

Acquisition and Persistence of Human Papillomavirus 16 (HPV-16) and HPV-18 Among Men With High-HPV Viral Load Infections in a Circumcision Trial in Kisumu, Kenya

Virginia Senkomago,¹ Danielle M. Backes,^{1,4} Michael G. Hudgens,² Charles Poole,¹ Kawango Agot,⁷ Stephen Moses,⁸ Peter J. F. Snijders,⁹ Chris J. L. M. Meijer,⁹ Albertus T. Hesselink,⁹ Nicolas F. Schlecht,⁵ Robert C. Bailey,⁶ and Jennifer S. Smith^{1,3}

¹Department of Epidemiology, and ²Department of Biostatistics, Gillings School of Global Public Health, and ³Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill; ⁴Department of Epidemiology, Brown Public Health, Brown University, Providence, Rhode Island; ⁵Department of Epidemiology and Population Health, Albert Einstein College of Medicine, New York, New York; ⁶Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois, Chicago; ⁷Impact Research and Development Organization, Kisumu, Kenya; ⁸Centre for Global Public Health, University of Manitoba, Winnipeg, Canada; and ⁹Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands

Background. Circumcision and lower human papillomavirus (HPV) viral loads in men are possibly associated with a reduced risk of HPV transmission to women. However, the association between male circumcision and HPV viral load remains unclear.

Methods. Swab specimens from the glans and shaft of the penis were collected from men enrolled in a circumcision trial in Kisumu, Kenya. GP5+/6+ polymerase chain reaction (PCR) was used to identify HPV DNA types. HPV-16 and HPV-18 loads were measured with a LightCycler real-time PCR and classified as high (>250 copies/scrape) or low (≤250 copies/scrape).

Results. A total of 1159 men were randomly assigned to undergo immediate circumcision, and 1140 men were randomly assigned to the control arm (these individuals were asked to remain uncircumcised until the study ended). The hazard of acquisition of high-viral load infections in the glans was lower in the circumcision arm, compared with the control arm, for HPV-16 (hazard ratio [HR], 0.32 [95% confidence interval {CI}, .20–.49]) and HPV-18 (HR, 0.34 [95% CI, .21–.54]). The 6-month risk of HPV persistence among men with high-viral load infections in the glans at baseline was lower in the circumcision arm, compared with the control arm, for HPV-16 (risk ratio [RR], 0.36 [95% CI, .18–.72]) and HPV-18 (RR 0.34 [95% CI, .13–.86]). Weaker and less precise results were obtained for shaft samples.

Conclusions. Male circumcision could potentially reduce the risk of HPV transmission to women by reducing the hazard of acquisition, and the risk of persistence of high-HPV viral load infections in the glans in men.

Keywords. male circumcision; human papillomavirus (HPV); viral load; HPV-16; HPV-18; men; randomized controlled trial (RCT), incidence; persistence; Kenya.

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Correspondence: Virginia Senkomago, PhD, MPH, Department of Epidemiology, University of North Carolina, 135 Dauer Drive, 2101 McGavran-Greenberg Hall, CB #7435, Chapel Hill, NC 27599 (epi.senkvirg@unc.edu).

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Persistent infections with high-risk human papillomavirus (HPV) type 16 (HPV-16) and HPV-18 are causes of approximately 70% of cervical cancer cases in women [1]. Women with higher HPV-16 or HPV-18 loads are more likely to have persistent infections and to progress to high-grade cervical intraepithelial neoplasia than those with lower viral loads [2–8]. Men with higher HPV-16 or HPV-18 loads have a higher prevalence of

flat penile lesions and greater HPV type concordance with their female partners than those with lower viral loads [9–11]. As a result, a higher HPV viral load in men is suggested to be associated with an increased risk of HPV transmission to their female partners [9].

Male circumcision has been found to be protective against human immunodeficiency virus (HIV) and penile HPV infections [12–18]. Three randomized controlled trials (RCTs) in Kenya, Uganda, and South Africa, as well as several longitudinal studies, have shown that high-risk HPV prevalence in circumcised men is lower than that in uncircumcised men [15–18]. RCTs in Kenya and Uganda also found that male circumcision reduces the acquisition of high-risk HPV infections and enhances clearance of HPV infections over 24 months [16, 17]. The prevalence of flat penile lesions after 24 months in circumcised men was lower than that in uncircumcised men in the Kenya trial [10]. Among HIV-negative men in the Uganda trial, a lower HPV prevalence was observed in female partners of circumcised men, compared with female partners of uncircumcised men [19]. Circumcision and lower HPV viral load in men are suggested to be associated with a reduced risk of HPV transmission to women, but the association between male circumcision and HPV viral load remains unclear [9].

To our knowledge, only the RCT in Uganda has examined the effect of male circumcision on penile HPV viral load [20]. This study, however, did not examine the association between male circumcision and the hazard of acquisition of high-HPV viral load infections, or the prevalence of high-viral load HPV infections versus low-viral load HPV infections among men with incident HPV infections. Also, the association between circumcision and the persistence of high-viral load and low-viral load HPV infections present at baseline (prevalent infections) has not yet been examined.

We present the results of an RCT examining the associations between male circumcision and the incidence and risk of persistence of high-viral load and low-viral load HPV-16 and HPV-18 infections in men from Kisumu, Kenya. We have previously shown that male circumcision reduces the incidence and increases the clearance of HPV infections in this population [16]; here we expand on those findings to examine the effect of male circumcision on HPV viral load.

