%, p=0.026). NG, CT, or TV co-infection occurred in 5 (9.6%) *M. genitalium* infections. There was one case of syphilis detected in an uninfected, uncircumcised participant.

Univariate Factors Associated with M. genitalium Infection

The reported age of men at follow-up ranged from 23–30 years, and did not differ by *M. genitalium* status (Table 1). There were no significant differences at the p<0.10 level between infected and uninfected men with regard to educational attainment at baseline, current marital status, or current residence in Kisumu district. The distributions of sexual risk behaviors were similar between infected and uninfected men with regards to number of sex partners, condom use at last sexual intercourse, having sex with a woman during her menses, and having sex with a woman the same day as meeting. Compared to uninfected men, a greater proportion of *M. genitalium*-infected men reported sex in exchange for money or gifts (10.2% vs. 4.5%, p=0.085), and a lower proportion reported cleaning the penis ≤ 1 hour after last sexual intercourse (24.0% vs. 39.7%, p=0.030). Infection with CT at trial enrollment (11.8% vs. 3.4%, p=0.005) and cumulative CT infection over trial follow-up CT (26.9% vs. 15.8%, p=0.043) were more common among *M. genitalium*-infected than uninfected men. Baseline and follow-up CT infection were correlated (r=0.41), and had similar magnitude of crude association with *M. genitalium* infection (Table 1). Likelihood ratio testing showed that a combined variable of baseline or incident CT infection explained more variance than either variable alone, and this combined variable was used in multivariable regression.

Multivariable Logistic Regression: Factors Associated with M. genitalium Infection

In multivariable logistic regression (Table 3), circumcision nearly halved the odds of *M. genitalium* [adjusted OR=0.54; 95% CI: 0.29–0.99]. Other variables significant at the p<0.05 level were HSV-2 infection [aOR=2.05; 95% CI: 1.05–4.00], CT infection [aOR=2.69; 95% CI: 1.44–5.02], and washing the penis ≤1 hour after sex [aOR=0.47; 95% CI:0.24–0.95]. There were no significant two-way interactions between any of these variables. Variables significant at the p<0.10 level from univariate analysis that were not

significant in multivariable analysis included exchange of sex for money or gifts and TV infection.

Discussion

The prevalence of *M. genitalium* in our cohort of young men in Kisumu, Kenya, was high (10%) and the majority was asymptomatic (98%). The odds of urethral *M. genitalium* were nearly halved for circumcised men compared to uncircumcised men, controlling for behavioral risks. We are not aware of other published reports of circumcision status and behavioral factors associated with *M. genitalium* in men to which we can compare our results.

In our trial [18] and the male circumcision trial in South Africa [19], male circumcision did not protect against urethral *N. gonorrhoeae*, *C. trachomatis*, or *T. vaginalis*. In men, *M. genitalium* is considered primarily a urethral infection. However, a recent report of men with non-gonococcal urethritis found that men with *M. genitalium* had more than a four times higher odds of balanoposthitis, controlling for CT and ureaplasma [20]. Horner et al. suggest that *M. genitalium* may be capable of infecting the poorly keratinized foreskin [20], as *in vitro* study has demonstrated that *M. genitalium* can infect vaginal epithelial cells [21]. Evaluation of the penile microbiome among participants in the circumcision trial in Uganda showed that circumcision led to a significant reduction in anaerobic and facultative anaerobic bacteria [22]. As *M. genitalium* is a facultative anaerobe, this may explain how *M. genitalium* would be recovered more frequently in uncircumcised than circumcised men. In our study, while *M. genitalium* was detected in urine, these two mechanisms (susceptibility of the foreskin to infection, anoxic microenvironment) provide explanation as to how the foreskin could provide a reservoir for urethral infection in uncircumcised men.

We found that men who reported cleaning their penis shortly after their last sexual intercourse were less likely to be infected with *M. genitalium*. One of the mechanisms by which circumcision is thought to protect against genital ulcer disease [23] and balanitis [24] is through improved genital hygiene. Thus men with better genital hygiene may be reducing the *M. genitalium* reservoir, decreasing likelihood of urethral infection, and this may be expected to have greater impact for uncircumcised men. While the interaction term was not statistically significant (p=0.19), for uncircumcised men, cleaning the penis ≤ 1 hour after intercourse had an OR of 0.28, and the OR was 0.68 for men who were circumcised.

