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Prevalence and Risk Factors of Human Papillomavirus Infection by Penile Site in Uncircumcised Kenyan Men

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Abstract

Human papillomavirus (HPV) prevalence was estimated from 2,705 sexually active, uncircumcised, human immunodeficiency virus seronegative men aged 17–28 years in Kisumu, Kenya. HPV prevalence was 51.1% (95% confidence interval: 49.2 – 53.0%) in penile cells from the glans/coronal sulcus and/or shaft. HPV prevalence varied by anatomical site, with 46.5% positivity in the glans/coronal sulcus compared with 19.1% in the shaft (p<.0001). High-risk HPV was detected in 31.2% of glans and 12.3% of shaft samples (p<.0001). HPV16 was the most common type and 29.2% of men were infected with more than one HPV type. Risk factors for HPV infection included presence of *C. trachomatis*, *N. gonorrhea*, self-reported sexually transmitted infections, and less frequent bathing. Lifetime number of sexual partners and herpes simplex virus type-2 seropositivity were also marginally associated with HPV infection.

Keywords

Human papillomavirus; HPV; Glans; Shaft; Men; Prevalence; Kenya

Introduction

Human papillomavirus (HPV) infection is the main cause of invasive cervical cancer (ICC) in women, and an important cause of vaginal, vulvar and anal cancer in women as well as anal and penile cancer in men. Given that men are important in transmitting infection to their female partners, understanding factors associated with HPV infections in men are important for control of HPV in both men and women. HPV prevalence data in men are limited, especially from Africa, despite ICC being the most common female cancer in this region 1. Type-specific data in men are needed to improve understanding of factors associated with male HPV infection and the role of penile high-risk HPV (i.e. oncogenic) types in transmission to female partners.

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Few data are available comparing HPV type-distribution from different anatomical sites in men 2,3. Although risk factors for penile HPV infections in men have been reported 4, data remain unreported on associations between penile HPV infection and laboratory-diagnosed sexually transmitted infections (STIs) including *C. trachomatis* and *N. gonorrhea*.

We previously reported penile HPV prevalence in three anatomical sites (shaft; glans/coronal sulcus; and urethra) from 98 men in Kisumu, Kenya 5. Here we assess type-specific HPV prevalence by anatomical site and estimate risk factors for prevalent HPV infection in over 2,700 young sexually active, uncircumcised men in Kenya, including the 98 men reported on previously.

Materials and Methods

Study Population and Enrollment

Uncircumcised men aged 17–28 years were screened between February 4, 2002 and September 6, 2005 in Kisumu, Kenya to participate in a randomized controlled trial (RCT) of male circumcision 6. The primary aim of the RCT was to determine the effectiveness of male circumcision in reducing human immunodeficiency virus (HIV) incidence. In brief, inclusion criteria included being uncircumcised, HIV seronegative, sexually active, and having hemoglobin of ≥9.0 g/100 mL. Study participants were recruited from STI clinics, workplaces, and community organizations.

This present analysis includes men screened for the RCT who consented to HPV sample collection and its shipment overseas for testing. A total of 6,686 men were screened for participation in the RCT 6. Of these, 4,489 were eligible to participate in the main RCT. The primary reasons for ineligibility included having age outside the required range (18–24) or unknown age, being a nonresident of Kisumu, refusing to consent to HIV testing, not being completely uncircumcised, being HIV seropositive (or with indeterminate results), not being sexually active in previous 12 months, or having an existing medical problem. Of the 4,489 eligible men, 2,705 consented to participate in the HPV study and had risk factor data collected at the baseline visit (2,228 men were enrolled into the RCT, 477 were not). The study protocol was approved by the Institutional Review Boards of all collaborating institutions and the University of North Carolina.

