BRIEF REPORT

# Male Circumcision and Herpes Simplex Virus Type 2 Infection in Female Partners: A Randomized Trial in Rakai, Uganda

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**Male circumcision reduces acquisition of herpes simplex virus type 2 (HSV-2) in men. We assessed whether male circumcision reduces HSV-2 infection among female partners. HSV-2–negative, human immunodeficiency virus–negative female partners of 368 males who were and 372 males who were not randomized to receive male circumcision were enrolled. The incidence of HSV-2 infection among females over a period of 2 years was 6.09 cases per 100 person-years in the intervention arm and 6.32 cases per 100 person-years in the control arm (incidence rate ratio [IRR], 0.96 [95% confidence interval {CI}, .62–1.49]; $P = .87$). Among female partners of HSV-2–positive males, the incidence of HSV-2 infection was 9.55 cases per 100 person-years in the intervention arm and 11.17 cases per 100 person-years in the control arm (IRR, 0.85 [95% CI, .44–1.67]; $P = .62$). Contrary to findings in males, male circumcision did not affect HSV-2 acquisition among female partners.**

Herpes simplex virus type 2 (HSV-2) is one of the most common sexually transmitted infections (STIs) and causes recurrent, painful genital lesions [1]. HSV-2 is also associated with a 3-fold increased risk of acquiring human immunodeficiency virus (HIV) in observational studies, but trials of HSV-2 suppression did not reduce HIV acquisition or transmission [1].

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Three randomized trials in South Africa, Kenya, and Uganda demonstrated that male circumcision (MC) significantly decreased HIV acquisition in men [2–4]. Two of these trials showed that MC reduces HSV-2 acquisition in men [5, 6]. The Ugandan trial also showed that female partners of circumcised men had decreased genital ulcer disease (GUD), *Trichomonas vaginalis* infection, bacterial vaginosis, and human papillomavirus infection [7, 8]. The decrease in GUD among females may be due to decreased HSV-2 acquisition among female partners of circumcised men. Two observational studies suggested that MC is associated with lower HSV-2 infection in female partners [9, 10], but these results may be due to confounding.

We used data from a randomized controlled trial of MC in HIV-negative males in Rakai, Uganda, to assess the efficacy of MC for reducing the incidence of HSV-2 infection among female partners.

## Materials and Methods

### *Participants, Study Design, and Randomization*

The Rakai Health Sciences Program in Rakai enrolled 4996 HIV-negative males in an MC trial for HIV/STI prevention [2, 6, 11]. Males were eligible for enrollment if they were uncircumcised, aged 15–49 years, had no medical indications or contraindications for MC, and provided written informed consent. Males were randomly assigned to receive immediate MC (intervention arm) or MC delayed for 24 months (control arm).

Consenting females who were married or in long-term consensual relationships with male trial participants were invited to participate in a separate parallel trial [7, 8, 12]. All female participants provided written informed consent. The effects of MC on female STIs were secondary trial outcomes. The primary objective of this analysis was to assess the efficacy of MC of HIV-negative males on the incidence of HSV-2 infection among female partners.

At each study visit (enrollment, year 1, and year 2), females were interviewed to ascertain sociodemographic characteristics, sexual risk behaviors, and health status. At each visit, females were asked to provide blood samples, which were maintained at 4°C–10°C for <6 hours and then at −80°C until assayed.

The trials were approved by the Uganda National Council for Science and Technology, the Science and Ethics Committee of the Uganda Virus Research Institute (Entebbe), the Committee for Human Research at Johns Hopkins University Bloomberg School of Public Health (Baltimore, Maryland), and the Western Institutional Review Board (Olympia, Washington) [2, 7, 8]. The trials were overseen by independent data and safety monitoring boards. The trials were registered with ClinicalTrials.gov (identifiers NCT00425984 and NCT00124878).

