Short Communication

Correlates of Human Papillomavirus Viral Load with Infection Site in Asymptomatic Men

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Abstract

Numerous studies have evaluated human papillomavirus (HPV) DNA load in women, especially HPV-16 viral load, and its role in cervical carcinogenicity. Few studies have examined HPV viral load in men, none among asymptomatic men. The aim of the current study is to quantify HPV-16 viral load in male anogenital specimens and to explore its correlates with anatomic sites. Two-hundred and ninety-four specimens from 42 men who tested positive for HPV-16 at one or more anatomic sites were evaluated. HPV DNA was detected with PGMY 09/11 primer and genotyped with reverse line blot assay followed by HPV-16 viral quantification using type-specific real-time PCR assay (TaqMan). The quantitative PCR assay showed a higher sensitivity in HPV-16 viral DNA detection compared with the reverse line blot assay. Viral load varied significantly by anatomic site (P = 0.019). Penile shaft specimens had significantly higher viral load than any other anatomic site evaluated except for the anal canal. HPV-16 viral load was positively correlated between proximal anatomic sites: perianal and anal canal (P = 0.003), perianal and scrotum (P = 0.011), scrotum and glans/corona (P = 0.045), and scrotum and penile shaft (P = 0.037). In conclusion, the penile shaft seemed to be the preferred site for HPV-16 viral replication. Viral load correlation between proximal sites suggested a possible autoinoculation in male HPV transmission. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3573–6)

Introduction

Our understanding of the natural history of human papillomavirus (HPV) infection in males is still very limited due to the small number of publications on the topic. Male HPV infection is important in male to female transmission of HPV and subsequent infection and disease in women (1, 2). Case-control studies of women with cervical cancer and their husbands have shown that men’s sexual behavior affects women’s risk of cervical neoplasia, even after controlling for female sexual activity (3, 4). HPV-16 viral load may be an important factor in the natural history of HPV in men as it may determine risk of infection persistence and subsequent disease in men and transmission to women (5-7).

In women, HPV viral load seems to be important in disease progression. Recent studies suggest that HPV-16 viral load may be used as a molecular biomarker to improve the prognostic value of HPV testing (8-10). Other studies found a positive correlation between high HPV-16 load and high-grade squamous intraepithelial lesions (11, 12). Retrospective studies observed that persistent infection with HPV-16 with persistently high viral load values are risk factors for the development of cervical cancer (13, 14). HPV-16 viral load has also been evaluated as a biomarker for the clearance of HPV infection in women (15). Unfortunately, there are few publications that have reported HPV viral load in men (5-7), and none that have examined viral load by anatomic site. The purpose of the current study is to determine HPV-16 viral load in male anogenital specimens and explore its possible association with anatomic site.

Subjects, Materials, and Methods

Study design, clinical sampling, and HPV testing for the current analysis have been previously described in detail (16-18). Briefly, a cross-sectional study of HPV infection in 463 men between 18 and 40 years old—359 in Tucson, Arizona and 104 in Tampa, Florida—was conducted from 2003 to 2006. Participants completed a risk factor questionnaire that included questions on sociodemographic, clinical, and sexual behavioral factors. Anogenital specimens were obtained independently by rubbing separate saline-wetted Dacron swabs over the entire surface of the (a) glans penis/coronal sulcus, (b) penile shaft (including the prepuce, if present), (c) scrotum, (d) perianal area, and (e) anal canal up to the anal verge. A calcium alginate or Dacron urethral swab was used to
sample the first 2 cm of the urethral epithelium. Semen specimens were self-collected in a sterile cup.

**HPV DNA Detection and Genotyping.** DNA extracted from swabbed cellular material and semen was used for HPV testing by PCR for amplification of a fragment of the L1 gene using the PGMY system (19). HPV genotyping was conducted using the reverse line blot method (20). This detection method uses the HPV L1 consensus PCR products labeled with biotin to detect 37 HPV types. The same DNA extracts were used for quantitation of viral load using HPV-16 type-specific real-time PCR.