Questionnaire, Clinical Examination, and Specimen Collection

After undergoing informed consent, participants were administered a standardized questionnaire on sociodemographic characteristics and sexual behavior by a trained male interviewer. Participants also underwent a clinical examination by a trained physician or clinical officer as described previously 5. Penile exfoliated cells were collected for HPV DNA detection from two anatomical sites using prewetted Dacron swabs in separate conical tubes: i. shaft and external foreskin tissue (shaft specimen) and ii. the glans, coronal sulcus and inner foreskin tissue (glans specimen). For the shaft specimen, shaft and external foreskin cells were collected from each of the 4 sides of the external shaft tissue, from the proximal to distal penile shaft, using a Type 3 Dacron swab prewetted in Tris buffer. For the glans specimen, a second penile exfoliated cell sample was collected using a second prewetted Type 3 Dacron swab as follows: the prepuce was gently retracted to collect exfoliated cells by swabbing the tip of the urethral opening, completely circling the urethral orifice 2–3 times; sampling the top to the bottom of the glans in a circular motion around the complete circumference of the penis; rotating the swab 3 times completely around the circumference of the coronal sulcus; and sampling from the inner foreskin tissue. Sampling of the penile exfoliated cell specimens for HPV testing did not exclude areas of the glans/coronal sulcus/inner foreskin or shaft that were affected by genital warts.

Both penile cell samples were placed in individual 15-mL centrifuge tubes containing 2-mL 10 mmol/L Tris-HCl, 0.01 mol/L, 7.4 pH, buffer, and processed on the day of collection at the UNIM (Universities of Nairobi, Illinois, and Manitoba) clinic laboratory by centrifugation at high speed (maximum, 3000g) for 10 minutes. Excess Tris-HCl buffer was discarded using a Pasteur pipette, and the remaining cell pellet was resuspended in the same volume of 0.1 mmol/L Tris-HCl buffer, and vortexed. Diluted cell pellets were then frozen at −75°C. All samples were sent using a dry shipper to the Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands, for HPV DNA laboratory testing.

HPV DNA Laboratory Testing

DNA was isolated from penile exfoliated cell samples using NucleoSpin 96 Tissue kit (Macherey-Nagel, Germany) and a Microlab Star robotic system (Hamilton, Germany) according to manufacturers' instructions. Presence of human DNA was evaluated by β-globin specific PCR, followed by agarose gel electrophoresis. HPV positivity was assessed by GP5+/6+ PCR followed by hybridisation of PCR products using an enzyme immunoassay readout with two HPV oligoprobe cocktail probes that, together, detect 44 HPV types: HPV6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71 (equivalent to CP8061), 72, 73, 81(equivalent to CP8304), 82(IS39 and MM4subtypes), 83 (equivalent to MM7), 84(equivalent to MM8), cand85, 86, cand89(equivalent to CP6108) and JC9710. Subsequent HPV genotyping was performed by reverse line blot hybridisation of PCR products, as described previously7‚8. Primers and probe sequences, as well as cycling and staining conditions were detailed previously 7‚8.

HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered high-risk types. HPV infections with multiple HPV types were considered high-risk if one or more high-risk HPV types were detected. Low-risk types included all other HPV types. HPV types detected by enzyme immunoassay but not by reverse line blot genotyping were designated as HPVX, indicating a type, sub-type or variant not detectable with probes used for reverse line blot hybridization.

Urine samples were tested for *N. gonorrhea* and *C. trachomatis* infections by PCR-based methods (Roche Diagnostics) and for *Trichomonas vaginalis* (Tv) by culture (Biomed Diagnostics). Serum specimens were tested for syphilis antibody and herpes simplex virus type 2 (HSV-2) antibody (Kalon). Positive Rapid Plasma Reagin (RPR) (Becton Dickinson) tests were confirmed by *Treponema pallidum* hemagluttination (TPHA) assay (Randox Laboratories).

