HUMAN PAPILLOMAVIRUS DNA DETECTION IN MALE SEXUAL PARTNERS OF WOMEN WITH GENITAL HUMAN PAPILLOMAVIRUS INFECTION

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ABSTRACT

Objectives. To determine the prevalence of human papillomavirus (HPV) DNA in the male partners of HPV-infected women, assess the concordance of the viral group in the infected pair, define the most affected sites in the male genitalia, and compare diagnostic methods in men.

Methods. Fifty male, stable sexual partners of women positive for HPV DNA by the Hybrid Capture 2 (hc2) test had material brushed from six different anogenital areas for hc2 testing. One week later, patients underwent classic peniscopy, and the lesions were biopsied for histologic analysis and hc2 testing.

Results. The brushings were HPV DNA positive in 35 (70%) of the 50 men: 32% in the high-risk HPV group, 14% in the low-risk HPV group, and 24% in both groups. HPV detection per anatomic site was 24% in the glans, 44% in the prepuce internal surface, 30% in the distal urethra, 24% in the prepuce external surface, 12% in the scrotum, and 8% in the anus. Acetowhite lesions were seen in 44 (88%) of the 50 patients. Overall, HPV DNA was detected in 27 (26%) of the 104 biopsy specimens, but histologic examination showed evidence of HPV infection in only 14 (13.5%) of 104 biopsy specimens. In 3 (6%) of 50 patients, hc2 was positive only in the histologic examination. Overall, the prevalence of detectable high-risk HPV DNA among male partners was 60% (30 of 50).

Conclusions. Of the 50 male partners studied, 76% were HPV DNA positive. Histologic examination was an inaccurate method to diagnose HPV DNA infection in men; however, brushings detected HPV in 92.1% of the infected men. UROLOGY 65: 251–255, 2005. © 2005 Elsevier Inc.

The etiologic role of human papillomavirus (HPV) in condyloma and cervical cancer is well defined. The virus is responsible for the most frequently diagnosed sexually transmitted diseases. More than 100 HPV types are now known, and about 30 are related to anogenital infection. Lórinz et al.,1 using Southern blot analysis, first established the relative risk of cervical neoplasia for the 15 most prevalent anogenital HPV types. Molecular biology and epidemiologic studies have led to a better understanding of the infection and its participation in the development of cervical carcinoma.2,3

The asymptomatic nature of HPV infection in men is believed to be responsible for the sustained transmission to female partners and thus the perpetuation of the infection in a given population. Therefore, the high prevalence of HPV infection in women, coupled with a lack of visible penile lesions in male partners, led investigators to search for more sensitive and specific diagnostic methods.

Initially, peniscopy with a colposcope of acetic-acid treated skin appeared to improve the clinical diagnostic accuracy in men.4 However, this method is now recognized to produce too many false-positive results secondary to nonspecific penile acetowhiteness that infrequently reveals abnormal histologic findings. Nicolau (data not published) found no consistent penile pattern of HPV-induced lesions. Only 23.5% had histologic findings suggestive of HPV infection. In an earlier study, brushings collected for cytologic
examination from the male sexual partners of women infected with HPV revealed koilocytosis in only 4.7% of men in the distal urethra and in 1.6% in the corona of the glans and the prepuce internal surface. Therefore, the pursuit of a more sensitive tool for detecting HPV infection in men is warranted.6–8

The objectives of the present study were to determine the prevalence of HPV DNA in the male partners of HPV-infected women, assess the concordance of viral group in infected pairs, define the partners of HPV-infected women, assess the concordance of viral group in infected pairs, define the partners of HPV-infected women, assess the con-

MATERIAL AND METHODS

The present cross-sectional study evaluated 50 heterosexual male, stable sexual partners of confirmed HPV-infected women seen at the outpatient unit of pathology of the lower genital tract at the university hospital using the Hybrid Capture 2 (hc2; Digene). The study was conducted between September 1997 and May 2000. All patients provided written informed consent, and the institutional review board approved the study. A stable relationship was defined as a duration of longer than 6 months, regardless of sexual intercourse with other partners.