In our study, HSV-2 seropositivity doubled the odds of *M. genitalium* infection. This association may represent an overlap in similar risk factors for these two sexually acquired infections. Another potential explanation is that *M. genitalium* was more likely to be *detected* among men with HSV-2, as men with HSV-2 may have greater inflammation, more persistent infection, or greater *M. genitalium* organism load. The effect of HSV-2 infection on detection of *M. genitalium* or on *M. genitalium* organism load has not been assessed. We are unaware of published studies reporting the effect of HSV-2 infection on the sensitivity and specificity of detection of NG, CT, or TV that may provide insight.

Infection with *M. genitalium* was associated with CT infection but not with NG or TV. This may be related to differences in risks for acquisition, with risks for CT and *M. genitalium* being more similar than risks for NG or TV. Two prospective cohort studies of female students in London [25] and sex workers in Nairobi [15] found more than a doubling of *M. genitalium* risk associated with incident CT adjusted for demographics, behavioral risks, and other STIs. In the study of sex workers in Nairobi, NG also increased the risk of *M. genitalium*. Among HIV-positive Kenyan women, *M. genitalium* organism burden was higher for women who were co-infected with CT but was not associated with NG infection

[16]. Broad acceptance of *M. genitalium* as a pathogenic STI is relatively recent. The overlapping epidemiology and potential biological synergism between *M. genitalium* and other STIs have not been studied extensively and are not well-understood.

Most *M. genitalium* infections (98%) were asymptomatic. The proportion of *M. genitalium* infections that were asymptomatic among male STI clinic attendees in Scandinavia is 34–39% [26–27], while results from a mobile STI service in South Africa found that 90% of *M. genitalium* infections were asymptomatic [6]. While the implications of pathogenesis and transmission of these asymptomatic infections in men are unclear, a high prevalence of *M. genitalium*, even if largely asymptomatic in men, is a public health concern due to associated risks of upper reproductive tract infection in women [3]. Determining the contribution of *M. genitalium* to upper genital tract infection among women in this region will be necessary for implementing the most efficacious treatment regimens.

TV was detected in 2.5% of men by urine TMA, in contrast to the one infection detected by urine culture. The sensitivity of culture for TV compared to TMA is ≤50% [28–29]. Throughout our trial and extended follow-up of the cohort, we used culture to identify TV. In our 24-month analysis [18], the incidence of TV was 2.5 per 100 person-years. It is clear from the current results that our previous analysis significantly underestimated the burden of TV infection. Future research measuring TV should invest in sensitive, nucleic acid-based methods of detection, as this will have a significant impact on prevalence and identifying risks for infection, as well as examining TV as a risk factor for other outcomes, such as HIV and adverse pregnancy outcomes.

Limitations

As this was a cross-sectional sample, temporal bias is a potential concern. However, most of the variables evaluated (circumcision status, HIV and HSV-2 status, and previous non-ulcerative STI infection status) were measured prior to detection of *M. genitalium* infection. Because 85% of circumcised men in this sample had been circumcised for at least 2 years prior to detection of infection, it is unlikely that men were circumcised in response to *M. genitalium* infection. While we have not identified significant differences between men who chose circumcision and those who remained uncircumcised after the trial ended with regard to age, number of recent sex partners, HSV-2 status, or non-ulcerative STI [30], it is possible that the protective association observed between being circumcised and *M. genitalium* infection is confounded by factors associated with choosing circumcision. Although we were unable to test all men in the cohort for *M. genitalium*, those tested did not differ from those who were not tested with regards to behavioral risks, HIV status, or STI history, indicating that resource constraints did not produce significant selection bias.

Conclusions

Male circumcision approximately halved the odds of *M. genitalium* infection, adding to the benefits of male circumcision in preventing several sexually transmitted infections. The effects of male circumcision in reducing HPV, penile carriage of anaerobic bacteria, and genital ulcers, are transferred to female sex partners [31–32]. Because of its association with urethritis, cervicitis, and PID, a reduction in MG through male circumcision is of public health importance to men and their female sex partners.