METHODS

Study Population and Enrollment

Uncircumcised men were screened between 4 February 2002 and 6 September 2005 in Kisumu to participate in an RCT of male circumcision (clinical trials registration NCT00059371) [14]. The main aim of the trial was to determine the effectiveness of male circumcision in reducing the incidence of HIV infection. Study methods have been previously described in detail [14]. Briefly, study inclusion criteria included being uncircumcised,

having an age of 18–24 years, testing negative for HIV, being sexually active within the past 12 months, and having a blood hemoglobin level of ≥ 90 g/L. Participants who met the study criteria were randomly assigned to either the circumcision arm and to undergo immediate circumcision or the control arm and to remain uncircumcised until the end of their 24 months of study participation. This study protocol was approved by institutional review boards of the universities of Illinois-Chicago, Manitoba, Nairobi, and North Carolina; RTI International; and VU University Medical Center.

This present analysis includes men from the RCT who consented to the collection of penile exfoliated cells and their shipment overseas for HPV DNA and HPV viral load testing. Of the 2784 men enrolled in the RCT, 2299 (83%) consented to provide penile swab samples and had baseline HPV data; 1159 men were in the circumcision arm, and 1140 were in the control arm (Figure 1). Eligibility for inclusion in incidence and persistence analyses was determined for each HPV type (HPV-16 or HPV-18) and anatomical site (glans or shaft) separately. Participants who were (1) positive for HPV-16 or HPV-18 at baseline, (2) had no HPV follow-up data, or (3) had missing HPV viral load data were excluded from incidence analyses for that specific HPV type and anatomical site. Similar proportions of samples in the circumcision and control arms were included in incidence analyses for HPV-16 (87% and 89%, respectively, in glans samples and 90% and 93%, respectively, in shaft samples) and HPV-18 (91% and 93%, respectively, in glans samples and 93% and 95%, respectively, in shaft samples). The persistence of prevalent high-HPV viral load and low-HPV viral load infections was examined in participants who were (1) positive for HPV-16 and/or HPV-18 at baseline, (2) had follow-up HPV data, and (3) had viral load data for that HPV type and anatomical site (for HPV-16, 144 [82.2%] and 67 [77.9%] had data for glans and shaft specimens, respectively; for HPV-18, 60 [75.9%] and 24 [80%] had data for glans and shaft specimens, respectively).

Follow-up and Specimen Collection

Participant follow-up visits were scheduled every 6 months for a period of 24 months. A standardized questionnaire on sociodemographic characteristics and sexual behavior was administered by a trained male interviewer at each visit [14]. Penile exfoliated cell specimens were collected by a trained physician or clinical officer at each study visit [21]. Samples were collected from two anatomical sites: (i) penile shaft and external foreskin (shaft specimen); and (ii) glans, coronal sulcus, and the internal tissue of the foreskin (glans specimen). Sampling of the foreskin was conducted only in uncircumcised men. Two pre-wetted type 3 Dacron swabs were used to collect penile exfoliated cells from the two separate anatomical sites. Samples were individually processed in the clinic laboratory on the same day they were collected and stored at -75°C [21]. All samples were transported via FedEx in a liquid nitrogen dry shipper from Kisumu to the

