Reduced Clearance of Penile Human Papillomavirus Infection in Uncircumcised Men

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The relationship between circumcision and the acquisition and clearance of human papillomavirus (HPV) infection was examined in a cohort of 357 men followed up at 2-month intervals for an average of 431 days. There were no differences in HPV acquisition by circumcision status. Clearance of HPV infection, including infection with oncogenic types, was slower in the glans/coronal sulcus of the penis of uncircumcised men than circumcised men. The median duration of HPV infection of the glans/coronal sulcus was significantly longer in uncircumcised men (154 days) than circumcised men (91 days) (P = .04). Circumcision may protect against HPV-associated disease by enhancing the resolution of infection.

There is growing evidence that circumcision may protect against human papillomavirus (HPV) infection and penile cancer [1–5]. Sex partners of uncircumcised men have an elevated risk of cervical cancer [3], which suggests that circumcision may also reduce the transmission of HPV. We recently demonstrated that, compared with circumcised men, men who are uncircumcised have a higher prevalence of HPV infection, specifically of the glans/coronal sulcus of the penis [6]. We pursued these findings further through an examination of the relationship between circumcision status and the acquisition and clearance of HPV infection of the external genitalia in a cohort of men.

Methods. This study was approved by the Committee on Human Studies of the University of Hawaii. Written informed consent was obtained from all study subjects. Study participants were primarily recruited from a university population in Hawaii. Eligible men were at least 18 years old, spoke English, and had no history of blood-clotting disorders (because of collection of blood for serological analysis). From July 2004 through December 2006, 445 men were initially enrolled, and 357 were subsequently followed up at 2-month intervals. The 88 men who did not complete >1 visit were excluded from the present analysis. Study visits were conducted at the University of Hawaii Health Services and the Cancer Research Center of Hawaii.

A structured questionnaire was administered by a trained interviewer at each study visit. The questionnaires covered demographic information and medical, sexual, and reproductive characteristics.

Trained clinicians collected exfoliated cell samples from the external genitals. Circumcision status was determined by the clinician obtaining the specimen. Separate specimens were collected from the glans penis and corona sulcus, penile shaft, and scrotum. For uncircumcised men, an additional specimen was obtained from the inner foreskin. Visible warts and lesions were avoided in sampling the genitals. Disposable gloves worn by clinicians were changed between the sampling of each site to minimize the risk of contamination between sites. Textured paper and saline-moistened swabs were used in the sampling procedure, which has been described elsewhere [6–8].

DNA was extracted from each specimen by means of commercial reagents (Qiagen). The polymerase chain reaction used PGMY09/PGMY11 primers to amplify a 450–base pair region of the L1 HPV genome. HPV-positive specimens were subsequently genotyped using a reverse line blot detection method for 37 HPV types. HPV-positive specimens that were subsequently found to be negative by the genotyping assay were considered to be unclassified HPV-positive specimens. All specimens were also tested using GH20 and PC04 primers to amplify a 268–base pair region of the human β-globin gene as an internal control for sample sufficiency. Specimens testing negative for β-globin were considered to be insufficient and were excluded from the analyses.

Twenty-eight men self-reporting to be positive for human immunodeficiency virus were excluded from all analyses because of the strong confounding effect of human immunodeficiency virus on HPV infection [9]. HPV DNA results were
evaluated by anatomic site (glans/coronal sulcus, shaft, and scrotum). HPV results from individual sites were also combined for analysis of any genital HPV infection. HPV results for the inner foreskin were excluded from the analysis of any genital infection because of lack of a comparable site in circumcised men. HPV genotypes were grouped as follows: (1) any HPV, including unclassified types; (2) oncogenic HPV; and (3) other HPV. Oncogenic HPV included types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 [10]. Other HPV included nononcogenic HPV and HPV of undetermined risk status: types 6, 11, 26, 40, 42, 53–55, 61, 62, 64, 66, 67, 69–73, 81–84, IS39 (subtype HPV-82), and CP6109 (HPV-89).

Analyses of HPV acquisition and clearance were limited to incident HPV infections, defined as the presence of an HPV genotype not identified during a previous visit. HPV clearance was defined as the acquisition of infection followed by the lack of detection of that HPV genotype at 2 or more subsequent visits. The timing of viral clearance was defined as the time of the first negative visit. We assessed the overall differences in the clearance of HPV infection between circumcised and uncircumcised individuals by the Kaplan-Meier (product-limit) method and the Wilcoxon test for the equality of strata.