Statistical Methods

Associations between categorical variables and anatomical site-specific HPV infection were assessed using Pearson's χ^2 test. Mantel–Haenszel methods were employed to account for multiple measures from each participant at the two anatomical sites. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for possible risk factors were estimated via age-adjusted, univariate logistic regression models, conditional on anatomical site. Multivariate logistic regression models were used to estimate associations between each variable and HPV infection, simultaneously adjusting for other possible risk factors. Multivariate models included risk factors with significant marginal associations with HPV or variables believed a priori to be potential confounders. Analyses were repeated with participants categorized as HPV-positive if i) HPV infection was detected at one or both anatomical sites; and ii) at least one high-risk type was detected. Analyses utilized data from all specimens collected regardless of β-globin positivity unless stated otherwise.

Results

HPV and β-Globin PCR Positivity by Anatomical Collection Site

The median age of 2,705 participating men was 20 (range of 17 – 28). HPV was detected in either the glans/coronal sulcus or shaft specimen of 1,382 men (51.1%, 95% CI: 49.2 – 53.0%). HPV prevalence varied by anatomical site, with 1,258 men (46.5%) having detectable viral DNA in penile exfoliated cells from the glans compared to 518 men (19.1%) with HPV in the shaft (p<.0001). β-globin positivity was 57.0% in the glans and 35.9% in the shaft (p<.0001). HPV DNA positivity was significantly higher in the glans compared to the shaft in both β-globin positive (50.1% vs. 24.5%; p<.0001) and β-globin negative (41.7% vs. 16.1% p<.0001) samples.

HPV Type-Specific Distribution

High-risk HPV16 was the most common type detected in either site (9.8% overall, 7.5% of glans samples, 3.7% of shaft samples, p<.0001). Among men with HPV in the glans or the shaft, 19.1% had detectable HPV16. The second most common type detected in either site was HPV56 (6.1% overall, 5.3% glans, 2.1% shaft, p<.0001). HPVX was detected at either anatomical site in 6.1% of males. The ten next most common types overall (i.e. detected in either site) were HPV42 (5.2%), HPV67 (5.1%), HPV66 (5.1%), HPV52 (4.7%), JC9710 (4.7%), HPV18 (4.3%), HPV35 (4.3%), HPV43 (4.2%), HPV40 (4.1%), and HPV31 (4.0%). All other types detected (HPV6, 11, 22, 25, 26, 30, 32, 33, 34, 39, 44, 45, 51, 53, 54, 55, 58, 59, 64, 68, 69, 70, 72, 73, 81, 82, 83, 84, 85, 86, and CP6108) were found in less than 4% of samples. Similar results were obtained when β-globin negative specimens were excluded. For example, the five most common types overall were HPV16, HPV56, HPV67, HPV42, and HPV66 among β-globin positive specimens.

At least one high-risk HPV type was detected in either the glans or shaft in 954 men (35.3%, 95% CI: 33.5–37.1). The prevalence of high-risk HPV infection varied by anatomical site (31.2% in the glans, 12.4% in the shaft, p<.0001). Detection of only low-risk HPV types in either the glans or shaft occurred in 428 men (15.8%, 95% CI 14.5–17.3), and was higher in the glans (15.3%) than in the shaft (6.8%), p<.0001. HPV infections with two or more types were detected in either the glans or shaft of 789 men (29.2%, 95% CI: 27.5–30.9), and also varied by site (25.1% in the glans, 7.2% in the shaft, p<.0001). Thus 57% (789/1382) of men with HPV in the glans or the shaft were infected with multiple HPV types.