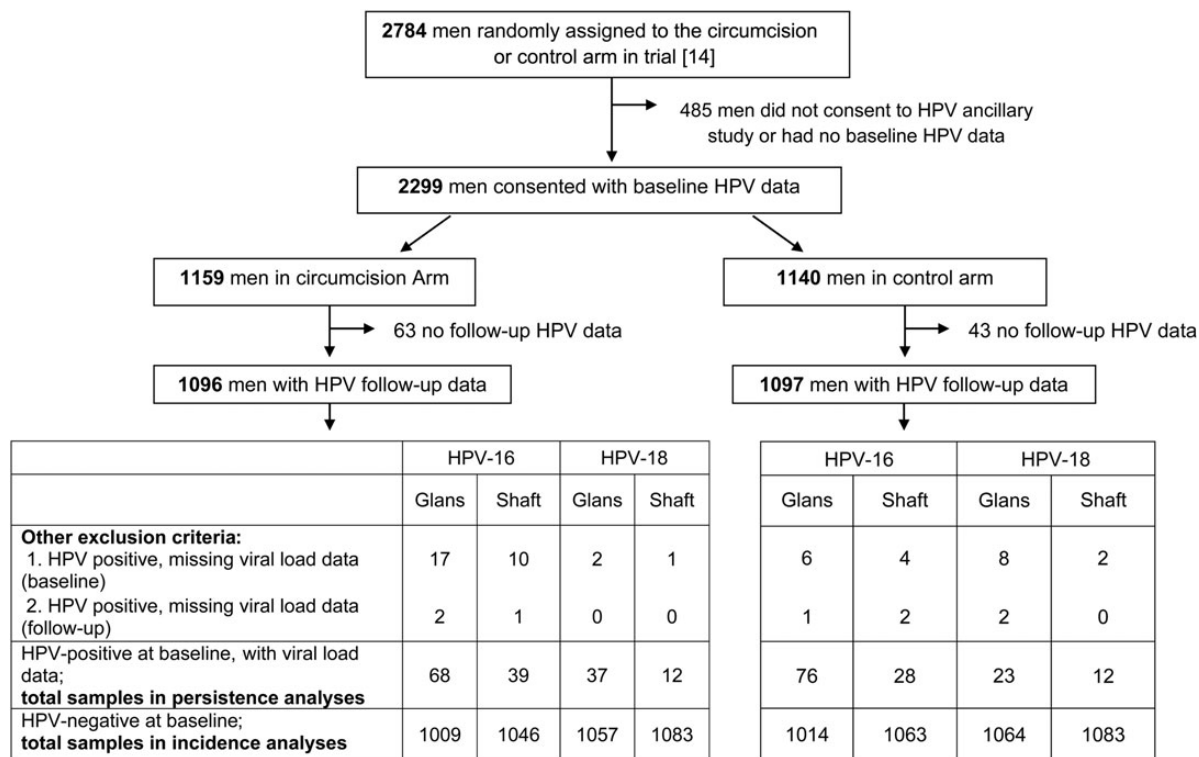


Figure 1. Study flow chart of human papillomavirus (HPV) samples included in analyses of the effect of male circumcision on penile HPV viral load.

Department of Pathology at VU University Medical Center in Amsterdam, Netherlands, for HPV DNA testing.

HPV DNA, HIV, and Sexually Transmitted Infection Testing

DNA was isolated from penile exfoliated cell samples, using the NucleoSpin 96 Tissue kit (Macherey-Nagel, Germany) and a Microlab Star robotic system (Hamilton, Germany) according to the manufacturers' instructions. The presence of human DNA was evaluated by β -globin-specific polymerase chain reaction (PCR), followed by agarose gel electrophoresis [22]. HPV positivity was assessed by GP5+/6+ PCR, followed by hybridization of PCR products, using an enzyme immunoassay readout with 2 HPV oligoprobe cocktail probes that detect 44 HPV types. Subsequent HPV genotyping was performed by reverse line blot hybridization of PCR products [22, 23].

HIV testing was performed at each visit, using 2 HIV antibody rapid tests (Determine, Abbott Diagnostic Division, Hoofddorp, the Netherlands; and Unigold, Trinity Biotech, Wicklow, Ireland), and confirmed by double enzyme-linked immunosorbent assay (ELISA; Adaltis, Montreal, Canada; Trinity Biotech, Wicklow, Ireland) at the University of Nairobi [14]. Urine samples at each visit were tested for *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis* infections by PCR-based methods (Roche Diagnostics), and serum was tested for herpes simplex virus 2 antibodies by a type-specific ELISA (Kalon).

HPV Viral Load Testing

HPV DNA viral load testing was performed on penile exfoliated cell samples that contained HPV-16 and/or HPV-18, using a real-time PCR assay and a LightCycler instrument (Roche, Mannheim, Germany) [24]. DNA extraction and purification were conducted according to manufacturer instructions, using 100 μ L of the remaining cell suspensions. All samples were run in duplicate in the same run, and resulting values were averaged. Dilutions of cloned HPV DNA were used to determine the standard curve for the HPV target. To calculate the number of copies per sample, the measured amount of detected HPV DNA from the standard curve (expressed in femtograms) was divided by 44 (since 1 femtogram of HPV DNA was considered to contain 44 viral DNA copies) and multiplied by the dilution factor [9]. The HPV viral load was categorized as low (≤ 250 copies/scrape) or high (> 250 copies/scrape). The 250 copies/scrape cut point for high versus low viral loads was chosen because with this cut point, high HPV viral loads (> 250 copies/scrape) in men have been associated with a higher prevalence of flat penile lesions, as well as with increased HPV type concordance in their female partners, compared with low HPV viral loads [9, 10].

Statistical Analyses

All analyses were performed for each HPV type (HPV-16 or HPV-18) and anatomical site (glans or shaft) separately. Intent-to-treat analyses were performed to examine the incidence

of high-HPV viral load infections in circumcision versus control arms. Men who were randomly assigned to the circumcision arm but were not circumcised (n = 23) and those randomly assigned to the control arm but were circumcised (n = 14) were included in the analysis in the group to which they were randomly assigned. Incident penile HPV infections were assumed to occur at the midpoint between the last HPV-negative test result and the first subsequent HPV-positive test result. Men who tested HPV negative at each visit were censored at their last observed visit. Only the first incident infections for each HPV type (HPV-16 or HPV-18) at each anatomical site (glans or shaft) were included in incidence analyses. The Kaplan–Meier method was used to estimate the 24-month cumulative risk of HPV-16 and HPV-18 in the glans or shaft for the circumcision versus control arms. Hazard ratios (HRs) comparing the rate of acquisition of high-viral load infections in circumcision versus control arms were estimated by fitting Cox proportional hazards models. For all incidence analyses, HR estimates accounting for interval censoring were obtained by fitting models assuming Weibull distributions for incidence times; results obtained were similar to those from the Cox models (data not shown).

An additional analysis was conducted among only participants with incident HPV-16 or HPV-18 infections to examine associations between circumcision and the prevalence of high versus low HPV viral loads at the time of HPV detection. Log binomial models were used to derive prevalence ratios (PRs) comparing the prevalence of high versus low HPV viral loads at the time of HPV detection for incident infections observed in the circumcision versus control arms. Potential confounders of the association between circumcision and HPV viral load were identified from the literature and analyzed using a directed acyclic graph [25].