The association between HPV acquisition or clearance and circumcision status was modeled through Cox regression, using the number of days of HPV-negative status (for acquisition) or the number of days since infection acquisition (for clearance) as the time metric. Hazard ratios (HRs) and 95% confidence intervals (CIs) were used as measures of association for the comparison of uncircumcised men with circumcised men. Separate infection paths were assigned to each HPV genotype detected. Estimation of risk associated with oncogenic types included specimens concurrently positive for other types. Likewise, risk estimation for other HPV included specimens concurrently positive for oncogenic types. Factors previously observed to be associated with circumcision and/or HPV infection [6] were included as covariates. These included age (continuous), race/ethnicity (white/nonwhite), birthplace (United States/non–United States), education (less than college education/college degree), lifetime number of female sex partners (continuous), history of sex with men (yes/no), condom use during prior 4 months (yes/no), and history of genital warts (yes/no). All P values were 2-sided; differences were considered to be statistically significant at $P < .05$.

**Results.** The cohort included 357 men followed up for at least 2 visits; 290 were circumcised, and 67 were uncircumcised. Men were followed up for an average of 431 days (range, 38–1262 days) over a mean of 7.2 visits (range, 2–9 visits). The mean age of the cohort was 29.2 years (range, 18–79 years). The majority of men were white (58%), were US born (81%), had never been married (82%), and were college students (64%). Seventy-five percent were heterosexual, with a median lifetime number of female sex partners of 6.5.

Overall, specimen sufficiency, measured by the presence of human β-globin, did not differ by circumcision status; for all genital specimens combined, 6068 (91%) of 6660 from circumcised men were positive for β-globin, compared with 1435 (92%) of 1557 from uncircumcised men ($P = .18$). For the glans/coronal sulcus specifically, β-globin detection was lower among circumcised men (1994/2220 [90%]), compared with that among uncircumcised men (481/519 [93%]) ($P = .05$). There were no differences in specimen sufficiency by circumcision status for shaft or scrotal specimens.

A total of 536 genotype-specific incident infections were observed across all genital sites during the follow-up period. There were no differences in the risk of HPV acquisition by circumcision status for any genital site (data not shown).

The duration and risk of clearance of incident HPV infections were evaluated (Table 1). The duration of infection did not vary by circumcision status for the penile shaft, scrotum, or all genital sites combined. There were also no differences in the risk of HPV clearance by circumcision status for these sites. For the glans/coronal sulcus, the median duration of HPV infection was greater among uncircumcised men (154 days) than circumcised men (91 days), although the 95% CIs overlapped. Product-limit estimates for the time to clearance of HPV infection of the glans/coronal sulcus were significantly longer among uncircumcised men than circumcised men ($P = .04$) (Figure 1). Uncircumcised men had a lower risk of HPV clearance in the glans/coronal sulcus than did circumcised men. The lower risk of clearance was observed for any HPV (HR, 0.59 [95% CI, 0.36–0.98]), oncogenic HPV (HR, 0.36 [95% CI, 0.14–0.91]), and other HPV (HR, 0.50 [95% CI, 0.25–0.98]).

**Discussion.** Our results demonstrate that, compared with circumcised men, uncircumcised men have a poorer ability to resolve HPV infections of the glans/coronal sulcus of the penis. The reduced viral clearance among uncircumcised men was observed for both oncogenic and nononcogenic HPV types. We previously observed a higher prevalence of any HPV and oncogenic HPV infection of the glans/coronal sulcus among uncircumcised men [6]. The present analysis suggests that the higher prevalence of HPV may be attributed to a longer duration of infection of the glans/coronal sulcus among uncircumcised men rather than to a greater rate of acquisition of infection.

Our results are consistent with 2 other longitudinal investigations of HPV infection in men, both of which demonstrated a reduced persistence and greater clearance of genital HPV among circumcised men [11, 12]. Lu et al [12] similarly observed greater clearance of oncogenic HPV as well as any HPV. In this study, however, site-specific estimations were not included, as individual genital specimens (glans/coronal sulcus, shaft, and scrotum) were combined for testing.

A major limitation of our study and other longitudinal in-
Table 1. Duration and Clearance of Genital Human Papillomavirus (HPV) Infection in Men (n = 357), by Circumcision Status—Hawaii, 2004–2008