At the first visit, a standardized questionnaire was administered to all patients, and material was brushed from six different anogenital areas: glans, prepuce internal surface (including sulcus and corona), distal urethra, prepuce external surface (in conjunction with cutis penis), scrotum, and anus. The material brushed from each area was carefully collected to avoid contamination and placed in separate specimen transport tubes for subsequent independent HPV DNA testing using hc2.

The brushings were collected after spraying the anogenital region with saline solution. The specimens were obtained using a vigorous motion of the conical brush included in the Digene kit, from distally to proximally and from right to left at each anatomic site. The distal urethra was anesthetized with plain 2% lidocaine using a cotton swab inserted and left for 5 minutes before brushing.

PENISCOPY

The second appointment took place 1 week later at which the patients underwent peniscopy, consecutively using 5% acetic acid, 1% toluidine blue, and Schiller’s iodine solution with enhanced visualization of the skin by a colposcope (D.F. Vasconcelos) under 4-fold, 7-fold, and 13-fold magnification, respectively. The examination strictly adhered to the following sequence of steps:

1. Examination of the entire penis and scrotum of the seated patient under magnification, initially without the solutions applied.
2. Examination of the distal 2 cm of the urethra, using a urethral speculum especially designed for this purpose.
3. The penis and scrotum were sprayed with 5% acetic acid. A cotton swab soaked with the same solution was inserted into the distal urethra. Peniscopy was performed after 5 minutes had elapsed.
4. Immediately thereafter, a cotton swab soaked with Schiller’s iodine solution was inserted into the distal 2 cm of the urethra, as described by Nicolau et al. followed by another examination with the speculum and colposcope.
5. If an acetowhite lesion was not observed, the penis and scrotum were soaked with 1% toluidine blue, removing the excess with 2% acetic acid after 3 minutes, and a final examination was done.

BIOPSY

Subclinical or clinically apparent lesions, suspicious for HPV infection, were biopsied, guided by peniscopy, before and after 5% acetic acid, 1% toluidine blue, and Schiller’s iodine solution administration in the distal urethra. Patients received local anesthesia (plain 2% lidocaine) and then underwent biopsy using a 2-mm dermateome. Hemostasis was achieved with a cotton swab soaked in concentrated metha-
cresolsulfonic acid applied to the raw area. Patients with an easily reached urethral lesion underwent biopsy with the aid of a Gaylor-Medina type forceps.

Each specimen was divided in two. One half was sent for conventional histologic analysis and the other for HPV hc2 testing. The specimens were labeled using the same anatomic description used for the brushings.

HISTOPATHOLOGIC EXAMINATION

Koilocytotic cells were considered indicative of HPV infec-
tion. Penile intraepithelial neoplasia grading was adapted from the cervical intraepithelial neoplasia classification proposed by Richart. The histopathologic examination results were reported according to the biopsied anatomic sites and recorded as negative or positive. The algorithm, characterized by peniscopy followed by biopsy and histologic examination of suspected lesions, when applicable, is referred to as the conventional method.

HYBRID CAPTURE

The specimens were sent to the Digene Brazil reference laboratory for masked HPV DNA testing according to the standard hc2 method employing the usual 1 pg/mL cutoff. hc2 is a signal-amplified hybridization microplate assay for the chemi-
luminescent detection of HPV types of low risk (6/11/42/43/44) and high risk (16/18/31/33/35/39/45/51/52/56/58/59/68) as described in the product insert.

The specimens with an RLU/cutoff value ratio of 1 or greater were considered positive.

STATISTICAL ANALYSIS

Men were considered positive for brush or biopsy specimens when any anatomically brushed site or biopsied lesion was positive for hc2. The significance of the differences using the various methods for diagnosing HPV infection among men was ascertained using the McNemar test (Statistical Package for Social Sciences, version 10.0, SPSS, Chicago, Ill). Exact binomial confidence intervals were calculated using StatExact (Cytel Boston).