Clearance of prevalent infections detected at enrollment was assumed to occur at the midpoint between the last HPV-positive test result and the first subsequent HPV-negative test result. Men were censored at their last observed visit if they tested positive at each consecutive visit for the same HPV type at the same anatomical site. HPV persistence was defined as the detection of the same HPV type at the same anatomical site at 6 months after baseline in participants who were positive for HPV-16 and/or HPV-18 at baseline. The Kaplan–Meier method was used to estimate the cumulative risk of clearance for prevalent high-HPV viral load and low-HPV viral load infections. Risk ratio (RR) estimates were calculated to compare the cumulative risk of HPV persistence for prevalent infections in the circumcision versus control arms at 6 months after baseline.

For all analyses of incidence and persistence, sensitivity analyses were performed by restricting analyses to samples that tested positive for the presence of human DNA by means of the β -globin test. We also conducted sensitivity analyses to examine incident HPV viral load as a continuous variable; specimens with nonquantifiable HPV viral loads (ie, <250 copies/

Table 1. Baseline Characteristics, by Intervention Arm

Characteristic	Circumcision Arm (n = 1096)	Control Arm (n = 1097)
Age, y	20 (19–22; 18–28)	20 (19–22; 17–24)
Education level		
Less than secondary	372 (33.9)	391 (35.6)
Any secondary or above	724 (66.1)	706 (64.4)
Employment status		
Employed and receiving a salary	94 (8.6)	98 (8.9)
Self-employed	303 (27.7)	294 (26.8)
Unemployed	699 (63.8)	705 (64.3)
Marital status		
Not married (no live-in partner)	1024 (93.8)	1018 (93.2)
Not married (with live-in partner)	7 (0.6)	7 (0.6)
Married (not living with wife)	5 (0.5)	15 (1.4)
Married (living with wife)	56 (5.1)	52 (4.8)
Sex partners, no.		
Lifetime history ^a	4 (3–7; 1–120)	4 (3–7; 1–86)
In previous 6 mo		
0	137 (12.5)	136 (12.4)
1	472 (43.2)	472 (44.1)
≥2	484 (44.3)	475 (43.5)
Used a condom with intercourse in previous 6 mo		
Always	210 (21.9)	208 (21.7)
Inconsistent	511 (53.3)	500 (52.1)
Never	238 (24.8)	251 (26.2)
Herpes simplex virus 2		
Positive	287 (27.3)	278 (26.5)
Negative	764 (72.7)	771 (73.5)
<i>Trichomonas vaginalis</i>		
Positive	21 (1.9)	24 (2.2)
Negative	1064 (98.1)	1059 (97.8)
<i>Neisseria gonorrhoeae</i>		
Positive	30 (2.8)	17 (1.6)
Negative	1053 (97.2)	1066 (98.4)
<i>Chlamydia trachomatis</i>		
Positive	57 (5.3)	42 (3.9)
Negative	1025 (94.7)	1041 (96.1)
Bathing frequency		
Less than daily	23 (2.1)	22 (2.0)
Daily	1063 (97.9)	1062 (98.0)

Data are no. (%) of subjects or median value (interquartile range; range). Numbers and percentages may not sum up to totals due to missing values and rounding.

^a Data are for 1004 subjects in the circumcision arm and 1016 subjects in the control arm.

scrape) were assumed to have HPV viral loads equal to 250 copies/scrape for these analyses. Differences in the distribution of

