Prevalence of human papillomavirus infection in the urinary tract of men with urethritis

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Objectives: To investigate the prevalence of human papillomavirus (HPV) in the genital and urinary tract of men with urethritis.

Methods: Cell samples were collected from the penis, urethra and urine of 142 men with urethritis. A HPV test was performed on the samples using the modified GP5+6+ polymerase chain reaction method, and the HPV genotype was determined using a HPV GenoArray test.

Results: Out of 142 urethritis patients, HPV was detected in 48% (68 cases), and high-risk HPV was found in 32% (46 cases) of patients, on their penis or in the urinary tract (urethra or urine). HPV was detected in 31% in the penis, 20% in the urethra and 24% in the urine, while high-risk HPV was identified in 23% in the penis, 12% in the urethra and 11% in the urine. Among the HPV-positive men, 66% had HPV infection in the urinary tract where the most common HPV types were HPV6, HPV16, HPV18 and HPV58. Single HPV-type infection was more frequently found in the urinary tract (89%) than in the penis (65%) (P < 0.05).

Conclusions: Similar to the penis, the urinary tract represents a common HPV infection site in men with urethritis.

Key words: HPV genotyping, human papillomavirus, urethritis, urinary tract.

Introduction

Cervical cancer is the second common cancer in women worldwide.1 Since the discovery of human papillomavirus (HPV) 16 and 18 DNA in cervical cancer tissue by zur Hausen’s group,2 more than 100 types of HPV have been isolated and at least 15 types of high-risk HPV have been identified.3

Cervical HPV infection appears to occur in more than 50% of young women within a few years of their sexual debut and 70–80% of women are likely to have the infection for all of their life.5 Thus, cervical HPV infection is thought to be one of the most common sexually transmitted infections in women. The incidence of cervical cancer in young women is increasing in many countries including Japan. In contrast, the role of HPV infection in men’s cancer is controversial and only 40% of penile cancer, which is most likely associated with HPV infection in men, is caused by HPV infection in men.6 Moreover, penile cancer is very rare and its incidence is estimated to range from 0.2 to 2.2 per 100 000 per year, and generally develops in men over 60 years of age.6 Therefore, sexually transmitted HPV infection in men has not attracted much attention from researchers.

Currently, the prophylactic anti-HPV16 and 18 vaccine has been available to prevent cervical cancer in young women around the world, and could lead to reducing the incidence of cervical cancer by approximately 70% in the future.7 On the other hand, vaccination of men is being discussed as a way of eliminating HPV infection in the generation who have been vaccinated, and some researchers have regarded the HPV prevalence in men only as a reservoir for HPV transmission, which could be a cause of cervical cancer.

Some recent studies have shown that HPV prevalence in men is equivalent to that of women.8,9 The most common infection sites for HPV in men are reported to be in the penile glans and shaft and the scrotum, but not common in the urinary tract.10,11 However, we have recently found that HPV prevalence in urine samples is much higher in men who have urethritis than in age-adjusted healthy controls.12 In the present study, we investigated the prevalence of HPV and common HPV genotypes in samples of urine, the urethra and penis of Japanese men with urethritis.
Methods

Subjects

The subjects were composed of 156 male patients with urethritis who visited the outpatient clinics of Kanazawa University Hospital (Kanazawa, Japan), Ishikawa Prefectural Central Hospital (Kanazawa, Japan), Taya Urological Clinic (Komatsu, Japan) and a sexually transmitted disease (STD) clinic in Osaka (Japan) between 2007 and 2008. Out of 156 patients with urethritis, 142 agreed to participate in the present study and written informed consent approved by the ethics committee of Kanazawa University Graduate School of Medicine was obtained from each of them. The patients with apparent lesions such as condyloma acuminate were eliminated in this study. The mean age ± standard deviation of the participants was 35.2 ± 9.9 years (range, 19–62 years).