Risk Factors for Penile HPV Infection

HPV penile prevalence in the glans and in the shaft did not appear to vary by age (Figure 1). Presence of laboratory-detected *C. trachomatis* and *N. gonorrhea*, self-reported STIs, and lower frequency of bathing had the strongest positive associations with penile HPV presence, both in glans/coronal sulcus and overall analyses (Table 1). Use of condoms with last sexual partner was negatively associated with HPV prevalence. Lifetime number of sexual partners was positively associated with HPV, but no association was found with years of sexual activity. Earning an income and lower educational attainment were associated with increased odds of HPV, although education was not significant after adjusting for other risk factors. In age-adjusted univariate models among 2,272 men with HSV-2 results (2,222 men enrolled in the main RCT, 50 not enrolled), HSV-2 was significantly associated with HPV presence in the glans (p=0.0004) and overall (p=0.02), but not in the shaft (p=0.59). In multivariate models, HSV-2 was marginally significant for HPV in the glans (OR=1.25, 95% CI 1.01–1.54, p=0.04) but not in the shaft (OR=0.89, 95% CI 0.68–1.16, p=0.38) or overall (OR=1.1, 95% CI 0.90–1.37, p=0.34). The following possible risk factors were also considered, but are not listed in Table 1 due to lack of association with HPV or high correlation with other risk factors: marital status, travel to Nairobi in last 6 months, age at

first intercourse, use of condom in the last six months, number of female partners in the last 12 months, paid money for sex in the last 6 months, ever tasted alcohol, presence of syphilis antibodies, positive Tv urine culture, and hours until washing penis after sex. Genital warts were uncommon, with only 1% (28/2705) of men having warts in the glans, coronal sulcus or foreskin, and no men having warts in the shaft. Similar results (data not shown) were found for associations with infection with at least one high-risk HPV type. Results were also similar when analyses were restricted to β-globin positive specimens, although some risk factors (e.g., bathing frequency) were no longer significantly associated with HPV infection due to smaller sample sizes relative to analyses using all specimens.

Discussion

HPV infection was detected in approximately half of participating men, with a higher HPV prevalence in the glans/coronal sulcus as compared to the shaft. Participating men were most commonly infected by HPV16, the most frequent HPV type detected in both invasive anal and penile cancers worldwide 9›10. The strongest risk factors for penile HPV infection included laboratory-diagnosed *C. trachomatis* or *N. gonorrhea* infections, less frequent bathing, and a self-reported history of STIs.

Penile HPV prevalence observed here is consistent with previous studies from Kenya that found 58% prevalence among 250 fishermen aged 18–63 years from Kisumu 11 and 57% prevalence among 49 young, high-risk men aged 18–29 years from Brazil 12. Our HPV prevalence results among men are somewhat higher than that reported among similarly aged women in Nairobi, Kenya 13, comparable to other reports that found HPV DNA prevalence among men as high as similarly aged women.

Penile HPV prevalence was stable across age. Albeit based on a relatively narrow age-band of 17 to 28 years, these data are consistent with similarly-aged young females where HPV prevalence generally peaks between 15 and 25 years of age 14. Associations observed here between HPV and infection with *C. trachomatis or N. gonorrhea* are consistent with global data on HPV risk from women 15 and are not unexpected given that these infections are all transmitted sexually. Inflammation induced by *C. trachomatis* or *N. gonorrhea* infections may increase the likelihood of HPV carriage among men. However, given the cross-sectional design, we are not able to investigate temporal relationships.

Main study advantages include the detection of HPV DNA using a sensitive assay to detect a broad range of types at two different anatomical sites among a large sample of men. To our knowledge, this is the first study to investigate the association between penile HPV infection and laboratory-diagnosed STIs. Our results of a higher prevalence of HPV infection in the glans/coronal sulcus than the shaft are in contrast with two previous U.S. studies 2›3, which both found a relatively higher prevalence of HPV infection in the shaft than the glans/coronal sulcus. Differences in study results may potentially be due to differences in hygienic practices, sampling or laboratory techniques between studies.

In our study, β-globin PCR positivity was relatively low. In reference to the relatively low rates of beta-globin positivity, especially in shaft samples, a possible explanation might be that the cells collected from the penis, in particular those of the shaft, are more keratinized and anucleated than those obtained from the cervix, and therefore may contain less human DNA. As such, beta-globin testing may not be the best control for HPV DNA testing among men. Nevertheless, HPV detection in the shaft, unlike that in glans, could have been somewhat underestimated. Differences in sampling quality as reflected by beta-globin positivity however, cannot fully account for differences in HPV positivity between the two anatomical sites since HPV DNA positivity was significantly higher in the glans compared

to the shaft in beta-globin positive specimens ($p<0.001$). Because overall HPV prevalence and risk factors were generally similar when all samples or only β-globin positive samples were included in statistical analyses, this suggests that amplifiable HPV copies often exceed those of the β-globin gene.

