Effect of Male Circumcision on the Prevalence of High-Risk Human Papillomavirus in Young Men: Results of a Randomized Controlled Trial Conducted in Orange Farm, South Africa

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(See the editorial commentary by Gray et al. and the articles by Nielson et al. and Warner et al., on pages 1–3, 7–13, and 59–65, respectively.)

Background. A causal association links high-risk human papillomavirus (HR-HPV) and cervical cancer, which is a major public health problem. The objective of the present study was to investigate the association between male circumcision (MC) and the prevalence of HR-HPV among young men.

Methods. We used data from a MC trial conducted in Orange Farm, South Africa, among men aged 18–24 years. Urethral swab samples were collected during a period of 262 consecutive days from participants in the intervention (circumcised) and control (uncircumcised) groups who were reporting for a scheduled follow-up visit. Swab samples were analyzed using polymerase chain reaction. HR-HPV prevalence rate ratios (PRRs) were assessed using univariate and multivariate log Poisson regression.

Results. In an intention-to-treat analysis, the prevalences of HR-HPV among the intervention and control groups were 14.8% (94/637) and 22.3% (140/627), respectively, with a PRR of 0.66 (0.51–0.86) ($P = .002$). Controlling for propensity score and confounders (ethnic group, age, education, sexual behavior [including condom use], marital status, and human immunodeficiency virus status) had no effect on the results.

Conclusions. This is the first randomized controlled trial to show a reduction in the prevalence of urethral HR-HPV infection after MC. This finding explains why women with circumcised partners are at a lower risk of cervical cancer than other women.

Trial registration. ClinicalTrials.gov identifier: NCT00122525.

A recent meta-analysis estimated the worldwide prevalence of human papillomavirus (HPV) among women to be 10.4% [1]. HPV genotypes are divided into high-risk and low-risk genotypes, on the basis of their association with cervical lesions. The high-risk HPV (HR-HPV) types are more frequently found in premalignant or malignant lesions and are associated with cancers of the cervix, vulva, vagina, anus, and penis [2–4]. A causal association between cervical cancer and HR-HPV has now been established [4–9], and the worldwide prevalence of HR-HPV in cervical carcinomas has been estimated at 99.7% [10]. Cervical cancer is the most common cancer affecting women in developing countries, and >70% of cases occurring in Africa have been attributed to HR-HPV genotypes 16 and 18 [1, 11, 12]. Thus, any factor reducing the probability of acquiring or transmitting HPV will also considerably reduce the burden of disease, especially in the developing world [4].

Observational studies have suggested that the prevalence of HPV is reduced among circumcised men compared with uncircumcised men [3, 4, 13–15]. Nevertheless, such an association has not yet been proved in a

randomized controlled trial. The objective of the present study was to analyze the effect of male circumcision (MC) on the prevalence of HR-HPV using data collected during a randomized controlled trial of MC conducted in Orange Farm, South Africa, which demonstrated a partial protective effect of MC on the acquisition of HIV by young men.

METHODS

Collection of data. The technical details of the trial (Agence Nationale de Recherches sur le SIDA [ANRS] study 1265) have been published elsewhere [16], and only a summary will be presented here. Between February 2002 and July 2004, 3274 uncircumcised men aged 18–24 years were recruited, randomized into 2 groups, and followed up. MC was offered immediately after randomization to the intervention group and after the end of the follow-up period to control group participants. During each follow-up visit at 3, 12, and 21 months, circumcision status was assessed by a nurse through genital examination. In addition, information on sexual behavior was collected, including the number of partners as a function of time, the number of sexual contacts with each partner, condom use, and age of partners.

During 262 consecutive days, from 7 March to 24 November 2005, a urethral swab sample was collected by a nurse from each participant coming for the 21-month visit. All participants signed a written consent form for this test. Because of limited funding, the collection of swab samples for HR-HPV testing was not started earlier. The urethra was chosen because the detection of HPV in this anatomical site is probably not affected by circumcision status. These swab samples were analyzed to assess the association between the prevalence of HR-HPV strains and MC. A urethral swab sample was also collected at a follow-up visit ~6 weeks after circumcision from all control group participants who took part in a nested study designed to compare 2 circumcision methods. To ascertain that the detection of HR-HPV was not affected by circumcision status, we used these swab samples to compare the prevalence of HR-HPV among the nested study participants before and after circumcision. Finally, study participants were asked to give a first-void urine sample to test for urogenital *Neisseria gonorrhea,* the presence of which was used as a biological marker of sexual behavior.