### *HSV-2 and HIV Detection*

HSV-2 infection was determined by an HSV-2 enzyme-linked immunosorbent assay (ELISA; Kalon Biological) [6, 13, 14]. HSV-2 seroconversion was defined as a negative result of serological testing at enrollment (optical density index value, ≤0.9), followed by a positive result of serological testing at follow-up (optical density index value ≥1.5) [14]. All positive cases by ELISA were confirmed by Western blotting (Euroimmun). Borderline results of Western blotting were considered positive. HIV status was determined using 2 separate ELISAs, and discordant results were confirmed by HIV-1 Western blotting [2].

### *Statistical Analysis*

Enrollment and follow-up characteristics, sexual risk behaviors, and STI symptoms in males and their female partners were tabulated by study arm, and differences were assessed by $\chi^2$ tests. All *P* values were 2-sided.

The primary assessment of MC efficacy for reduced HSV-2 infection incidence among females used an intention-to-treat analysis. An as-treated analysis was also carried out, in which intervention arm females were classified as crossover exposures if their male partner remained uncircumcised at the annual follow-up visit, and partners of control arm males were classified as crossover exposures if the male partner underwent MC from other sources during the follow-up interval in which the procedure was performed.

For incidence rate and person-time calculations, it was assumed that HSV-2 infection occurred at the midpoint between the last negative and first positive serological test. Time from enrollment was accumulated to the 24-month follow-up visit or the last available visit, and HSV-2 infection incidence was estimated as cases per 100 person-years (py). Incidence rate ratios (IRRs) and 95% confidence intervals (CIs) of HSV-2 acquisition in the intervention group as compared with the control group were estimated using a Poisson log-linear model. Because some females were in polygamous marriages with the same male partner, generalized estimating equation (GEE) exchangeable correlation structure was used to account for the potential correlation between females with the same partner. Multivariate Poisson regression with GEE robust variance was used to estimate adjusted IRRs after adjustment for enrollment covariates that differed significantly between the arms at $P > .15$ and included marital status, self-reported dysuria, and known risk factors for HSV-2 (ie, age, number of sex partners [1 vs >1], condom use, and alcohol use before sex).

Analyses were performed using Stata 11.2 (StataCorp), R 2.8.1, and SAS 9.2 software (SAS Institute).

## Results

A total of 4996 HIV-negative males were enrolled and randomized. Of the 2474 intervention arm males at enrollment, 1167 (47.2%) reported being married or in consensual union with 1264 female spouses (1.08 female partners per male). Of 2522 control arm males at enrollment, 1173 (46.5%) reported being married or in consensual union with 1239 female spouses (1.06 female partners per male). The number of linked females exceeded the number of enrolled males because of polygamous relationships. At the time of female enrollment, 1203 female partners of intervention arm males (95.2%) were HIV negative, and 1171 female partners of control arm males (94.5%) were HIV negative. Among these HIV-negative married females, 835 (69.4%) were enrolled concurrently with their husbands in the intervention arm, and 803 (68.6%) were enrolled concurrently with their control arm husbands. Of these females, 368 were HSV-2 negative at enrollment in the intervention arm (44.1%) and 372 were HSV-2 negative at enrollment in the control arm (46.3%). These HIV-negative, HSV-2 negative females who enrolled concurrently with their husbands constituted the primary analysis population for this study.

The female retention rates at the year 1 follow-up visit were 95.4% (351 of 368) in the intervention arm and 94.9% (353 of 372) in the control arm. At the year 2 follow-up visit, female retention rates were 91.0% (335 of 368) in the intervention group and 89.0% (331 of 372) in the control group.

Male baseline sociodemographic characteristics, sexual behaviors, and symptoms of STIs were similar between study arms (Table 1). At enrollment, the HSV-2 prevalence among males was 16.8% (59 of 352) in the intervention arm and 13.2% (48 of 363) in the control arm ($P = .41$) (Table 1). The female enrollment characteristics were similar between study arms, except there were more female partners in polygamous relationships in the intervention arm, compared with the control arm ($P = .038$) (Table 1). During the 2-year trial, 1 male in the intervention arm (0.3%) and 3 males in the control arm (0.8%) acquired HIV ($P = .33$). In addition, 5 females in the intervention arm (1.4%) and 3 females in the control arm (0.8%) acquired HIV ($P = .47$).

