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Higher HPV16 and HPV18 Penile Viral Loads are associated with Decreased HPV Clearance in Uncircumcised Kenyan Men

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Abstract

Background—Whether higher penile human papillomavirus(HPV) viral load is associated with a lower rate of HPV clearance remains unknown.

Objectives—We examined the association between penile HPV16 and HPV18 viral load and subsequent HPV clearance in uncircumcised Kenyan men.

Study Design—Participants were HIV-seronegative, sexually active, 18–24 year old men randomized to the control arm of a male circumcision trial in Kisumu, Kenya. Men provided exfoliated penile cells from two anatomical sites (glans/coronal sulcus and shaft) every 6 months for 2 years. GP5+/6+ polymerase chain reaction(PCR) was used to identify 44 HPV-DNA types. HPV viral load testing was conducted using a LightCycler real-time PCR assay; viral load was classified as high(> 250 copies/scrape) or low(≤ 250 copies/scrape), for non-quantifiable values. The Kaplan-Meier method and Cox regression modelling were used to examine the association between HPV viral load and HPV clearance.

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Conflicts of Interests: V. Senkomago has analyzed data for other projects in collaboration with Trovagene Inc. and Gen-Probe. DM. Backes, MG. Hudgens, C. Poole, SR. Meshnick, N. Schlecht, S. Moses, K. Agot, AT. Hesselink, and R.C. Bailey do not have a conflict of interest with this manuscript. J. S. Smith has received consultancy and/or research grants from GSK and Merck Corporation over the past five years. P. Snijders has received speaker fees from Roche, Qiagen, Gen-Probe, Abbott Seegene, and has minority stake in Self-screen, and CJL Meijer has received speaker fees from GSK, Roche, Qiagen, SPSMD/Merck, Seegene, and has minority stake in Diassay and Self-screen.

Ethics Approval

The study protocol was approved by Institutional Review Boards of the Universities of Illinois at Chicago, Manitoba, Nairobi and North Carolina; RTI International and VU University Medical Center. The trial is registered at clinicaltrials.gov, NCT00059371.

Results—A total of 1097 men, with 291 HPV16 and 131 HPV18 cumulative infections over 24 months were analyzed. HPV clearance at 6 months after first HPV detection was lower for high versus low viral load HPV16 infections in the glans [adjusted hazard ratio (aHR) = 0.65(95% confidence interval, 0.46-0.92)] and shaft (aHR=0.44(0.16-0.90), and HPV18 infections in the glans [aHR=0.05(0.01-0.17)].

Discussion—High versus low HPV viral load was associated with reduced HPV clearance for HPV16 infections in the glans and shaft, and for HPV18 infections in the glans, among young uncircumcised men. Reduced clearance of high viral load HPV16 and HPV18 infections in men may increase HPV transmission to their female partners as well as enhance the development of penile lesions in comparison to men with low viral load HPV infections.

SUMMARY

Two-year study of 313 uncircumcised Kenyan men found that high versus low penile HPV viral load was associated with reduced HPV16 clearance (glans and shaft), and reduced HPV18 clearance (glans).

Keywords

Human Papillomavirus (HPV); viral load; Natural history; Clearance; Men; Kenya

BACKGROUND

Persistent infections with high-risk HPV types 16 and 18 cause approximately 70% of cervical cancer in women and one third of penile squamous cell carcinomas in men^{1–3}. HPV viral load is an important factor in the natural history of HPV infection; in women, higher HPV16 or HPV18 viral loads are associated with decreased HPV clearance and increased progression to high grade cervical intraepithelial neoplasia in comparison to lower HPV viral loads^{4–11}.

In men, higher penile HPV16 or HPV18 viral loads have been associated with greater HPV type concordance with female sexual partners as well as a greater prevalence of flat penile lesions in comparison to lower HPV viral loads^{12–14}. It is suggested that higher versus lower HPV viral load infections in men increase HPV transmission to their female partners and enhance the development of penile lesions by reducing the rate of HPV clearance^{12,13}. However, the association between penile HPV viral load and the subsequent rate of HPV clearance in men remains unknown. To our knowledge, only one study in Uganda has examined the association between penile HPV viral load and HPV persistence; however, this study did not examine type-specific HPV viral load and only examined HPV infections in the glans anatomical site¹⁵.