Laboratory methods. Specimens were frozen at −20°C immediately after collection and kept frozen until processing. DNA was extracted from the urethral swabs by means of the MagNA Pure LC instrument (Roche) with the Roche MagNA Pure LC DNA Isolation Kit I. Swabs were lysed in 500 μL of the kit lysis buffer for 30 min at room temperature. The MagNA Pure external lysis protocol was used to extract DNA from the lysis buffer into 100 μL of eluate; 50 μL of the eluate was used for screening (Roche Amplicor HPV Test), and 50

μL was used for genotyping (Roche Linear Array HPV Genotyping Test). This standardized polymerase chain reaction (PCR)–based method can detect 13 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Because of the combined probe of the assay for HPV-52 and to be conservative, samples were classified as being positive for HPV-52 only when they were negative for genotypes 33, 35, and 58. Negative results with a negative internal β-globin PCR control were excluded. Genotyping was performed for all positive samples. An HPV-positive sample was defined as a sample in which at least 1 HR-HPV was detected. In some analyses we also considered multiple-HPV samples, defined as samples in which at least 2 HR-HPV genotypes were detected. Urine specimens were tested for *N. gonorrhea* by PCR (Roche Cobas Amplicor PCR).

Data analysis. The intention-to-treat and as-treated prevalence rate ratios (PRRs) for HR-HPV positivity and *N. gonorrhea* positivity were estimated using univariate log Poisson regression These analyses were repeated multivariately by controlling for ethnic group, age, education, lifetime number of sex partners, marital status, number of nonspousal partners in the past 12 months, condom use in the past 12 months, number of sex acts in the past 12 months, and HIV status.

To assess the potential impact of HIV acquisition, which is reduced by MC and is associated with HPV infection [17], these analyses were repeated after excluding subjects who underwent HIV seroconversion during the follow-up period ($n = 25$). To evaluate a possible imbalance between the groups, analyses were repeated after controlling for propensity score coded in quintiles [18]. Statistical analyses were performed using the statistical package SPSS for Windows (version 8; SPSS) and the R programming language (version 2.6.1) [19].

Ethics. The research protocol was reviewed and approved by the University of Witwatersrand Human Research Ethics Committee (Medical) on 22 February 2002 (protocol study M020104). The trial was also approved by the ANRS Scientific Commission (protocol study 1265; 2002, decision 50), and authorization was obtained from the City of Johannesburg, Region 11, on 25 February 2002.

RESULTS

Table 1 shows the baseline characteristics of the 1264 participants from whom a urethral swab sample was collected at the 21-month visit, reported by randomization group. These characteristics were similar in the 2 groups; the only significant difference was in HIV status. The mean (median) durations of follow-up in the intervention and control groups were 644 (637) and 649 (637) days, respectively.

Table 2 presents the intention-to-treat univariate association between the prevalence of HR-HPV and MC at the scheduled 21-month visit. HR-HPV prevalence was signifi-

Table 1. Background characteristics, reported sexual behaviors, and HIV prevalence at the 21-month visit.

Characteristic	Control group (n = 627)	Intervention group (n = 637)	P
Ethnic group			.77[a]
Sotho	55.7	54.9	
Zulu	29.2	28.4	
Other	15.2	16.6	
Age <21 years	34.0	29.8	.12[a]
Primary level of education completed	98.9	98.6	.80[a]
Married or living as married[b]	4.0	5.2	.49[a]
Reported sexual behavior			
Lifetime no. of sex partners, mean (median)	4.7 (4.0)	4.2 (4.0)	.10[c]
No. of nonspousal sex partners,[b] mean (median)	0.9 (1.0)	0.9 (1.0)	.48[c]
No. of sex acts,[b] mean (median)	10.0 (5.0)	11.6 (5.0)	.98[c]
Consistent condom use with nonspousal sex partners[b,d]	25.0	26.0	.84[a]
HIV positive	7.3	3.9	.010[a]

NOTE. Data are percentage of subjects, unless otherwise indicated.
[a] χ^2 or Fisher's exact test, as appropriate.
[b] During the past 12 months.
[c] Kruskal-Wallis test.
[d] Among those having had sexual intercourse during the past 12 months.

cantly lower among men in the intervention group. As indicated in figure 1, the percentage of each of the 13 HR-HPV genotypes was always lower in the intervention group than in the control group. In the intention-to-treat comparison, the differences were significant for genotypes 18, 31, 45, 52, 56, 58, and 68.