In the intention-to-treat analysis, HSV-2 infection was detected in 40 female partners of males in the intervention group and in 41 female partners of males in the control group over the 2-year follow-up period. The incidence of HSV-2 infection among female partners at year 2 was 6.09 cases per 100 py in the intervention arm and 6.32 cases per 100 py in the control arm (IRR, 0.96 [95% CI, .62–1.49]; $P = .87$) (Table 2). Some females were in polygamous marriages with the same male partner, but adjustment for the potential correlation between females with the same partner did not change the efficacy estimate (IRR, 0.96 [95% CI, .62–1.49]; $P = .86$). After adjustment for female age, marital status, dysuria, number of sexual partners, condom use, and alcohol use before sex, the adjusted IRR of HSV-2 acquisition in female partners of intervention relative to control arm males was 1.00 (95% CI, .65–1.55; $P = .99$).

In an as-treated analysis, the incidence of HSV-2 acquisition was 6.14 cases per 100 py (41 cases per 667.0 py) in female

**Table 1. Baseline Characteristics, Risk Behaviors, and Sexually Transmitted Infection Symptoms Among Males and Their Female Partners, by Study Arm**

| | Males, No (%), by Study Group | | | Females, No. (%), by Study Group | | |
|---|---|---|---|---|---|---|
| | Intervention | Control | *P* Value | Intervention | Control | *P* Value |
| | (n = 353) | (n = 365) | | (n = 368) | (n = 372) | |
| Age | | | .82 | | | .84 |
| 15–19 y | 8 (2.3) | 7 (1.9) | | 80 (21.7) | 72 (19.6) | |
| 20–24 y | 85 (24.1) | 93 (25.5) | | 139 (37.8) | 148 (39.8) | |
| 25–29 y | 127 (36.0) | 120 (32.9) | | 99 (26.9) | 104 (28.0) | |
| 30–49 y | 133 (37.7) | 145 (39.7) | | 50 (13.6) | 48 (12.9) | |
| Marital status | | | .11 | | | .038 |
| Monogamous | 317 (89.8) | 340 (93.2) | | 320 (87.0) | 341 (91.7) | |
| Polygamous | 36 (10.2) | 25 (6.8) | | 48 (13.0) | 31 (8.3) | |
| Religion | | | .66 | | | .99 |
| Catholic | 225 (63.7) | 244 (66.8) | | 224 (60.9) | 223 (59.9) | |
| Protestant | 105 (29.7) | 104 (28.5) | | 106 (28.8) | 108 (29.0) | |
| Saved/ Pentecostal/other | 21 (5.9) | 16 (4.4) | | 28 (7.6) | 30 (8.1) | |
| Muslim | 2 (0.6) | 1 (0.3) | | 10 (2.7) | 11 (3.0) | |
| Education | | | .78 | | | .46 |
| No education | 29 (8.2) | 30 (8.2) | | 49 (13.3) | 40 (10.8) | |
| Primary | 249 (70.5) | 256 (70.1) | | 266 (72.3) | 266 (71.5) | |
| Secondary | 56 (15.9) | 53 (14.5) | | 43 (11.7) | 56 (15.1) | |
| Postsecondary | 19 (5.4) | 26 (7.1) | | 10 (2.7) | 10 (2.7) | |
| Sex partners in past year, no.[a] | | | .78 | | | .35 |
| 0 | 0 | 0 | | 0 | 0 | |
| 1 | 215 (60.9) | 213 (58.4) | | 359 (97.6) | 358 (96.2) | |
| 2 | 99 (28.0) | 110 (30.1) | | 7 (1.9) | 13 (3.5) | |
| ≥3 | 39 (11.0) | 42 (11.5) | | 2 (0.5) | 1 (0.3) | |
| Nonmarital relationships | | | .56 | | | .80 |
| No | 308 (87.3) | 313 (85.8) | | 362 (98.4) | 365 (98.1) | |
| Yes | 45 (12.7) | 52 (14.2) | | 6 (1.6) | 7 (1.9) | |
| Condom use in past 12 mo | | | .25 | | | .32 |
| None | 238 (67.4) | 231 (63.3) | | 309 (84.0) | 302 (81.2) | |
| Consistent or inconsistent use | 115 (32.6) | 134 (36.7) | | 59 (16.0) | 70 (18.8) | |
| Alcohol use with sex in past 6 mo | 186 (52.7) | 201 (55.1) | .52 | 107 (29.1) | 104 (28.0) | .74 |
| Transactional sexual intercourse[b] | 3 (0.8) | 5 (1.4) | .51 | 2 (0.5) | 3 (0.8) | .66 |
| Self-reported symptoms of STIs | | | | | | |
| Genital ulcer disease | 14 (4.0) | 24 (6.6) | .12 | 31 (8.4) | 33 (8.9) | .83 |
| Urethral discharge | 8 (2.3) | 9 (2.5) | .86 | 169 (45.9) | 156 (41.9) | .27 |
| Dysuria | 10 (2.8) | 20 (5.5) | .08 | 77 (20.9) | 62 (16.7) | .14 |
| HSV-2 status[c] | | | .41 | | | 1.00 |
| Negative | 254 (72.2) | 272 (74.9) | | 368 (100.0) | 372 (100.0) | |
| Indeterminate | 39 (11.1) | 43 (11.8) | | 0 | 0 | |
| Positive | 59 (16.8) | 48 (13.2) | | 0 | 0 | |