OBJECTIVE

We present the results of a longitudinal study in uncircumcised men in Kisumu, Kenya, examining: (i) the association between HPV16 or HPV18 viral load at baseline and participant characteristics; and (ii) the association between penile HPV16 and HPV18 viral load and the subsequent rate of HPV clearance in the glans and in the shaft anatomical sites

over 24 months. This analysis was restricted to uncircumcised men since male circumcision is strongly associated with a higher prevalence of low versus high viral load HPV16 and HPV18 infections as well as increased HPV clearance^{17–19}. This analysis focusses on further understanding the natural history of HPV infection in uncircumcised men, and implications for development of penile lesions in these men as well as HPV transmission to their female partners.

STUDY DESIGN

Study population and enrollment

Uncircumcised men in Kisumu, Kenya were invited to participate in a randomized controlled trial (RCT) estimating the effect of male circumcision on HIV incidence¹⁶. Briefly, inclusion criteria were: being uncircumcised, aged 18–24, HIV negative, sexually active within the past 12 months and having blood hemoglobin ≥ 90 g/l. The study protocol was approved by Institutional Review Boards of the Universities of Illinois at Chicago, Manitoba, Nairobi and North Carolina; RTI International and VU University Medical Center.

An ancillary HPV study was conducted among men in the RCT who consented to the collection of penile exfoliated cells and their shipment overseas for HPV DNA and HPV viral load testing. Uncircumcised men who: i) had no HPV follow-up data; ii) were HPV16- or HPV18-negative throughout follow-up; iii) had their first HPV16- or HPV18-positive result at their last study visit; or iv) had missing HPV viral load data (mainly due to missed visits) were excluded from clearance analyses for that specific HPV type (HPV16 or 18) and anatomical site (glans or shaft) (Fig.1).

Follow-up and specimen collection

Participant follow-up visits were scheduled at 6-month intervals over 24 months. A standardized questionnaire on sociodemographic characteristics and sexual behavior was administered by a trained male interviewer at each visit¹⁶. Penile exfoliated cell specimens were collected by a trained clinical officer at each study visit 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)²⁰. Details of the processing and freezing of penile swab samples have been described in detail elsewhere^{13,21}. All frozen samples were transported to the Department of Pathology at VU Medical Center in Amsterdam, Netherlands, for HPV DNA testing.

HPV DNA and Sexually Transmitted Infection (STI) Testing

DNA was isolated from cell samples using NucleoSpin 96 Tissue kit (Macherey-Nagel, Germany) and a Microlab Star robotic system (Hamilton, Germany) according to manufacturers' instructions. The presence of human DNA was evaluated by β -globin – specific PCR, followed by agarose gel electrophoresis^{22,23}. HPV positivity was assessed by GP5+/6+PCR, followed by hybridization of PCR products using an enzyme immunoassay readout with two HPV oligoprobe cocktail probes that detect 44 HPV types. Subsequent

HPV genotyping was performed by Reverse Line Blot (RLB) hybridization of PCR products^{12,22,23}.

HIV testing was performed at each visit using two HIV antibody rapid tests (Determine, Abbott Diagnostic Division, Hoofddorp, 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 Nairobi¹⁶. A physician or clinical officer conducted a genital exam during which a swab (if symptomatic) or urine sample were collected for PCR detection of *C. trachomatis* and *N. gonorrhea* (Roche Diagnostics). Serum specimens were tested for herpes simplex virus type 2 (HSV-2) ELISA (Kalon).

HPV Viral Load Testing

HPV DNA viral load testing was performed on all cells samples that contained HPV-16 and/or HPV-18 (regardless of beta-globin results) 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 µl of the remaining cell suspensions. All samples were run in duplicate in the same run and resulting HPV viral load 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 amount of HPV DNA in one femtogram was multiplied by the dilution factor and divided by the weight of the cloned pHPV viral genome.

HPV viral load was categorized as high (> 250 copies/scrape), or low (< 250 copies/scrape); 250 copies/scrape was the limit of quantification of HPV viral load measurements, samples with non-quantifiable DNA and quantifiable DNA at or below 250 copies/scrape were classified as low viral load. Previous studies using this cut-point have found that high versus low HPV viral load is associated with increased HPV type concordance with female partners and a higher prevalence of flat penile lesions^{12,14}.

Statistical Analyses

The distribution of participant characteristics by HPV viral load was examined among all HPV16- or HPV18-positive men at baseline; categorical proportions were compared using [chi]² test and continuous variables were assessed using the Wilcoxon rank-sum test. For these comparisons, HPV viral load results from the glans and shaft were combined; high viral load was defined as >250 copies/scrape in the glans or shaft, and low viral load was defined as < 250 copies/scrape in both the glans and shaft.