Table 2 shows that the protective effect of MC on HR-HPV was higher in the as-treated analysis than in the intention-to-treat analysis. The protective effect was also higher in both analyses when potential confounders were controlled for, including HIV status and reported sexual behavior cofactors. HR-HPV was associated with HIV status in both analyses,

with adjusted PRRs (aPRRs) of 2.2 (95% confidence interval [CI], 1.5–3.3) and 2.2 (95% CI, 1.5–3.2), respectively. When those who underwent HIV seroconversion during the follow-up period were excluded from the analysis, the results remained practically unchanged from those shown in table 2, with P values <.009 and a relative variation in PRRs and aPRRs of <5.2%. This suggests that the effect of MC on HR-HPV is independent of the effect of MC on HIV. The aPRRs were almost identical when the analyses were adjusted for propensity score in addition to the other covariates.

The prevalence of multiple HR-HPV types was 7.0% (89/1267; 95% CI, 5.7%–8.6%). It was significantly lower in the

Table 2. Association between the prevalence of high-risk human papillomavirus (HR-HPV) and male circumcision.

Group	HR-HPV prevalence, % (proportion positive)	PRR (95% CI) [P]	
		Unadjusted	Adjusted[a]
Randomization group			
Control	22.3 (140/627)	1.00 (reference)	1.00 (reference)
Intervention	14.8 (94/637)	0.66 (0.51–0.86) [.002]	0.68 (0.52–0.89) [.004]
Circumcision status			
Uncircumcised	23.2 (144/621)	1.00 (reference)	1.00 (reference)
Circumcised	14.0 (90/643)	0.60 (0.46–0.79) [<.001]	0.62 (0.47–0.80) [<.001]

NOTE. CI, confidence interval; PRR, prevalence rate ratio.
[a] Adjusted for ethnic group, age, education, lifetime no. of sex partners, marital status, no. of nonspousal partners in the past 12 months, condom use in the past 12 months, no. of sex acts in the past 12 months, and HIV status.
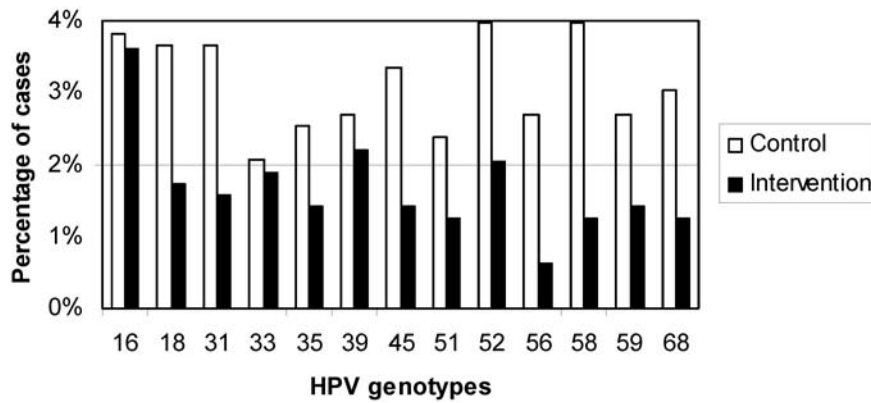
Figure 1. Distribution of high-risk human papillomavirus (HPV) genotypes as a function of randomization group.

intervention group than in the control group (4.2% vs. 9.9%; PRR, 0.43 [95% CI, 0.28–0.66]; $P < .001$). Among men with at least 1 HR-HPV, the multiple HR-HPV prevalence was also lower among those in intervention group (44.3% vs. 28.7%; PRR, 0.64 [95% CI, 0.45–0.94]; $P = .020$).

As indicated in table 3, the prevalence of *N. gonorrhea* was similar in the 2 groups. Among men in the control and intervention groups, the median lifetime numbers of sex partners were 4.1 and 4.2 ($P = .49$, Kruskal-Wallis test), and the proportions of subjects who consistently used condom were 17.4% and 19.7%, respectively ($P = .45$, Fisher's exact test). These findings suggest that the protective effect of MC on HR-HPV cannot be attributed to a difference in sexual behavior between the 2 groups.

During the study period, 371 men in the control group were circumcised and underwent urethral swab sampling before and after MC. The average (median) duration between the 2 swab collections was 59 (43) days. As expected, the HR-HPV prevalences were not different for the 2 samplings (23.7% vs. 23.9%; $P > .99$, sign test). The proportions of men with multiple HR-HPV infections did not differ significantly (10.2% vs. 12.1%; $P = .40$, sign test). These results indicate that the as-treated effect of MC on HR-HPV prevalence shown in table 2 cannot be attributed to easier detection of HR-HPV by urethral swab sampling in uncircumcised men.