RESULTS

The mean age of the 50 men was 31 years (range 19 to 53 years). On average, the current sexual relationship lasted for 71 months (range 6 to 360 months). The mean age at first sexual intercourse was 14.9 years (range 12 to 19 years). On average, couples reported sexual intercourse three times per week (range one to seven). A previous episode of a sexually transmitted disease was reported by 11 (22.4%) of 49 men and regular condom use by 9 (18.4%) of 49. Of the 50 men, 20 (40%) reported sexual relationships with more than one concurrent partner; 4 (8%) of 50 were circumcised.

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Of the 50 men, 35 (70%) were positive for HPV DNA in at least one brushed specimen, distributed as follows: high-risk group in 16 (32%), low-risk in 7 (14%), and both high-risk and low-risk in 12 (24%).

According to the anatomic site, HPV was detected by brushing the glans in 24% (95% confidence interval [CI] 13% to 38%) of the patients, prepuce internal surface in 44% (95% CI 30% to 59%), distal urethra in 30% (95% CI 18% to 45%), prepuce external surface in 24% (95% CI 13% to 38%), scrotum in 12% (95% CI 5% to 24%), and anus in 8% (95% CI 2% to 19%). In none of the cases was HPV exclusively detected in the scrotum or anus (Table I).

The collection from the prepuce internal surface, glans, and prepuce external surface combined yielded a 58% detection rate that increased to 70% by adding the brushing from the distal urethra (P = 0.01). Brushings of the scrotum or the anus did not improve the detection rate.

According to the peniscopic findings, the prepuce internal surface was the site most frequently biopsied (57 of 104). Of the 57 biopsy specimens, only 8 had histologic evidence of HPV infection, although 17 were hc2 positive (P = 0.07). Overall, HPV DNA was detected in 27 (26%) of the 104 biopsy specimens, and histopathologic examination showed evidence of HPV infection in only 14 (13.5%) (P = 0.03; Table II).

Table III compares the incidence of HPV infection detected by conventional morphology, hc2 testing on brushed material, and hc2 testing on the biopsy material. Positivity for HPV infection increased from 22% with morphology to 76% when hc2 was performed on the biopsy fragments and brushings. Three men had evidence of HPV infection by hc2 only on the biopsy fragments. Positivity of the hc2 test performed on brushed material was significantly greater than when performed on biopsy fragments (P <0.0001). In addition, all 11 cases positive for koilocytosis were also hc2 positive on brush or biopsy specimens.

Complete concordance of the HPV group in matched couples was observed in only 18 (36.7%; 95% CI 23% to 52%) of 49 couples. Of the 20 male partners of high-risk HPV women, 10 (50%) were not positive for high-risk HPV. Conversely, 20 (69%) of 29 male partners of women positive for both high-risk and low-risk HPV had high-risk HPV. Overall, 11 (22.5%) of the 49 male partners were uninfected with HPV. Furthermore, the female HPV viral load did not increase the likelihood for the same HPV group to be detected in the male partner (data not shown).
The results of this study have demonstrated that the best strategy to diagnose HPV infection in men is HPV DNA testing. In that regard, HPV DNA positivity in brushed material was greater than in biopsy fragments of the same affected area. Although the prepuce internal surface was the penile area where HPV DNA was most frequently detected, collecting from the urethra contributed significantly to HPV detection. The HPV group (high-risk or low-risk) of the female partner did not predict the male HPV group.