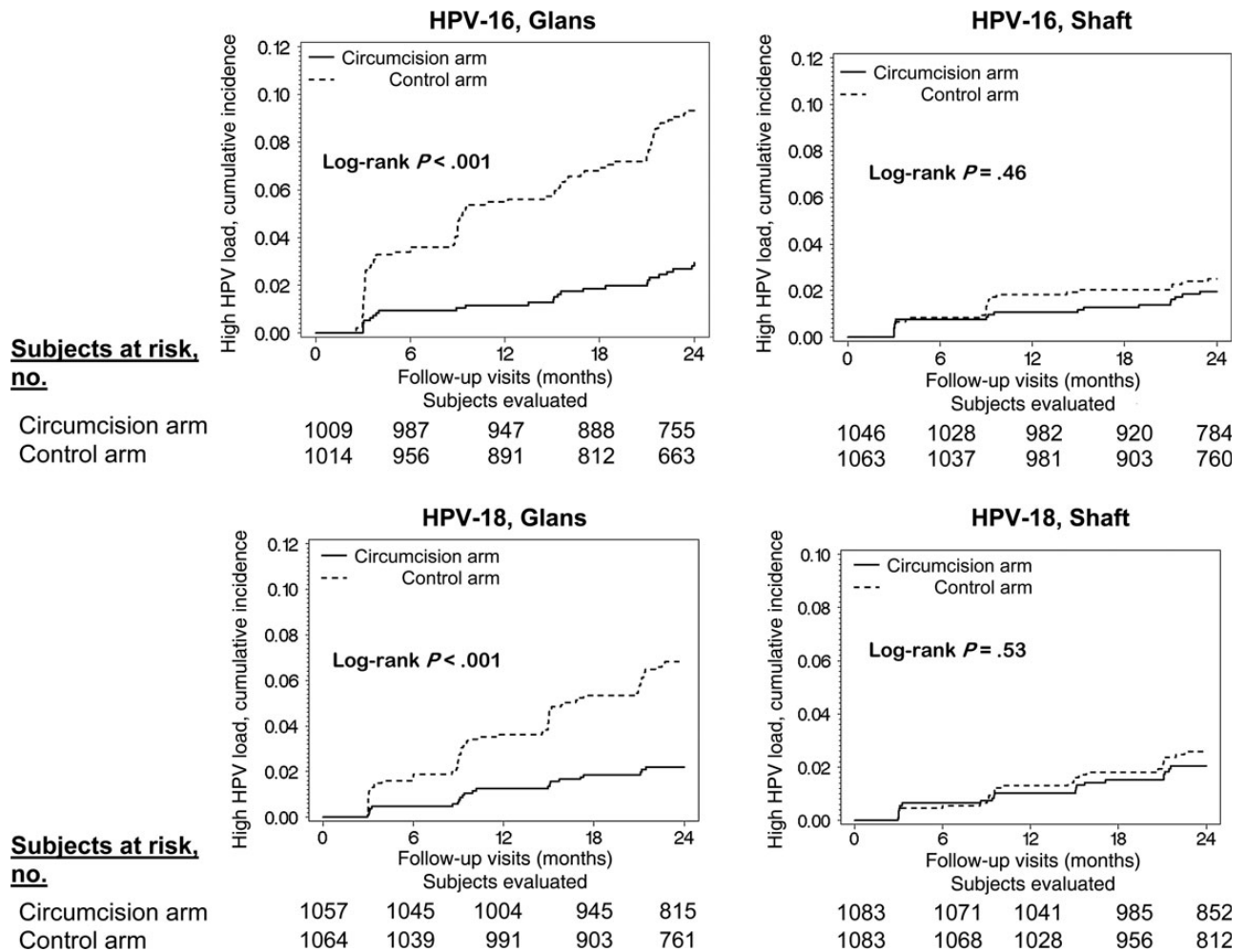


Figure 2. Estimated cumulative incidence of high-viral load human papillomavirus (HPV) infections, by anatomical site over 24 months in Kenyan men.

incident HPV-16 and HPV-18 loads in the circumcision versus control arms were assessed using the Wilcoxon rank sum test.

RESULTS

Characteristics of men enrolled in this RCT have been previously described [14, 16, 21]. Briefly, the median age of eligible study participants was 20 years (range, 18–24 years). Most men were unemployed (65%) and unmarried (93%). There were no appreciable differences in sociodemographic characteristics, sexual history, or results of laboratory tests for sexually transmitted infections at baseline between eligible participants randomly assigned to the circumcision and control arms (Table 1).

Incidence of High-HPV Viral Load Infections

The 24-month cumulative risk of high-viral load infections in the glans was lower in the circumcision arm, compared with control arm, for HPV-16 (2.7% vs 9.5%; $P < .001$, by the log-rank

test) and HPV-18 (2.2% vs 6.8%; $P < .001$, by the log-rank test; Figure 2). In the shaft, the estimated 24-month cumulative risk of high-viral load infections was comparable in the circumcision and control arms for HPV-16 (2.0% and 2.6%, respectively; $P = .46$, by the log-rank test) and HPV-18 (2.0% and 2.6%, respectively; $P = .53$, by the log-rank test). Few men had incident infections in both the glans and shaft (19% for HPV-16 and 25% for HPV-18), and in all of these cases the viral load category (high or low viral load) was the same in both anatomical sites.

Of incident HPV infections observed, 151 (39.7%) with a high HPV-16 load and 135 (69.2%) with a high HPV-18 load occurred in either the glans or shaft (Table 2). The rate of acquisition of high-viral load infections in the glans was lower in the circumcision arm, compared with the control arm, for both HPV-16 (HR, 0.32 [95% confidence interval (CI), .20–.49]) and HPV-18 (HR, 0.34 [95% CI, .21–.54]). Weaker results were observed in the shaft for both HPV types (HRs, 0.79 [95% CI, .42–1.45] for HPV-16 and 0.87 [95% CI, .48–1.54] for HPV-18).

Table 2. Estimated Effect of Circumcision on Incidence of High-Viral Load Human Papillomavirus 16 (HPV-16) and HPV-18 Infections Over 24 Months in Kenyan Men

HPV Type, Anatomical Site, Treatment Arm	All Samples		β-globin-Positive Samples	
	Incident High-Viral Load Infections, ^a No.	HR ^b (95% CI)	Incident High-Viral Load Infections, ^a No.	HR ^b (95% CI)
HPV-16				
Glans				
Circumcision	26	0.32 (.20–.49)	19	0.29 (.17–.56)
Control	83	1	72	1
Shaft				
Circumcision	18	0.79 (.42–1.45)	9	0.76 (.31–1.76)
Control	24	1	13	1
HPV-18				
Glans				
Circumcision	22	0.34 (.21–.54)	15	0.38 (.21–.70)
Control	66	1	45	1
Shaft				
Circumcision	21	0.87 (.48–1.54)	11	0.67 (.31–1.42)
Control	26	1	17	1

Abbreviation: CI, confidence interval.

^a Incident infection was defined as the first type-specific HPV-positive result in men who were negative for that HPV type at the same anatomical site at baseline. A high HPV viral load was defined as >250 copies/scrape for the given HPV type.

^b Hazard ratios (HRs) reflect comparisons of the risk of incident high-HPV viral load infections for the circumcision arm vs that for the control arm.