All analyses were performed separately for each HPV type (HPV-16 or HPV-18) and anatomical site (glans or shaft), since HPV clearance has been shown to vary by anatomical site¹⁷. For each participant, only the first detected HPV16 or HPV18 infection (prevalent or incident) was included. A prevalent infection was defined as an HPV16- or HPV18-positive result at baseline, and an incident infection was defined as an HPV16- or HPV18-positive result in participants who were negative for that specific HPV type at baseline. Time-to-clearance since HPV detection was calculated starting at the visit date of first HPV detection; for prevalent infections it was baseline, and for incident infections it was the visit

date of first HPV detection. Clearance was assumed to occur at the mid-point between the last HPV-positive result and the first subsequent HPV-negative result. Men were censored at their last observed visit if they remained HPV-positive for the same HPV type at the same anatomical site, for each observed consecutive visit. For all clearance analyses, we combined incident and prevalent infections for each HPV type at each anatomical site and adjusted for HPV infection type (incident versus prevalent) as a covariate. Actual circumcision status at each visit was reviewed to verify that men remained uncircumcised during HPV detection and clearance. Twelve men assigned to the control arm were circumcised over follow-up, but in each case circumcision was performed after HPV clearance so these men were included in analyses.

The Kaplan-Meier method was used to estimate HPV clearance of high versus low viral load HPV infections over 24 months. Cox regression models were fitted to estimate the adjusted hazard ratios (aHRs) for clearance of high versus low viral load HPV infections at 6 months after HPV detection. An interaction term between HPV viral load category and time-to-clearance was included in all the Cox regression models to allow for non-proportional hazards in clearance of high and low viral load HPV infections. We examined clearance at 6 months after HPV detection since this was the first follow-up time point. Potential confounders were identified from the literature, and analyzed using a directed acyclic graph. All models were adjusted for: educational attainment and bathing frequency at baseline; HPV infection type (incident or prevalent infection), HIV infection status and infection with multiple HPV types at detection of HPV16/HPV18; and condom use, number of sex partners, and infection with other STIs in the previous 6 months²⁵. aHRs could not be obtained in cases where all high or low viral load HPV infections had cleared at 6 months after detection.

Additional analyses restricted to high viral load infections (with quantifiable viral load values >250 copies/scrape) were performed to examine the association between the natural log HPV viral load at detection of HPV16 or HPV18 infection, and the rate of HPV clearance after 6 months at each site. Sensitivity analyses were conducted restricting analyses to beta-globin-positive results.

RESULTS

Of the 2299 uncircumcised men with baseline HPV and viral load results, 1140 men were randomized to the control arm, and 1097(96%) had follow-up HPV data (Fig. 1). Over 24 months, HPV16-positive results were obtained for 238(22%) men for glans samples and 120(11%) men for shaft samples. A total of 115(10%) men had an HPV 18-positive result for glans samples and 54(5%) for shaft samples. HPV16 or HPV18 infections that had missing viral load results or that were first detected at the participants' last study visit were excluded from clearance analyses [HPV16 glans=44(18%), HPV16 shaft=23(19%), HPV18 glans=25(22%) and HPV18 shaft=13(24%)] (Fig.1).

Distribution of Participant characteristics by baseline HPV viral load

Of the 2299 uncircumcised men enrolled in RCT, 239(10%) men were HPV16-positive and 103(5%) were HPV18-positive in either the glans or shaft (Table 1). Infection with multiple

HPV types at baseline was greater in men with high versus low viral load infections for both HPV16 (67.9% versus 57.7%, respectively) and HPV18 (80% versus 60.5%, respectively). The prevalence of STIs in HPV18-positive men was greater in those with high versus low viral load infections (36.9% versus 18.4%, respectively), however this association was not observed for HPV16 infections. Men with high versus low viral HPV16 or HPV18 viral load appeared to have similar distributions of all other variables examined.