<table>
<thead>
<tr>
<th>Site, circumcision status</th>
<th>HPV duration (any HPV(^a)), median (95% CI), days</th>
<th>HPV clearance, adjusted(^b) HR (95% CI)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Any HPV(^a)</td>
<td>Oncogenic HPV(^c)</td>
</tr>
<tr>
<td>Any genital site(^e)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circumcised</td>
<td>109 (89–126)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Uncircumcised</td>
<td>106 (64–146)</td>
<td>1.04 (0.76–1.40)</td>
<td>0.90 (0.48–1.68)</td>
</tr>
<tr>
<td>Penis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Foreskin</td>
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<td></td>
<td></td>
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<tr>
<td>Circumcised</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Uncircumcised</td>
<td>112 (70–184)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Glans/coronal sulcus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circumcised</td>
<td>91 (71–111)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Uncircumcised</td>
<td>154 (68–248)</td>
<td>0.59 (0.36–0.98)</td>
<td>0.36 (0.14–0.91)</td>
</tr>
<tr>
<td>Shaft</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Circumcised</td>
<td>119 (108–176)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Uncircumcised</td>
<td>105 (91–182)</td>
<td>1.06 (0.71–1.58)</td>
<td>0.60 (0.24–1.49)</td>
</tr>
<tr>
<td>Scrotum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circumcised</td>
<td>91 (75–112)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Uncircumcised</td>
<td>70 (63–106)</td>
<td>0.95 (0.67–1.35)</td>
<td>1.24 (0.48–3.22)</td>
</tr>
</tbody>
</table>

NOTE. Boldface type indicates a statistically significant lower risk of clearance. CI, confidence interval; HR, hazard ratio.

\(^a\) Includes untyped HPV-positive specimens.
\(^b\) Adjusted for age (continuous), race/ethnicity (white/nonwhite), birthplace (United States/non–United States), education (less than college education/college degree), lifetime number of female sex partners (continuous), history of sex with men (yes/no), condom use during prior 4 months (yes/no), and history of genital warts (yes/no).
\(^c\) Oncogenic HPV types are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.
\(^d\) Includes nononcogenic HPV types and HPV types of undetermined risk status (6, 11, 26, 40, 42, 53–55, 61, 62, 64, 66, 67, 69, 70–73, 81–84, IS39 [subtype HPV-82], and CP6109 [HPV-89]).
\(^e\) Excludes foreskin specimens.

vestigations of HPV is the inability to distinguish clearance of infection from failure to detect an infection. Lack of viral detection may result from fluctuations in viral levels, sampling variability, and limitations in assay sensitivity.

It is not likely that the greater specimen sufficiency for the glans/coronal sulcus of uncircumcised men compared with that of circumcised men explains the observed differences in viral clearance. Measurement of HPV acquisition and clearance was limited to specimens that were positive for β-globin. Moreover, a lower percentage of sufficient glans/coronal sulcus samples among circumcised men would make detection of a clearance event less likely, which may therefore lead to a longer estimated duration of HPV infection among circumcised men. However, the opposite was observed in our data—that is, circumcised men had a shorter duration of infection of the glans/coronal sulcus.

A major strength of this investigation is the short interval between visits (2 months), which enhanced our ability to examine the acquisition of new infections and the duration of these incident infections. Estimates of viral duration and clearance events were made more robust by limiting these observations to incident infections and excluding prevalent HPV infections detected at baseline. We were able to account for sexual history, including condom use and number of sex partners, and for other potential confounders related to either circumcision status or HPV infection. Site-specific sampling and testing allowed us to separately evaluate genital subsites.

Figure 1. Clearance of human papillomavirus (HPV) infection of the glans/coronal sulcus of the penis, by circumcision status. The Wilcoxon test for equality of time-to-clearance rates between circumcised and uncircumcised men indicated \(P = .04\).
Our study demonstrates that the apparent protective influence of circumcision against genital HPV infection may not involve a reduction in new infections but rather the enhanced ability to resolve existing HPV infections. Similar to what has been observed in females, HPV infections are generally transient in males [11]. It is not understood how circumcision facilitates greater clearance of HPV. It is possible that HPV persists more efficiently within the mucosal surface to the inner foreskin of uncircumcised men compared with the keratinized penile surface of circumcised men. Thorough washing of the genitals may be more difficult for uncircumcised men, because this requires retraction of the foreskin to expose the inner surface [13]. To what extent genital washing influences HPV clearance is not known. Nonetheless, we did not observe a longer duration of HPV infection of the foreskin compared with that of the glans/coronal sulcus among uncircumcised men, indicating that the infections did not persist longer in the mucosal surface.

Our observation of a reduced clearance time for oncogenic HPV infection specifically in the glans/coronal sulcus of uncircumcised men has clinical significance. Uncircumcised men have an increased risk of penile cancer [1, 5], and the glans penis is the primary subsite of penile cancer [14, 15]. Partners of uncircumcised men have an increased risk of cervical cancer [3], underscoring the possibility that transmission of HPV to sex partners is more efficient among uncircumcised men because of the greater duration of their infections.

Strategies to minimize the persistence of HPV infection combined with those to prevent acquisition of infection may be the most effective means of controlling HPV-associated disease. Whether circumcision is an effective means of facilitating HPV clearance has yet to be demonstrated.

Acknowledgments

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References