Sensitivity analyses restricted to β-globin-positive samples yielded similar results (Table 2). Sensitivity analyses examining incident HPV viral load as a continuous variable also found that

the median HPV viral load was lower in the circumcision arm, compared with the control arm, for incident HPV-16 infection (250 vs 450 copies/scrape) and incident HPV-18 infection (250

Table 3. Association Between Circumcision and Prevalence of High Versus Low Viral Load at Detection of Incident Human Papillomavirus (HPV) Infections

HPV Type, Anatomical Site, Treatment Arm	All Samples, by HPV Viral Load ^a			β-globin-Positive Samples, by HPV Viral Load ^a		
	High	Low	PR ^b (95% CI)	High	Low	PR ^b (95% CI)
HPV-16						
Glans						
Circumcision	26	43	0.70 (.50–.99)	19	26	0.72 (.53–.98)
Control	83	72	1	72	51	1
Shaft						
Circumcision	18	52	0.93 (.55–1.57)	9	22	0.92 (.45–1.86)
Control	24	62	1	13	28	1
HPV-18						
Glans						
Circumcision	22	24	0.59 (.43–.82)	15	16	0.59 (.40–.87)
Control	66	16	1	45	10	1
Shaft						
Circumcision	21	6	1.20 (.88–1.62)	11	3	1.25 (.84–1.86)
Control	26	14	1	17	10	1

Abbreviation: CI, confidence interval.

^a Incident infection was defined as the first type-specific HPV-positive result in men who were negative for that HPV type at the same anatomical site at baseline. A high HPV viral load was defined as >250 copies/scrape for the given HPV type; low viral load was defined as ≤250 copies/scrape for the given HPV type.

^b Prevalence ratios (PRs) reflect comparisons of the prevalence of high vs low viral load at HPV detection in men with incident HPV infections in the circumcision arm vs the control arm.

Table 4. Persistence of Baseline Prevalent Human Papillomavirus (HPV) Infections, by HPV Type, Anatomic Site, HPV Viral Load, and Treatment Arm, in Kenyan Men

HPV Type, Anatomical Site, HPV Viral Load, ^a Treatment Arm	Cleared Infection, Proportion ^b	Person-Years	Estimated 6-mo Risk of HPV Persistence ^c (95% CI)	Estimated 6-mo RR ^d of HPV Persistence (95% CI)
HPV-16				
Glans				
High				
Circumcision	35/35	14.4	0.20 (.09–.34)	0.36 (.18–.72)
Control	40/44	23.8	0.55 (.39–.68)	1
Low				
Circumcision	33/33	9.2	0	...
Control	31/32	9.3	0.13 (.04–.26)	
Shaft				
High				
Circumcision	5/5	1.3	0	...
Control	3/3	2.2	0.67 (.05–.94)	
Low				
Circumcision	34/34	12.1	0.09 (.02–.19)	1.06 (.20–5.53)
Control	25/25	7.8	0.08 (.01–0.22)	1
HPV-18				
Glans				
High				
Circumcision	24/24	9.1	0.17 (.05–.34)	0.34 (.13–0.86)
Control	16/16	10.8	0.50 (.35–.71)	1
Low				
Circumcision	13/13	3.9	0.08 (.00–.29)	0.57 (.05–6.44)
Control	7/7	2.5	0.14 (.01–.46)	1
Shaft				
High				
Circumcision	4/4	1.0	0	...
Control	6/6	2.3	0.15 (.01–.52)	
Low				
Circumcision	8/8	2.5	0	...
Control	6/6	1.5	0	

Abbreviation: CI, confidence interval.

^a High HPV viral load was defined as >250 copies/scrape, and low HPV viral load was defined as ≤250 copies/scrape.

^b Data are no. of cleared HPV infections/total no. of HPV infections at baseline.

^c Persistence was defined as an HPV-positive result in participants who were positive for that given HPV type at the same anatomical site in the previous visit. Estimates for risks of persistence and duration since baseline visit were obtained from Kaplan–Meier graphs.

^d Risk ratio (RR) comparing estimated risk of HPV persistence in the circumcision arm vs that in the control arm. Ellipses denote RRs that could not be calculated because all HPV infections in at least 1 group had cleared at 6 months after HPV detection.

vs 7326 copies/scrape) in the glans but not for incident infection in the shaft (Supplementary Table 1).

Prevalence of High Versus Low HPV Viral Load at Detection of Incident HPV Infections

Among men with incident HPV infections in the glans, the prevalence of high versus low viral load at the time of HPV detection was lower in the circumcision arm, compared with the control arm, for HPV-16 (PR, 0.70 [95% CI, .50–.99]) and HPV-18 (PR, 0.59 [95% CI, .43–.82]; Table 3). However, this association was not observed in the shaft for HPV-16 (PR, 0.93 [95% CI, .55–1.57]) or HPV-18 (PR, 1.20 [95% CI, .88–1.62]).

PRs obtained after adjustment for HIV infection, condom use, number of sex partners, bathing frequency, presence of sexually transmitted infections, and multiple HPV type infection were similar to the reported unadjusted HRs (results not shown). Sensitivity analyses restricted to β-globin–positive samples showed similar results as the main analysis (Table 3).

Persistence of High-Viral Load and Low-Viral Load HPV Infections Prevalent at Baseline

For both HPV types, the prevalence at baseline was higher in the glans (6.6% for HPV-16 and 3.1% for HPV-18), compared with the shaft (2.8% for HPV-16 and 1.1% for HPV-18).