**HPV-16 Viral Load.** Specimens from seven anatomic sites of those men who were HPV-16–positive at one or more anatomic sites by the reverse line blot method were evaluated for HPV-16 viral load using a quantitative real-time PCR assay (TaqMan) as previously described (21). Briefly, the standard curve for absolute quantification of HPV-16 was generated using the E6-E7 region of HPV-16 that had been cloned into plasmid vectors (21). Real-time PCR reactions were done in a 20 μL volume using the ABI PRISM1 7900HT instrument (Applied Biosystems). A picogreen fluorescence assay to determine DNA concentration was used to normalize viral load values with cell equivalents (CE) in exfoliated skin cells (Invitrogen). HPV-16 standard curves were generated by plotting the cycle threshold (Ct) values against known concentrations of input HPV-16 copy number. The calculated CE based on the picogreen assay was used to determine the viral copy number per CE for each unknown sample as described by van Duin et al. (15). Serial dilutions of CaSki DNA (HPV-16–positive cell line) ranging from 2.7 to 10.8 ng were used as positive controls to determine inter-run variability, as well as non-template controls as negative control.

**Statistical Analysis.** Viral load (per 10⁶ CE) was log₁₀-transformed and summarized in box plots and scatter plots. The Kruskas-Wallis test and the Wilcoxon rank-sum test were applied to compare viral load by anatomic site and by reverse line blot genotyping results. The Spearman rank correlation was used to determine the correlation in viral load between anatomic sites.

### Results

Of 463 participants enrolled in the cross-sectional study, 42 participants who tested positive for HPV-16 at one or more anatomic sites by the reverse line blot method were

<table>
<thead>
<tr>
<th>Anatomic site</th>
<th>Reverse line blot genotyping result no. (%)</th>
<th>Median viral load (range)</th>
<th>HPV-16 negative Median viral load (range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites</td>
<td>82 (67.2)</td>
<td>782 (3-373,782)</td>
<td>40 (32.8)</td>
<td>19 (0.8-990)</td>
</tr>
<tr>
<td>Anal canal</td>
<td>6 (62.5)</td>
<td>1,315 (665-43,797)</td>
<td>3 (37.5)</td>
<td>4 (0.8-9)</td>
</tr>
<tr>
<td>Perianal area</td>
<td>6 (50.0)</td>
<td>5,667 (26-82,742)</td>
<td>6 (50.0)</td>
<td>12 (2-178)</td>
</tr>
<tr>
<td>Scrotum</td>
<td>17 (68.0)</td>
<td>138 (3-373,782)</td>
<td>8 (32.0)</td>
<td>78 (17-588)</td>
</tr>
<tr>
<td>Men</td>
<td>18 (60.0)</td>
<td>251 (7-38,212)</td>
<td>12 (40.0)</td>
<td>28 (4-332)</td>
</tr>
<tr>
<td>Urethra</td>
<td>2 (50.0)</td>
<td>114 (59-170)</td>
<td>2 (50.0)</td>
<td>40 (5-76)</td>
</tr>
<tr>
<td>Penile shaft</td>
<td>32 (64.2)</td>
<td>2,486 (12-75,701)</td>
<td>6 (15.8)</td>
<td>156 (3-990)</td>
</tr>
<tr>
<td>Urethra</td>
<td>2 (40.0)</td>
<td>590 (229-991)</td>
<td>3 (60.0)</td>
<td>2 (1-8)</td>
</tr>
</tbody>
</table>

*Denotes statistical significance (α = 0.05).
included in this analysis, contributing a total of 294 specimens. The mean age of the 42 participants was 27.5 years (SD, 6.3). The majority of men were nonsmokers (57%) or former smokers (17%), and circumcised (86%).

Of the 294 specimens evaluated for HPV-16 DNA, 122 specimens were HPV-16–positive by quantitative PCR method, whereas only 82 of 122 specimens were HPV-16–positive by reverse line blot method. Of 122 specimens with a detectable viral load, significantly higher viral load was found in reverse line blot HPV-16–positive specimens than that in reverse line blot HPV-16–negative specimens ($P < 0.001$), and the differences remained significant for individual sites: anal canal ($P = 0.037$), perianal ($P = 0.013$), scrotum ($P = 0.005$), and penile shaft ($P = 0.006$; Table 1).