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Table 1

Factors Associated with *Mycoplasma genitalium* Infection.

variable	*M. genitalium* Infection Status	
	Positive, N=52 n (%)	**Negative, N=474 n (%)**
Circumcised^	29 (44.8)	325 (68.6)
Age at follow-up (years)		
23–25	22 (42.3)	169 (35.7)
26–28	26 (50.0)	234 (49.4)
29–31	4 (7.7)	71 (15.0)
Married or Cohabiting	35 (70.0)	272 (58.8)
Education completed at Baseline		
Primary, None	22 (42.3)	166 (35.0)
Some secondary	12 (23.1)	105 (22.2)
Secondary or higher	18 (34.6)	203 (42.8)
District of residence Kisumu (vs. Other)	32 (62.8)	290 (61.7)
Sexual Behaviors+		
Number of sex partners		
0–1	33 (66.0)	309 (66.9)
2 or more	17 (34.0)	153 (33.1)
Used a condom at last vaginal intercourse	20 (40.0)	203 (43.8)
Cleaned penis ≤ 1 hour after last sex*	12 (24.0)	184 (39.7)
Exchanged gifts or money for sex^	5 (10.2)	20 (4.5)
Sex with a woman the same day as meeting	12 (24.0)	106 (22.9)
Had sex with a woman during menstruation	3 (6.4)	29 (6.7)
HIV and Sexually Transmitted Infections		
HIV seropositive	4 (7.7)	27 (5.7)
HSV-2 seropositive*	37 (71.2)	261 (55.1)
Non-ulcerative STIs at Baseline		
*C. trachomatis**	6 (11.8)	16 (3.4)
N. gonorrhoeae	0 (0.0)	13 (2.8)
T. vaginalis	2 (3.9)	9 (1.9)
Non-ulcerative STIs over Follow-Up (cumulative incidence post-baseline)		

variable	*M. genitalium* Infection Status	
	Positive, N=52 n (%)	**Negative, N=474 n (%)**
C. trachomatis[*]	14 (26.9)	75 (15.8)
N. gonorrhoeae	6 (11.5)	51 (10.8)
T. vaginalis[*]	5 (9.6)	12 (2.5)
Current Co-infection		
C. trachomatis[*]	3 (7.0)	4 (1.0)
N. gonorrhoeae	0 (0.0)	6 (1.5)
T. vaginalis	2 (3.8)	12 (2.5)
Signs and Symptoms		
Urethral Discharge, Current complaint and exam	1 (1.9)	7 (1.5)
Dysuria, Current complaint	1 (1.9)	8 (1.7)

[+] Recall period for sexual behaviors and practices is past 6 months unless otherwise specified.

[^] Indicates 0.05< Chi-square or Fisher's exact p-value <0.10;

[*] indicates Chi-square or Fisher's exact p-value <0.05

Table 2

Number and Percent of Co-Infections by Pathogen.

Co-Infections	**Total, N=526 n (%)**	***M. genitalium* Positive n/N (%)**	***M. genitalium* Negative n/N (%)**	**Fisher's exact p-value**
C. trachomatis	7 (1.6)	3/45 (7.0)	4/400 (1.0)	0.023
N. gonorrhoeae[*]	6 (1.4)	0/45 (0.0)	6/400 (1.5)	1.000
T. vaginalis[^]	14 (2.7)	2/52 (3.8)	12/474 (2.5)	0.639
C. trachomatis, N. gonorrhoeae, or T. vaginalis	26 (4.9)	5/52 (9.6)	21/474 (4.4)[+]	0.165

[*] All *N. gonorrhoeae* infections were detected by PCR; none were positive by culture.

[^] Among 14 *T. vaginalis* infections detected by TMA, one was also positive by culture.

[+] Includes one dual NG-TV infection.

Table 3

Results of Univariate and Multivariable Logistic Regression: Factors Associated with *Mycoplasma genitalium* infection, n=513.

	Crude Odds Ratio [95% CI]	Adjusted Odds Ratio [95% CI]
Circumcised	0.58 [0.32–1.03]	0.54 [0.29–0.99]
Exchanged gifts or money for sex	2.40 [0.86–6.73]	
Cleans penis ≤ 1 hour after sex	0.48 [0.24–0.94]	0.47 [0.24–0.95]
HSV-2 seropositive	2.01 [1.08–3.77]	2.05 [1.05–4.00]
Chlamydia infection at baseline or over follow-up	2.63 [1.44–4.81]	2.59 [1.39–4.85]
Trichomonas infection over follow-up	4.10 [1.38–12.1]	

CI = Confidence Interval

Crude odds ratio presented for variables significant at the $p<0.10$ level from univariate analyses.

Adjusted Odds Ratio adjusted for all variables presented.