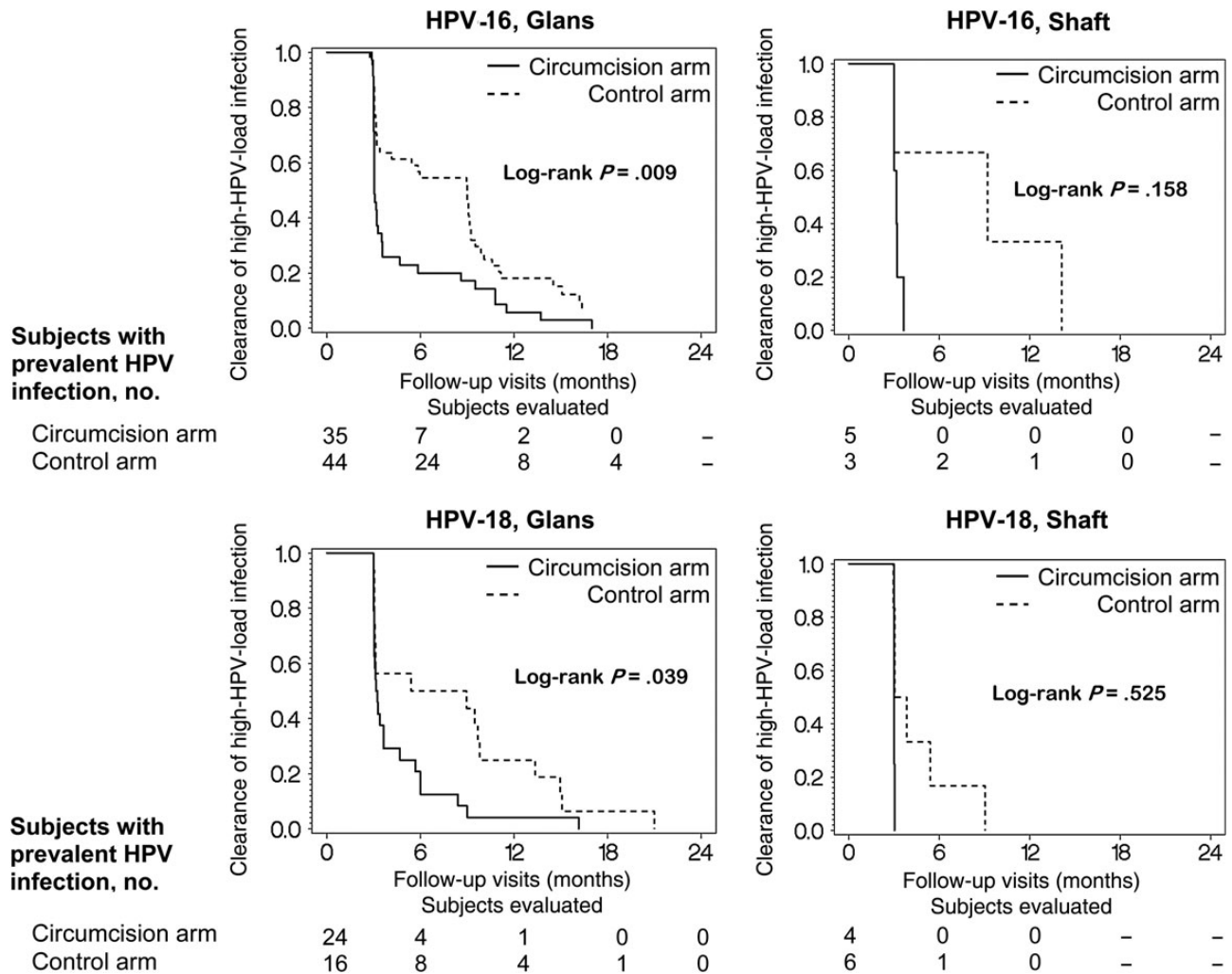


Figure 3. Estimated clearance of prevalent high-viral load human papillomavirus (HPV) infection, by circumcision status, in Kenyan men.

Prevalent infections in the glans at baseline were more likely to have high HPV viral loads (54.9% [79] for HPV-16 and 66.7% [40] for HPV-18) than prevalent infections at baseline in the shaft (11.9% [8] for HPV-16 and 41.7% [10] for HPV-18; Table 4). Similar proportions of men with prevalent HPV-16 and HPV-18 infections at baseline were assigned to the circumcision and control arms (Figure 1).

Nearly all prevalent infections at baseline cleared during the 24 months of follow-up, with the exception of 5 HPV-16 glans infections (6.6%) in the control arm (4 high-viral load infections censored at 18 months and 1 low-viral load infection censored at 12 months after baseline). For both HPV types, most prevalent infections in the glans or shaft cleared within 6 months (Figure 3 and Supplementary Figure 1). The estimated risk of persistence to 6 months for prevalent HPV infections in the glans was lower in the circumcision arm, compared with the control arm, for HPV-16 (RR, 0.36 [95% CI, .18-.72]) and HPV-18 (RR, 0.34 [95% CI, .13-.86]; Table 4). The estimated

risk of persistence to 6 months for high-viral load infections in the circumcision arm (0.20 [95% CI, .09-.34]) was similar to that for low-viral load infections in the control arm (0.13 [95% CI, .04-.26]) for prevalent HPV-16 infections in the glans. A similar trend was observed for prevalent HPV-16 infections in the shaft and for prevalent HPV-18 infections in the glans and shaft. Sensitivity analyses of HPV persistence restricted to prevalent infections that were β -globin positive found results similar to those from the main analysis (data not shown).