Clearance of high versus low viral load HPV16 or HPV18 infections

Over 24 months, a greater proportion of high versus low viral load HPV16 infections were observed in the glans than shaft for both HPV16 (56.2% versus 23.7%, respectively) and HPV18 (77.8% versus 61.0%, respectively) (Table 2). Whereas the majority of HPV18 infections (glans and shaft) and HPV16 infections in the glans had high viral loads, a smaller proportion of HPV16 infections in the shaft had high viral load (23.7%). Most HPV16 and HPV18 infections in the glans or shaft cleared within 6 months of detection (Fig.2). Reduced HPV clearance for high versus low viral load infections was observed for HPV16 infections in the glans [aHR=0.65(0.46-0.92)] and shaft, [aHR=0.44(0.16-0.90)], and for HPV18 infections in the glans [aHR=0.05(0.01-0.17)] at 6 months after HPV detection. For these three models, p-value for the interaction of HPV viral load category and time to clearance were <0.001, 0.014 and <0.001, respectively. The interaction term parameter estimates were all negative, indicating that the association between viral load and clearance decreased over time. All low viral load HPV18 infections in the shaft had cleared within 6 months of detection. Sensitivity analyses restricted to beta-globin samples (56.7% in glans and 48.4% in shaft) produced similar, but less precise results (Table 2).

HPV Clearance per log HPV viral load among high viral load infections

When analyses were restricted to high viral load infections, a log increase in HPV viral load was associated with lower HPV clearance in the glans at 6 months after HPV detection for HPV18 [aHR=0.62(0.49-0.78)] but less precise estimates were obtained for HPV16 [aHR=0.93(0.83-1.05)] (Table 3). For both models, p-value for the interaction of log HPV viral load and time to clearance was <0.001. aHRs for HPV clearance per log HPV viral load at 6 months could not be obtained for HPV16 and HPV18 infections in the shaft due to the small number of high viral load infections present at 6 months (HPV16=3; HPV18=2 infections).

DISCUSSION

Among uncircumcised Kenyan men, reduced HPV clearance, which may also be considered as a greater risk of HPV persistence, was observed for high versus low viral load HPV16 and HPV18 infections in the glans, and for HPV16 infections in the shaft at 6 months after HPV detection. All low viral load HPV18 infections in the shaft had cleared within 6 months of detection. Analyses restricted to high viral load infections with quantifiable viral load measurements found that a log increase in HPV viral load was associated with decreased clearance for HPV18 but less so for HPV16 infections in the glans at 6 months after HPV detection.

To our knowledge, only one study in Ugandan men has examined the association in penile HPV viral load and subsequent HPV clearance¹⁵. This study found that prevalent high viral load infections for high-risk HPV types were more likely to persist to 6 months and 12 months in comparison to low viral load infections in the glans. These findings are similar to our results that showed reduced HPV clearance at 6 months after HPV detection for high versus low viral load HPV16 and HPV18 infections in the glans.

We found similar, but attenuated results for the associations between log HPV viral load and HPV clearance, in comparison to the respective analyses performed with high versus low HPV viral load categories for HPV16 and HPV18 glans infections. This suggests that the larger distinction between high versus low HPV viral load (> 250 versus ≤ 250 copies/scrape) may be more indicative of reduced HPV clearance than smaller changes in log HPV viral load.

Reduced HPV clearance of high versus low viral load infections may be explained by the greater number of virus-infected cells the immune system has to eliminate²⁶. Alternatively, reduced HPV clearance of high versus low viral load infections may reflect a weaker immune response to the HPV virus and virus-infected cells²⁶. Our baseline risk factor analyses found that high HPV16 or HPV18 viral load is associated with a higher prevalence of multiple HPV types; this suggests that high HPV viral loads are possibly more likely to occur in men with weaker immune systems in comparison to low HPV viral loads.

Main strengths of this study include the extensive longitudinal data on HPV viral load collected every 6 months for 2 years, and quantitative HPV viral load measurements by real-time PCR. Our study is also the first to examine the association between type-specific HPV viral load and HPV clearance in both the glans and shaft anatomical sites. One limitation is that HPV viral load measurements were not normalized to account for number of cells in each swab sample (viral load copies/cell). However, the copies/scrape units have been previously used to show the associations between high HPV viral load (> 250 copies/scrape) and higher prevalence of flat penile lesions as well as increased HPV type concordance with female partners^{12,14}. It is also possible that HPV-negative results may reflect HPV viral loads below detection level rather than HPV clearance; however this is unlikely since the GP5+/6+PCR-RLB procedure has been shown to be highly sensitive and specific for the detection of 44 HPV types²⁰. The lower reproducibility of PCR assays when examining low viral copy numbers is a possible limitation of this study; however, our examination of duplicate samples analyzed in the same run found high agreement of viral load measurements, including categorization of low viral load measurements (< 250 copies/scrape) (Pearson $r = 0.90$). We also observed fairly low beta-globin positivity (56.7% in glans and 48.4% in shaft) and it is possible that HPV viral load measurements in this study may be underestimated. However, all the clearance analyses restricted to beta-globin-positive samples found similar results as the main analyses, and a similar prevalence of beta-globin positivity have been observed among men in a similar study in Uganda.¹⁸ Other limitations of this study include possible length bias in prevalent infections, small number of HPV infections, and the inability to know when incident infections occurred or when infections cleared.