Peniscopy followed by biopsy and histologic examination (conventional method) was positive in only 11 (22%) of the 50 men. However, the hc2 test on brushed material detected HPV in 35 (70%) of the 50 men ($P < 0.0001$). Such a significant improvement in diagnostic efficiency was associated with a considerable reduction in cost, patient discomfort, and time required for performing the male examinations. Performing the hc2 test on biopsied material, in addition to the brushed material, of male genitalia increased HPV detection from 70% to 76% ($P < 0.005$). Therefore, our data suggest that when HPV DNA tests are available, it is unwarranted to continue performing peniscopy with subsequent biopsy of suspected lesions as a strategy to diagnose HPV in men. However, peniscopy has been recommended by many investigators, particularly when lesions are subclinical or not visible.4,12–16

HPV was detected in 58% of the men when the specimens were collected from the prepuce internal surface, glans, and prepuce external surface combined. The detection rate increased significantly to 70% when brushings from the distal urethra were added ($P = 0.01$).

In contrast, Baldwin et al.17 tested the urine of men attending a sexually transmitted disease clinic and did not find any contribution when testing urethral specimens from the distal urethra for HPV DNA by polymerase chain reaction. The material was collected with a Dacron-tipped urethral swab that was inserted, rotated, and removed from the distal urethra. The urethral samples yielded adequate DNA in only 65.6% of participants, and their laboratory was unable to develop an adequate method for the detection of HPV in urine; therefore, they did not provide the urethral results.

Moreover, Weaver et al.18 tested for HPV DNA by polymerase chain reaction in specimens collected from multiple sites of the male genitalia in two different populations, one from a sexually transmitted disease clinic and the other from university students, and found only an additional 7% of men with HPV when the urine samples were analyzed. As above, they also found that more data are needed with regard to the optimal method to process urine samples for the purpose of HPV DNA detection.

Although physicians have been reluctant to collect material from the urethra because of patient discomfort, the use of lidocaine before and after brushing made the collection procedure much less painful. Postcollection dysuria was mild, short-lived, and self-limited. No diagnostic gain was realized by collecting material from the scrotum or the anus.

Our data also did not suggest any clearcut effect of the female viral load on male HPV positivity. The data were not indicative of any consistent HPV transmission or detectable infection pattern among stable couples in which all the women were HPV infected. Other studies in published medical reports have demonstrated similar results.19–22 It is likely that both partners were at some point exposed to an infectious level of the HPV type detected at the time of this study in one partner, but not in the other. However, differences in susceptibility related to factors such as pre-existing or subsequent host immunity probably led to variable control and expression of the infection, which were translated as poor concordance of HPV detection. Thus, the impact of female HPV DNA detectability and of viral load in the dynamics of HPV transmission warrants additional study.

In this study, even though 76% of the men partners were HPV infected, penile intraepithelial neo-

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**TABLE III. Evidence of HPV infection among 50 men according to diagnostic method used**

<table>
<thead>
<tr>
<th>Method</th>
<th>HPV DNA</th>
<th>Koilocytosis</th>
<th>HPV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peniscopy, biopsy, and histologic examination</td>
<td>NA</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Biopsy (hc2)</td>
<td>18</td>
<td>32*</td>
<td>18</td>
</tr>
<tr>
<td>Brush (hc2)</td>
<td>35</td>
<td>NA</td>
<td>35</td>
</tr>
<tr>
<td>Brush or biopsy (hc2)</td>
<td>38</td>
<td>NA</td>
<td>38</td>
</tr>
</tbody>
</table>

**Notes:**

- NA = not available; other abbreviations as in Table II.
- * Six men with no visible lesions at colposcopy were considered negative.
- † Biopsy vs. brush; $P < 0.0001$ (McNemar).
plasia was very rare. As with the vagina and vulva, the penile skin frequently hosts HPV, but cancer develops very rarely. Also, only a fraction of visible abnormalities are related to HPV, with the remainder related to chronic inflammation. Sometimes they appear to be precancerous microscopically, but the risk of invasion is quite low.

CONCLUSIONS

Although examination of the male partners of women with identified HPV infections is of unclear value, our data underscored the role of HPV DNA tests. Brushed material collected from the distal urethra, in addition to the external surface of the penis, seems to be the optimal approach for diagnosing HPV infection in men.

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REFERENCES