DISCUSSION

Using data collected during the MC trial conducted in Orange Farm, South Africa, we have demonstrated an independent and partial protective effect of MC on the prevalence of HR-HPV. We demonstrated the effect on HR-HPV prevalence and not incidence because of the available biological samples in this MC trial. This effect remained unchanged when the analysis was adjusted for possible confounding factors, such as sexual behavior and condom use. Given the randomization, the results of the propensity analysis, and the absence of obvious differences between groups in gonorrheal prevalence or sexual behavioral characteristics, the difference in HR-HPV prevalence between the 2 groups is likely attributable to MC. In this light, the difference observed is probably the consequence of a difference in HR-HPV incidence between circumcised and uncircumcised men. Indeed, in the present study HR-HPV prevalence is likely a proxy for HR-HPV incidence, because among young men HPV prevalence rises as a function of age [20].

The present study has some limitations. First, biological samples were not collected throughout the follow-up period, so the HR-HPV status at inclusion is unknown. This information would have allowed us to compare HR-HPV incidence as a function of MC status and HR-HPV prevalence between interven-

Table 3. Association between the prevalence of *Neisseria gonorrhea* and male circumcision.

Group	*N. gonorrhea* prevalence, % (proportion positive)	PRR (95% CI) [P]	
		Unadjusted	Adjusted[a]
Randomization group			
Control	10.3 (62/601)	1.00 (reference)	1.00 (reference)
Intervention	9.1 (56/613)	0.89 (0.62–1.27) [.51]	0.87 (0.60–1.26) [.46]
Circumcision status			
Uncircumcised	10.0 (60/598)	1.00 (reference)	1.00 (reference)
Circumcised	9.4 (58/616)	0.94 (0.65–1.35) [.73]	0.93 (0.64–1.34) [.69]

NOTE. CI, confidence interval; PRR, prevalence rate ratio.

[a] Adjusted for ethnic group, age, education, lifetime no. of sex partners, marital status, no. of nonspousal partners in the past 12 months, condom use in the past 12 months, no. of sex acts in the past 12 months, and HIV status.

tion groups at inclusion. Because some participants were certainly already infected by HR-HPV at inclusion, the effect on prevalence that we measured underestimates the true effect of MC. Second, that participants were not blind to the intervention may have led to sexual behavior change and bias. Finally, HR-HPV was detected in urethral swab samples, a method that is likely to miss infections [21]. Thus, the prevalence of HR-HPV infections in our cohort is likely underestimated, because the rate of detection in the urethra is significantly lower than that in the glans, corona sulcus, or penis shaft [21, 22]. However, we believe that there is no risk of nondifferential misclassification, because we did not find any difference when we compared the urethral HR-HPV prevalences before and after circumcision in a subsample of participants. Hence, we believe that HR-HPV infections would be underestimated equally in the 2 arms and that this underestimation would have no effect on PRRs. Despite this loss of power, our study found a significant protective effect of MC against HPV infection.

We could not detect an effect of MC on some HR-HPV genotypes, such as 16 and 33. The apparent variation in the effect of MC according to genotype can be the result of true variation or random variation. This possible variation according to genotype should be further investigated, for example, by combining the results of the present study with those of other MC trials conducted in Kenya and Uganda [23, 24].

The protective effect corresponds in magnitude to what could have been expected from observational studies. Castellsague et al. [13] reported in their meta-analysis an odds ratio of 0.56 (95% CI, 0.39–0.82), whereas Baldwin et al. [3] found an adjusted relative risk of 0.44 (95% CI, 0.23–0.81). Similarly, Hernandez et al. [14] found that uncircumcised men had nearly a 2-fold (relative risk, 1.96 [95% CI, 1.02–3.75]) increased risk of oncogenic HPV infections. Given the results of our randomized trial, there is now clear evidence that MC decreases the risk of heterosexual HR-HPV acquisition among men.

HR-HPV is a major public health problem because of its causal association with malignancies, especially cervical cancer in women. Our findings illustrate why MC has long been thought to be protective against cervical cancer [9]. Indeed, as shown in the present study, MC reduces the risk of HR-HPV infection among men and consequently reduces the exposure of women to HR-HPV. Thus, the risk of cervical cancer is lowered because of the causal link between HR-HPV and cervical cancer among women [4–9].

Because 3 randomized controlled trials have shown that MC has a partial protective effect on the acquisition of HIV by males in Africa [16, 23, 24], the effect of MC on HR-HPV reinforces the recommendation of the World Health Organization and the Joint United Nations Programme on HIV/AIDS for the implementation of MC programs in countries with a high prevalence of HIV infection, a low prevalence of MC, and a high acceptance of MC [25]. These countries, mainly in southern and eastern Africa, are those in which the affordability of the HPV vaccine remains a problem. Moreover, the protective effect of MC may supplant HPV vaccines in terms of genotype coverage and target-group age range.

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