In conclusion, this study found that high HPV16 and HPV18 viral load in the glans was associated with a reduced rate of HPV clearance in young uncircumcised men. Reduced clearance of high viral load penile HPV infections in men may increase HPV transmission to their female partners as well as the development of penile lesions in comparison to men with low viral load HPV infections.

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Figure 1.
Study flowchart showing samples included in analyses of HPV viral load and HPV clearance

Figure 2.
Clearance of HPV16 and HPV18 infections by HPV viral load in uncircumcised Kenyan men a
aHigh viral load was defined as > 250 copies/scrape, and low viral load was defined as ≤ 250 copies/scrape at the time of HPV detection.

Table 1

Baseline participant characteristics, stratified by HPV16 and HPV18 viral load in men in Kisumu, Kenya

Demographic characteristics ^b	HPV16 Prevalent Infections (N =239) ^a		HPV18 Prevalent Infections (N=103) ^a	
	High HPV16 Viral Load (N =109)	Low HPV16 Viral Load (N =130)	High HPV18 Viral Load (N =65)	Low HPV18 Viral Load (N =38)
Age (years)	20 (19-21;18-24;109)	20 (19-22;18-24;130)	20 (19-22;18-24;65)	20 (20-22;18-24;38)
Education level				
Less than secondary	40 (36.7)	55 (42.3)	23(35.4)	15 (39.5)
Any secondary or above	69 (63.3)	75 (57.7)	42(64.6)	23 (60.5)
Employment status				
Employed and receiving a salary	6 (5.5)	7(5.4)	7 (10.8)	3 (7.9)
Self-employed	38 (34.7)	41 (31.5)	21 (32.3)	12 (31.6)
Unemployed	65 (59.6)	82 (63.1)	37 (56.9)	23 (60.5)
Marital status				
Not married (no live-in partner)	103 (95.4)	125 (96.1)	61 (93.8)	34 (89.5)
Married (not living with wife)	2 (1.8)	0	4 (6.2)	3 (7.9)
Married (living with wife)	3 (2.8)	5 (3.9)	0	0
Infection with Multiple HPV types ^c				
Yes	74 (67.9)	75 (57.7)	52 (80.0)	23 (60.5)
No	35 (32.1)	55 (42.3)	13 (20.0)	15 (39.5)
Infection with sexually transmitted infections ^d				
Positive	32 (29.3)	45 (34.6)	24 (36.9)	7 (18.4)
Negative	77 (70.7)	85 (65.4)	41 (63.1)	31 (81.6)
Number of partners in previous 6 months				
0	12 (11.1)	12 (9.3)	8 (12.3)	4 (10.5)
1	41 (38.0)	63 (48.8)	27 (41.5)	19 (50.0)
2+	55 (50.9)	54 (41.9)	30 (46.2)	15 (39.5)
Used condom with intercourse in previous 6 months				
Always	17 (17.5)	32 (27.1)	11 (18.6)	5 (14.7)
Inconsistent	54 (55.7)	59 (50.0)	33 (55.9)	18 (52.9)
Never	25 (25.7)	27 (22.9)	14 (23.7)	11 (32.4)
Bathing Frequency				
Less than daily	3 (2.8)	3 (2.3)	2 (3.1)	2 (5.3)
Daily	105 (97.2)	127 (97.7)	63 (96.9)	36 (94.7)

^aThis table includes only men with detected HPV16 DNA or HPV18 DNA at baseline. High viral load was defined as >250 copies/scrape in the glans or shaft; low viral load was defined as ≤ 250 copies/scrape in the both the glans and shaft at the time of HPV detection. Numbers and percentages may not sum up to totals due to missing values and rounding.

^bData are median (IQR; range; n) for continuous data, or n (%) for categorical data.

^cInfection with multiple HPV types was defined as co-infection with at least one other HPV type

^dPositive result for HSV-2, *Trichomonas vaginalis*, *Chlamydia trachomatis* or *Neisseria gonorrhoeae* was examined.