Diagnosis of urethritis was based on pyuria that showed more than five white blood cells in high-power fields and/or the presence of urinary symptoms such as pain on urination, urethral discomfort and pus discharge. All patients with urethritis were tested for Neisseria gonorrhoeae and Chlamydia trachomatis in first-catch urine or urethral swab, based on the Aptima Combo 2 Assay (Gen-Probe, San Diego, CA, USA) or Amplicor STD-1 PCR (Roche Diagnostics, Basel, Switzerland).

Sampling

Samples from the penis, distal urethra and urine were collected from all 148 participants. To obtain a sample from the penis, cells were collected by rubbing the entire surface of the glans, coronary sulcus and prepuce with a saline-wetted cotton tip. The urethral samples were collected with a saline-wetted cotton tip by insertion approximately 3 cm into the urethra, and rubbing up and down. For the urine samples, 50 mL of urine was centrifuged at 1500 r.p.m. (approximately 504 g) for 10 min and the sediment was stored at −30°C until DNA analysis. The cells collected from the penis and urethra were placed into a separate tube containing 2.5 mL of preservative solution for liquid-based cytology (Liqui-PREP; LGM International Inc., Fort Lauderdale, FL, USA) and were stored at 4°C until use.

HPV-DNA test and HPV genotyping

Aliquots of 1 mL of preservative solution containing cell samples were centrifuged at 5000 r.p.m. (approximately 504 g) for 10 min, and the supernatant was discarded. The cell pellet was washed twice with 300 μL of 10 mmol/L Tris-Cl (pH 8.0). DNA was extracted from the cells using a DNA extraction kit (SMI test; G & G Science Co., Fukushima, Japan) according to the manufacturer’s instructions. DNA quality was confirmed by amplifying the β-globin gene as an internal control by polymerase chain reaction (PCR), as described previously. In the present study, only β-globin gene-positive samples were tested for HPV. The presence of HPV-DNA was examined using modified GP5+/GP6+ PCR, as reported previously. HPV-DNA was amplified using about 10–50 ng of sample DNA in 10 μL of PCR solution containing 8 mmol/L MgCl₂, 200 μmol/L deoxynucleotide triphosphate, 10X PCR buffer (Toyobo, Tokyo, Japan), 20 pmol of each primer and 0.25 units of KOD Dash DNA polymerase (Toyobo, Tokyo, Japan). PCR was performed according to the following program: one cycle of 5 min at 95°C, 30 s at 55°C and 30 s at 74°C, followed by 40 cycles of 30 s at 95°C, 30 s at 48°C and 30 s at 74°C. HPV was defined as positive when a band of approximately 140 bp was observed after electrophoresis of 8 μL of PCR product on 2.5% agarose gels and staining with ethidium bromide.

HPV genotyping was performed in HPV-positive samples using a HPV GenoArray Test Kit (HybriBio Ltd., Chaozhou, China). This assay is able to determine 21 HPV types including 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), 5 low-risk HPV types (6, 11, 42, 43 and 44) and two unknown risk types (53 and CP8304), by the flow-through hybridization technique using HPV DNA amplified by PCR. Aliquots of 25 μL of heat-denatured PCR solution were used for flow-through hybridization analysis according to the manufacturer’s protocol. The HPV type was determined by visualization of blue spots at the position of each HPV type probe on the membrane (Fig. 1). Some samples that were positive on the HPV screening test that did not show any positive signals in the genotyping test were classified as infection with unknown HPV types.
Statistical analysis

All statistical analyses were performed using the $\chi^2$ test or Fisher’s exact probability test to compare categorical variables. $P < 0.05$ was considered as significant.

Results

Among 142 urethritis patients, 29 had gonococcal infection, 34 had chlamydial infection, one had both infections and 78 had non-gonococcal and non-chlamydial urethritis. There were no significant differences in HPV prevalence according to the causative microorganisms for urethritis. No apparent lesions such as condyloma acuminata were observed in the 142 subjects.