The number of men is less than the number of female partners enrolled, because of polygynous unions.

Abbreviations: HSV-2, herpes simplex virus type 2; STI, sexually transmitted infection.

[a] Includes long-term partners.

[b] Defined as sexual intercourse in exchange for money or gifts.

[c] Could not be determined for 1 male in the intervention arm and for 2 males in the control arm.

**Table 2. Incidence of Herpes Simplex Virus Type 2 Infection Among Female Partners, by Study Arm and Follow-up Interval**

| Follow-up Interval, Variable | Intervention Group | Control Group | Incidence Rate Ratio (95% CI) | *P* Value |
|---|---|---|---|---|
| 0–12 mo | | | | |
| Participants, No. | 351 | 353 | | |
| Incident events, No. | 17 | 26 | | |
| Person-years, No. | 342.5 | 340.0 | | |
| Incidence[a] | 4.96 | 7.65 | 0.65 (.35–1.20) | .17 |
| 12–24 mo | | | | |
| Participants, No. | 310 | 297 | | |
| Incident events, No. | 22 | 13 | | |
| Person-years, No. | 299.0 | 290.5 | | |
| Incidence[a] | 7.36 | 4.48 | 1.64 (.83–3.26) | .16 |
| 0–24 mo[b] | | | | |
| Participants, No. | 8 | 10 | | |
| Incident events, No. | 1 | 2 | | |
| Person-years | 15.0 | 18.0 | | |
| Incidence per 100 person-years | 6.67 | 11.11 | 0.60 (.05–6.62) | .73 |
| Overall | | | | |
| Participants, No. | 359 | 363 | | |
| Incident events, No. | 40 | 41 | | |
| Person-years, No. | 656.5 | 648.5 | | |
| Incidence[a] | 6.09 | 6.32 | 0.96 (.62–1.49) | .87 |

Abbreviation: CI, confidence interval.

[a] Defined as the number of infections per 100 person-years.

[b] Females with only baseline and year two follow-up samples.

partners of circumcised males and 6.18 cases per 100 py (39 cases per 630.5 py) in female partners of uncircumcised males (IRR, 1.01 [95% CI, .65–1.56]; $P = .98$).