Table 2
Clearance of high versus low viral load HPV16 and HPV18 infections in uncircumcised Kenyan Men

HPV Type	Site	Viral Load ^a	Total Infections over 24 months, N (%)	Cleared infections over 24 months (n/N) ^b	Total Person-Years over 24 months	aHR for clearance at 6-months (95%CI) ^c
All Samples						
HPV 16	Glans	High	109 (56.2)	92/109	56.14	0.65 (0.46 - 0.92)
		Low	85 (43.8)	76/85	26.6	1
	Shaft	High	23 (23.7)	23/23	8.07	0.44 (0.16 - 0.90)
		Low	74 (76.3)	67/74	20.95	1
HPV 18	Glans	High	70 (77.8)	61/70	30.71	0.05 (0.01 - 0.17)
		Low	20 (22.2)	18/20	5.03	1
	Shaft	High	25 (61.0)	23/25	7.43	---- ^d
		Low	16 (39.0)	15/16	3.90	
Beta Globin-Positive Samples						
HPV 16	Glans	High	69 (62.7)	60/69	30.83	0.49 (0.29 - 0.82)
		Low	41(37.3)	40/41	13.64	1
	Shaft	High	11 (23.9)	11/11	3.90	---- ^e
		Low	35 (76.1)	34 /35	9.41	
HPV 18	Glans	High	44 (74.6)	38/44	18.33	0.09 (0.02 - 0.30)
		Low	15 (25.4)	14/15	4.14	1
	Shaft	High	13 (56.3)	12/13	3.55	---- ^d
		Low	7 (43.7)	7/7	1.87	

^a High viral load was defined as >250 copies/scrape; low viral load was defined as ≤ 250 copies/scrape at time of HPV detection

^b Clearance was defined as an HPV-negative result in participants who were positive for that given HPV type at previous visit; n= number of cleared HPV infections; N=total number of HPV infections observed

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Adjusted Hazard Ratio (aHR) comparing clearance of high versus low viral load infections at 6 months after HPV detection. HR adjusted for educational attainment and bathing frequency at baseline; HPV infection type (incident or prevalent infection), HIV infection status and infection with multiple HPV types at detection of HPV16/HPV18; and condom use, number of sex partners, and infection with other STIs in the previous 6 months

Adjusted hazard ratio (aHR) could not be obtained because all low viral load HPV infections had cleared at 6 months after HPV detection

Adjusted hazard ratio (aHR) could not be obtained because all high viral load HPV infections had cleared at 6 months after HPV detection

Table 3
Description and HPV clearance of high viral load HPV16 and HPV18 infections in uncircumcised Kenyan Men

HPV Type	Site	HPV Clearance over 24 months	High viral load infections ^a	HPV Viral Load (copies/scrape)			aHR for log clearance per log viral load at 6 months (95% CI) ^b
				Minimum	Maximum	Median Interquartile Range	
HPV16	Glans	Cleared	92	255.75	5499146.97	7369.93 2455.61 - 67039.23	0.93 (0.83 - 1.05)
		Not cleared	17	365.74	5190308.15	7054.00 913.39 - 115683.80	
	Shaft	Cleared	23	307.11	374494.80	2526.00 660.86 - 15796.75	-- ^c
		Not cleared	0	-	-	-	
HPV 18	Glans	Cleared	61	320.72	8847926.00	22151.09 2213.30 - 87972.02	0.62 (0.49-0.78)
		Not cleared	9	836.98	411792.90	34350.93 24997.25 - 76519.61	
	Shaft	Cleared	23	284.01	4885641.00	3261.15 1260.93 - 13191.94	-- ^c
		Not cleared	2	722.39	69416.42	35069.40 722.38 - 69416.42	

^a High viral load was defined as >250 copies/scrape; only viral load >250copies/scrape was accurately quantifiable and examined as a continuous variable

^b Adjusted Hazard Ratio (aHR) comparing clearance per log HPV viral load at 6 months after HPV detection. HR adjusted for educational attainment and bathing frequency at baseline; HPV infection type (incident or prevalent infection), HIV infection status and infection with multiple HPV types at detection of HPV16/HPV18; and condom use, number of sex partners, and infection with other STIs in the previous 6 months

^c Adjusted hazard ratio (aHR) could not be obtained because model did not converge due to the small number of high viral load infections present at 6 months after detection (HPV16 shaft=3 and HPV18 shaft=2 infections)