The distribution of HPV-16 viral load by anatomic site is shown in Fig. 1. Among all specimens with a detectable viral load, the viral copy number ranged from $<1$ to $4.65 \times 10^4$, with the highest median viral copy of 133 copies detected in anal canal specimens and the lowest median viral copy of 1 copy observed in urethra specimens. After cellular normalization, viral load (per CE) showed a similar pattern in distribution. Normalized viral load ranged from $<1$ to 373 copies per CE with the highest median viral load of 1.6 copies per CE (range, $<1$-75 copies per CE) found on the penile shaft followed by the anal canal and perianal area, and the lowest median viral load found in the urethra. Viral load varied significantly by anatomic site ($P = 0.019$). The penile shaft had a significantly higher viral load than any other anatomic sites except for the anal canal. Figure 2 presents the correlation of viral load between anatomic sites. HPV-16 viral load was significantly correlated between specimens from anatomic sites in close proximity: perianal and anal canal ($P = 0.003$), perianal and scrotum ($P = 0.011$), scrotum and glans/corona ($P = 0.045$), and scrotum and penile shaft ($P = 0.037$).

Discussion
To our knowledge, this is the first study to examine HPV-16 viral load at seven different anogenital sites using type-specific real-time PCR in men. In this study, the real-time PCR assay detected HPV-16 viral copies in 122 specimens. Of 122 specimens, 40 (32.8%) specimens with significantly lower viral load had been classified as HPV-16–negative by reverse line blot genotyping assay. This may indicate that the threshold of detection in the real-time PCR assay could be lower than the one observed for the reverse line blot assay. Alternatively, our quantitative PCR assay could be less sensitive to PCR inhibitors present in skin exfoliated cells of the male genital area. Although PCR-based genotyping assays are the method of choice to detect HPV DNA, the use of more sensitive assays such as quantitative real-time PCR may be advantageous in minimizing false negatives in HPV testing. HPV-16 viral load in male genital specimens in the present study seems to be much lower compared with HPV-16 viral load found in female cervical specimens, as shown in a previous study by our group (22). This finding is consistent with the results reported by Bleeker and colleagues, in which 61.4% of male specimens had viral load values that were below the detection threshold of the assay compared with 0% of female cervical specimens (5).

In the present study, the penile shaft seems to be the preferred site for HPV-16 replication among all the sites evaluated. We observed significantly higher viral loads in the penile shaft than in most anatomic sites. In addition, we have previously shown that infections with
multiple HPV types were most frequently detected on the penile shaft of men enrolled in this cross-sectional study (17). We observed significant positive correlations in viral load between infection sites that were in close proximity such as perianal and anal canal, or perianal and scrotum, or the sites that are in regular contact with each other at a resting position such as the scrotum and glans/corona, or scrotum and penile shaft. This observation suggests that previously HPV-uninfected genital sites may be inoculated by existing infections from proximal anatomic sites through direct contact between the sites and facilitated by environmental factors such as humidity, temperature, and possibly poor hygiene. Autoinoculation has been observed in the transmission of other viruses such as the herpes simplex virus (23, 24). In the case of herpes simplex virus, active virions can be transported through scratching or body fluid such as saliva and lead to manual inoculation of proximal sites. This hypothesis is supported by the lack of correlation of HPV-16 viral load among distant sites such as the perianal area and the glans/corona or penile shaft. However, simultaneous acquisition of HPV infection in the correlated anatomic sites is also possible. The relationship between proximal sites observed in the present study needs to be confirmed in future studies.

In conclusion, our findings suggest that the penile shaft seems to be the preferred site for HPV-16 viral replication and autoinoculation may play a role in the transmission of HPV in men.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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References
Correction: Correlates of Human Papillomavirus Viral Load with Infection Site in Asymptomatic Men

In this article (1), which was published in the December 2008 issue of Cancer Epidemiology, Biomarkers & Prevention, an error resulted in an author's name being printed in the byline incorrectly as Lu Beibei. The author's name is Beibei Lu.

Reference


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