The $\beta$-globin gene was positive in 89% of samples from the penis, 93% from the urethra and 65% of the urine samples (Table 1). Any type of HPV was detected in 48% (68 cases) of urethritis patients, and high-risk HPV was detected in 32% (46 cases). The prevalence of any type of HPV was 31% from the penis, 20% from the urethra and 24% in the urine samples, indicating that the penis was the most common site. When we counted the urine and urethral samples together, as being from the same resource (the urinary tract), the prevalence was almost the same between the penis and the urinary tract. HPV was positive in the urinary tract in 66% (45 cases) of 68 HPV-positive patients. Therefore, the urinary tract was a common HPV infection site in men with urethritis. The high-risk HPV type was detected in 23% of the penis and 18% of the urinary tract (12% of the urethra and 11% of the urine). Although multiple HPV-type infection was frequently identified on the penis (14/40; 35%), single HPV-type infection was more frequent (40/45; 89%) in the urinary tract ($P < 0.05$).

In all of the sites, HPV16 was the most common type, followed by HPV types 6, 18, 58, 43, 52, 68 and 39 (Table 2). HPV16, 18 and 58 were common high-risk types, while HPV6 and 43 were common low-risk types. Low-risk HPV types were detected in 26 urethritis patients who had no condyloma acuminata, suggesting that many asymptomatic infections occur even in HPV6 and HPV11. Most of the high-risk HPV types and one low-risk type, HPV43, did not induce apparent lesions on the penis. The HPV type could not be determined (unknown type) in three patients.

Discussion

A HPV vaccine has been available to prevent cervical cancer in young women around the world. On the other hand, there have been some arguments about whether a vaccination for men would be necessary to eliminate HPV infection in the population who have been vaccinated. However, nobody knows the details of HPV transmission from men to women, and what parts of the male genital tract are the source of HPV transmission. Furthermore, there is another important question as to whether HPV induces malignant tumor of the male genital tract or not. Therefore, the epidemiology of HPV infection in men is an important issue for men’s health.

We demonstrated that overall HPV prevalence was 48% in men with urethritis in the present study. Some previous reports indicate that the prevalence of HPV infection on the male external genitalia in 17- to 70-year-old men visiting STD clinics ranges from 28% to 45%.14–16 It has recently been reported that HPV prevalence among 1160 healthy men (18 to 70 years old) from Brazil, Mexico and the USA was 65.2% in samples combined from the penile glans, coronal sulcus, penile shaft and scrotum.17 Although it is difficult to compare that data directly, because the target age, population and the site of samples differs, the prevalence observed in the present study is a little less than that reported in Mexico and the USA. On the other hand, a previous study in another Japanese group has shown that HPV prevalence on the penile glans is 18.5% in 130 patients (17 to 49 years) with urethritis,18 showing a lower HPV-positive rate than that in the present study. The hybrid capture-2 method, which is detectable for both high-risk and low-risk HPV, was used in the previously mentioned Japanese study. Although

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<th>Table 1</th>
<th>Prevalence of human papillomavirus (HPV) infection in 142 men with urethritis</th>
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<tr>
<td>$\beta$-globin positive rate</td>
<td>Glans ($n = 127$)</td>
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<tr>
<td></td>
<td>n (%)</td>
</tr>
<tr>
<td>Any HPV positive</td>
<td>40 (31%)</td>
</tr>
<tr>
<td>High-risk HPV positive</td>
<td>29 (23%)</td>
</tr>
<tr>
<td>Low-risk HPV positive</td>
<td>15 (12%)</td>
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<tr>
<td>Multiple HPV infection</td>
<td>14 (11%)</td>
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Overall indicates the number and rate of HPV detection at any site. Urinary tract includes HPV detection from urethral sample or urine sample. $n$, number of adequate samples.
hybrid capture assays are widely used to detect clinically relevant HPV infection, limitations of this method include an inability to identify specific HPV types, and lower analytical but not necessarily clinical sensitivity compared with the PCR method. A higher HPV prevalence in the present study might be due to higher sensitivity and broader ranges of HPV type detection using the present PCR method. The HybriBio HPV GeniArray Kit used in the present study is a commercial kit that has been recently introduced into the market, and this assay is able to determine 21 HPV types by the flow-through hybridization technique. This array has shown good agreement (93.8%) in detection of HPV types with the results by the Amplicor HPV test. According to a systematic review on the prevalence of HPV infection among men, external genitalia including the penile shaft, glans, coronal sulcus and prepuce are the sites with a high prevalence of HPV infection in asymptomatic men. Giuliano et al. has reported that asymptomatic HPV infection is frequently observed in the penile shaft (49.9%), followed by the glans (35.8%), scrotum (34.2%), perianal area (20.0%), anal area (17.6%) and the urethra (10.1%), indicating that HPV prevalence is lowest in the urethra. Other reports have also indicated HPV prevalence rates of urethral samples ranging from 8.7% to 30% and HPV prevalence in urine samples ranging from 0.9% to 6.9%, even in analysis of adequate samples in which the internal control gene is detected, suggesting that urinary tract HPV infection is not so common in sexually active men. However, we have shown that HPV-DNA is detected in 1.9% (3/160) of healthy men, whereas it is in 24% (7/29) of the urine from urethritis men. In the present study, we have clearly shown that the urinary tract is a common HPV infection site, and the prevalence of HPV infection is equivalent to that in the penis of men with urethritis.