Our results may not be generalized to a broader population of similarly aged men, given that participants in this study were from one region in Kenya, uncircumcised, sexually active, and met criteria to participate in a trial that entailed circumcision. Bailey et al11 provide additional discussion of the generalizability of results from the RCT to the general population of young Kenyan men. For the subjects reported on here, 82% (2221/2696) were enrolled in the RCT. There was no difference in the overall prevalence of HPV between men who were and were not enrolled in the RCT ($p=0.16$). Risk factors considered in Table 1 were similar between men enrolled and not enrolled in the RCT, although enrolled men tended to have higher education ($p=0.01$) and were more likely to have used a condom with their last sexual partner ($p=0.02$).

Men reporting less frequent washing had a higher prevalence of penile HPV infection. Since all the participants in this baseline study were uncircumcised, it remains to be seen if the association between lower bathing frequency and HPV infection is modified by circumcision status. This will be assessed in our analysis of incident and persistent HPV infections over study follow-up. Although prophylactic vaccines targeting oncogenic types HPV16 and 18 may soon be approved for the prevention of HPV persistence and genital warts among young men, current vaccines do not cover all high-risk HPV types, and may prove too costly for implementation among men in many less-developed countries. Meanwhile, in light of our current findings, interventions promoting penile hygiene may be worth consideration.

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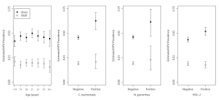

Figure 1.
Estimated human papillomavirus (HPV) prevalence with 95% confidence intervals by anatomical site.

Table 1

Estimated odds ratios (ORs) and 95% confidence intervals (CIs) for human papillomavirus (HPV) by anatomical site and overall[†].

		Glans								Shaft								Overall							
		Univariate model				Multivariate model				Univariate model				Multivariate model				Univariate model				Multivariate model			
Variable	**N**	**OR**	**95%**	**CI**	**p-value[‡]**	**OR**	**95%**	**CI**	**p-value**	**OR**	**95%**	**CI**	**p-value**	**OR**	**95%**	**CI**	**p-value**	**OR**	**95%**	**CI**	**p-value**	**OR**	**95%**	**CI**	**p-value**
Presence of N. gonorrhea																									
Yes	52	1.87	1.06	3.28		1.97	1.03	3.75		1.26	0.65	2.41		1.09	0.53	2.25		2.40	1.31	4.40		2.32	1.17	4.58	
No	2610	ref	-	-	0.03	ref	-	-	0.04	ref	-	-	0.49	Ref	-	-	0.81	ref	-	-	0.005	ref	-	-	0.02
Presence of C. trachomatis																									
Yes	126	2.01	1.39	2.91		1.87	1.24	2.81		1.09	0.70	1.69		1.04	0.64	1.68		1.77	1.22	2.58		1.66	1.09	2.50	
No	2535	ref	-	-	0.0002	ref	-	-	0.003	ref	-	-	0.71	Ref	-	-	0.89	ref	-	-	0.003	ref	-	-	0.02
Presence of STI (self-reported)																									
Yes	239	1.95	1.48	2.55		1.78	1.30	2.43		1.44	1.05	1.96		1.31	0.92	1.87		1.94	1.47	2.56		1.82	1.32	2.51	
No	2466	ref	-	-	<0.0001	ref	-	-	0.0002	ref	-	-	0.02	Ref	-	-	0.13	ref	-	-	<0.0001	ref	-	-	0.0002
Bathing frequency																									
Less than daily	59	2.10	1.23	3.60		2.71	1.37	5.36		1.21	0.65	2.25		1.18	0.57	2.43		2.62	1.47	4.67		2.90	1.41	5.97	
Daily	2620	ref	-	-	0.01	ref	-	-	0.004	ref	-	-	0.55	Ref	-	-	0.66	ref	-	-	0.001	ref	-	-	0.004
Use of condom last partner																									
Yes	1302	0.77	0.66	0.90		0.80	0.68	0.95		0.84	0.69	1.02		0.86	0.69	1.06		0.78	0.67	0.91		0.80	0.67	0.94	
No	1396	ref	-	-	0.001	ref	-	-	0.01	ref	-	-	0.08	Ref	-	-	0.15	ref	-	-	0.001	ref	-	-	0.01
Lifetime # of female partners																									
1	208	ref	-	-		ref	-	-		ref	-	-		Ref	-	-		ref	-	-		ref	-	-	
2–5	1426	1.55	1.14	2.10		1.40	1.02	1.93		1.57	1.03	2.38		1.54	1.00	2.39		1.66	1.23	2.23		1.53	1.11	2.10	
6+	842	1.75	1.28	2.40	0.002[‡]	1.44	1.02	2.04	0.14[‡]	1.62	1.05	2.51	0.10[‡]	1.51	0.94	2.41	0.34[‡]	1.76	1.29	2.41	0.002	1.48	1.05	2.08	0.03
Years of sexual activity																									
0–2	521	ref	-	-		ref	-	-		ref	-	-		Ref	-	-		ref	-	-		ref	-	-	
3–4	650	0.99	0.78	1.25		0.86	0.67	1.11		0.85	0.64	1.14		0.77	0.57	1.05		0.93	0.74	1.17		0.79	0.61	1.01	
5–6	671	1.12	0.88	1.42		1.02	0.79	1.32		0.86	0.64	1.16		0.80	0.58	1.10		1.02	0.80	1.29		0.92	0.71	1.19	