DISCUSSION

The 2-year cumulative incidence of high-viral load HPV-16 and HPV-18 infections in the glans was lower in the male circumcision arm, compared with the control arm, in young men from Kisumu. Male circumcision reduced the rate of acquisition of high-viral load HPV-16 and HPV-18 infections in the glans over 24 months. Among men with incident HPV-16 or HPV

18 infections, those who were circumcised were more likely than uncircumcised men to have low-viral load infections, rather than high-viral load infections, in the glans over 24 months. We also found that for prevalent high-viral load infections detected at baseline, the risk of persistence to 6 months was lower in the circumcision arm, compared with the control arm, for both HPV-16 and HPV-18 in the glans. Weaker and less precise results were observed for the estimated effect of male circumcision on the incidence of high-load HPV-16 and HPV-18 infections in the shaft samples.

To our knowledge, only one other study, the male circumcision trial conducted in Rakai, Uganda, has examined the effect of male circumcision on the HPV viral load in men [20]. The study found that the combined HPV viral load for HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, and HPV-52 in the glans was lower in the circumcision arm, compared with the control arm, at 24 months after randomization. Their estimated protective effect of circumcision on the point prevalence of high-viral load versus low-viral load HPV infections in the glans (PR, 0.54 [95% CI, .21–1.42]) was similar to the 24-month period prevalence estimates we obtained for incident high-viral load infection, compared with incident low-viral load infection of HPV-16 (0.70 [95%, .50–.99]) and HPV-18 (0.59 [95% CI, .43–.82]) in the glans.

Our study examined HPV viral load in both the glans and shaft of the penis. The estimated effects of male circumcision in reducing the incidence and risk of persistence of high-viral load infections in the shaft were weaker and less precise than estimated effects for the glans. This may be due to a lower proportion of high-HPV viral load infections detected in the shaft, compared with the glans. Stronger effects of male circumcision on HPV infection have been previously observed in the glans, compared with anatomical sites more distal to the foreskin [18].

The biological mechanism by which male circumcision reduces the viral load of HPV is unclear. It has been hypothesized that the keratinized skin surface and scar tissue in circumcised men reduces the entry of HPV and subsequent HPV replication in the basal epidermal cells of the penis [26]. In uncircumcised men, the moist subpreputial cavity may promote HPV survival and subsequently increase the risk of HPV persistence [26, 27]. Male circumcision has been previously shown to reduce the prevalence of flat penile lesions; our findings are consistent with the current understanding that male circumcision reduces the prevalence of flat penile lesions by reducing the HPV viral load in men [9–11]. Furthermore, the effect of male circumcision in reducing the HPV viral load may explain why fewer HPV infections and lower HPV viral loads are observed in female partners of circumcised men, compared with those of uncircumcised men [19, 27]. It is plausible that male circumcision reduces the risk of transmission of HPV infections to women by reducing incidence and persistence of high-HPV viral load infections in their male partners.

The main strengths of this study include the extensive longitudinal data on HPV viral load collected every 6 months for 2 years and the performance of quantitative HPV viral load measurements by real-time PCR. Our study is also the first to examine the type-specific HPV viral load in both the glans and shaft of the penis. Our main study limitation is that HPV viral load measurements were not normalized to account for the number of cells in each swab sample (viral load copies/cell). However, the copies/scrape units have been previously used to show that a high HPV viral load (ie, >250 copies/scrape) in men is associated with a higher prevalence of flat penile lesions and greater HPV type concordance in their female partners [9, 10]. Furthermore, sensitivity analyses examining the HPV viral load in incident infections as a continuous variable found similar results as the main analyses that used the 250 copies/scrape cut point. We also observed fairly low β -globin positivity in our samples (61.5% in glans samples and 43.5% in shaft samples). A possible explanation is that penile cells, particularly in shaft samples, are more keratinized and anucleated than those in the cervix, and therefore may contain less human DNA [28]. A lower frequency of β -globin positivity in penile swab samples has also been documented in a similar study [17]. Because of the fairly low frequency of beta-globin positivity in our study, it is possible that the HPV viral load measurements in this study may be underestimated. However, all of the incidence and persistence sensitivity analyses restricted to β -globin-positive samples found results similar to those of the main analyses.

Interestingly, high-viral load infections appeared to have a higher risk of HPV persistence than low-viral load infections, irrespective of circumcision status. This may be due to the greater number of virus-infected cells that the immune system has to eliminate for high-viral load infections, compared with low-viral load infections, although this needs to be confirmed in a larger study that accounts for other factors associated with HPV clearance.

In conclusion, this study showed that male circumcision reduces the hazard of acquisition and risk of persistence of high-HPV viral load penile infections in the glans in Kenyan men. High HPV viral load in men is suggested to be associated with an increased risk of HPV transmission to female partners [9]. Therefore, male circumcision could potentially reduce the risk of HPV transmission to women by reducing the hazard of acquisition and risk of persistence of high-HPV viral load infections in men. These findings support the effectiveness of male circumcision as an intervention to reduce the incidence and risk of persistence of high-viral load HPV infections in men and, potentially, the incidence of subsequent HPV infection in their sexual partners.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary

data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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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