We could identify high HPV detection in the urinary tract of men with urethritis. Some studies suggested that urine specimens could be of value in the assessment of HPV infection in cases with intrameatal warts or urethral infection. Because inflammation induced by some microorganisms can contribute to cell exfoliation, urethritis leads to easier detection of asymptomatic HPV infection in men with urethritis than healthy men. Alternatively, urethritis itself might increase the risk of HPV infection in the urinary tract, because micro-injury is necessary for HPV to access the basal cells of the epithelium. However, the detection rate of β-globin gene in urine samples by the PCR-based method has been poorer than that of penile and urethral rubbed samples. Improvement in DNA detection by the PCR-based method should be required if we test HPV-DNA using urine samples.

Some studies indicated a high prevalence of low-risk HPV infection such as HPV6 and HPV11 (range, 8–20%) in

<table>
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<th>Glans (n = 40)</th>
<th>Urethra (n = 26)</th>
<th>Urine (n = 22)</th>
<th>Urinary tract (n = 45)</th>
<th>Overall (n = 68)</th>
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<td><strong>High-risk type</strong></td>
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<tr>
<td>16</td>
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<td>11</td>
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<td>43</td>
<td>4</td>
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<td>6</td>
<td>9</td>
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<tr>
<td><strong>Unknown type</strong></td>
<td>1</td>
<td>ND</td>
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</table>

Urinary tract includes HPV detection from urethral sample or urine sample. ND, not detected.
men without condyloma acuminata in their genitalia. Some lial lesions, while single-type infection is known as a sign of infection was single-type infection. Generally, multiple-type external genitalia. In contrast, 89% of urinary tract HPV type infections might also be commonly observed in male hypothesis, and we are going to start further HPV studies to explore the role of HPV infection in the development of precursor lesions, if the infection is persistent for a long time. However, further study is needed to warrant this hypothesis, and we are going to start further HPV studies to explore the role of HPV infection in the development of urogenital tumor.

References


16 Lazcano-Ponce E, Herrero R, Muñoz N et al. High prevalence of human papillomavirus infection in Mexican


**Editorial Comment**

**Editorial Comment to Prevalence of human papillomavirus infection in the urinary tract of men with urethritis**

In this article, the authors clearly showed that the detection rate of human papillomavirus (HPV) in the urethra was 22% and that in urine it was 24% in patients with male urethritis. The authors concluded that the urinary tract is a common HPV infection site in such men and that HPV infection in the urinary tract is equivalent to that in the penis. In the era of HPV vaccination, there have been some arguments as to whether vaccination for men is necessary or not. Therefore, the details of male HPV infection or colonization must be clarified. On that point, the authors presented invaluable discussion and suggestions.

From another angle, some urologists believe that “asymptomatic” HPV infection must be a common disease in humans because most cases of HPV infection are transient, even though many men and women have HPV infection or colonization in the external genitalia due to sexual intercourse. In addition, some urologists indicate that they do not necessarily care whether such infection develops or not.