		Glans								Shaft								Overall							
		Univariate model				Multivariate model				Univariate model				Multivariate model				Univariate model				Multivariate model			
Variable	**N**	**OR**	**95%**	**CI**	**p-value‡**	**OR**	**95%**	**CI**	**p-value**	**OR**	**95%**	**CI**	**p-value**	**OR**	**95%**	**CI**	**p-value**	**OR**	**95%**	**CI**	**p-value**	**OR**	**95%**	**CI**	**p-value**
7+	742	1.08	0.84	1.39	0.39‡	0.90	0.68	1.20	0.80‡	1.01	0.74	1.38	0.84‡	0.86	0.61	1.21	0.53‡	1.01	0.79	1.30	0.73‡	0.83	0.62	1.10	0.41‡
Employment status																									
Income	1239	1.37	1.17	1.60		1.23	1.03	1.48		1.28	1.05	1.55		1.26	1.00	1.58		1.35	1.16	1.57		1.23	1.03	1.48	
No Income	1457	ref	-	-	<0.0001	Ref	-	-	0.03	ref	-	-	0.01	Ref	-	-	0.05	ref	-	-	0.0002	ref	-	-	0.03
Education																									
Primary or none	975	1.48	1.11	1.98		1.24	0.88	1.73		1.36	0.94	1.99		1.05	0.69	1.61		1.44	1.08	1.92		1.20	0.86	1.67	
Secondary	1487	1.05	0.80	1.40		1.04	0.76	1.42		1.03	0.71	1.49		0.89	0.60	1.31		0.99	0.75	1.31		0.96	0.71	1.31	
Tertiary	243	ref	-	-	<0.0001‡	ref	-	-	0.09‡	ref	-	-	0.02	Ref	-	-	0.35‡	ref	-	-	<0.0001	ref	-	-	0.07‡

†Results are based on all specimens, regardless of β-globin positivity. Analyses restricted to β-globin positive specimens gave similar results.

‡Ordinal variables were treated as continuous with consecutive integer values if the likelihood ratio goodness-of-fit test did not reject the assumption of linearity.