A subanalysis was also performed of female partners of males who had positive or indeterminate results of HSV-2 serological testing at enrollment or males who seroconverted to HSV-2 during the follow-up period. In an intention-to-treat analysis, HSV-2 infection was detected in 20 female partners of these males in the intervention group and in 20 female partners of males in the control group over the 2-year follow-up period. Female partner HSV-2 infection incidence at year 2 was 9.55 cases per 100 py (20 cases per 209.5 py) in the intervention arm and 11.17 cases per 100 py (20 cases per 179.0 py) in the control arm (IRR, 0.85 [95% CI, .44–1.67]; $P = .62$). The rate of HSV-2 seroconversion was higher among female partners of male HSV-2 seroconverters (27.3 cases per 100 py; 15 cases per 55.0 py) than among female partners of males with prevalent HSV-2 infection or indeterminate serological test results (7.5 cases per 100 py; 25 cases per 333.5 py) (IRR, 3.64 [95% CI, 1.78–7.18]; $P < .001$). However, the efficacy of MC for HSV-2 prevention among female partners was not statistically significant among partners of male HSV-2 seroconverters (IRR, 0.98 [95% CI, .30–3.08]; $P = .97$) or prevalent-positive males (IRR, 0.89 [95% CI, .37–2.12]; $P = .76$).

Self-reported rates of female partners' sexual behaviors and STI symptoms were assessed by the male partner's circumcision status and follow-up interval. There were no differences among the female partners between study arms in self-reported number of sexual partners, nonmarital relationships, condom use, GUD, vaginal discharge, or dysuria at either year 1 or year 2 (data not shown). During the first year of follow-up, 1.1% of females (4 of 351) in the intervention arm and 2.8% of females (10 of 351) in the control arm reported nonmarital relationships ($P = .11$). During the second year of follow-up, 1.9% of females (6 of 315) in the intervention arm and 3.0% of females (9 of /303) in the control arm reported nonmarital relationships ($P = .39$).

## Discussion

We found that circumcision of HIV-negative males did not affect HSV-2 incident infection among female partners. The year 1 results suggest that MC may protect female partners from acquiring HSV-2. However, when combined with year 2 data, MC has no impact on female partner HSV-2 acquisition, suggesting the difference in efficacy between years 1 and 2 is likely due to chance. Overall, the data are in contrast to previous studies in which MC reduced GUD among female partners, suggesting that MC might decrease ulcerative STIs such as HSV-2 [8]. Also, 2 observational studies of men circumcised during childhood found that their female partners had a decreased HSV-2 prevalence [9, 10], and observational studies and 2 randomized trials demonstrated that MC reduces HSV-2 acquisition among men [5, 6, 15].

The lack of MC efficacy for prevention of HSV-2 infection in female partners of HIV-negative males is likely due to multiple factors. Approximately 3.6% of females reported nonmarital relationships; however, this rate may be low because of recall or social desirability bias. HSV-2 acquisition from outside relationships could dilute the potential efficacy of MC. The enrollment prevalence of HSV-2 was relatively low among married HIV-negative males (Table 1), thus the potential exposure to HSV-2–infected male partners was very low. The study lacked the power to detect an effect of MC on incident HSV-2 infections among females, particularly among the minority of couples in which the male partner was known to be HSV-2 seropositive. Thus, the study results do not rule out a possibility that MC may reduce the HSV-2 incidence among female partners.

This study had other limitations. Some females were lost to follow-up or did not attend all follow-up visits, although the >90% retention rate during the 2-year trial is quite robust. The females were all in stable partnerships with HIV-negative males and may represent a self-selected population of more-compliant lower-risk participants in both arms. The risk behaviors and symptoms of STIs are self-reported, and the data were potentially vulnerable to recall and reporting bias.

MC did not affect HSV-2 acquisition among female partners over the short term in this study. However, the study lacked power, and there was a modest and nonstatistically significant reduction in incidence among female partners of HSV-2–positive males. In addition, it is possible that the 28%–34% reduction in HSV-2 acquisition among males [5, 6] could reduce exposure to HSV-2 infection in women over the longer term.

## Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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