Human Papillomavirus DNA Is Found in the Vas Deferens

Marjut A. M. Rintala,1 Pasi P. Pöllänen,1,2
Vesa P. Nikkanen,1 Seija E. Grönman,1
and Stina M. Syrjänen3

The role of the male reproductive tract as a reservoir for human papillomavirus (HPV) infection is poorly understood. To analyze the presence of HPV DNA, 27 samples, comprising postvasectomy semen samples and pre- and postejaculation urine samples, were obtained from 18 men recalled for follow-up. HPV DNA was analyzed by nested polymerase chain reaction, confirmed with Southern blot hybridization, cloned, and sequenced. Multiple HPV types were found in different DNA samples of the same men. Five (18.5%) of 27 vas deferens samples contained HPV type 6, 11, or 16. Five (27.8%) of 18 seminal plasma samples (secretions without semen cells) were HPV DNA positive. None of the men had both vas deferens and semen plasma samples HPV positive. Several HPV types can be detected in the male reproductive tract at the same time. This is the first report to show HPV DNA in the vas deferens.

Human papillomaviruses (HPVs) are small DNA viruses that can induce proliferating epithelial changes in infected skin and mucosa. Both low-risk and high-risk HPV types exist, and the latter pose a significant risk for development of cervical precancerous lesions and invasive cancer [1]. Genital HPV infection has been viewed as a sexually transmitted disease, although other routes of viral spread have also been implicated recently [1]. The natural history of HPV infection and the possible reservoir of HPV in men are less well understood than those in women. Similarly, little is known about the role of healthy men as potential transmitters of HPV infection. HPV DNA was detected in urine samples from 76% of men with urethral warts [2] but in only 7.1% of clinically healthy men [3]. However, intraurethral reservoirs of HPV in association with subclinical infections have been reported to be much more common in men [4]. HPV DNA was also detected in semen samples [5], in which it was localized to spermatozoa [6]. Furthermore, HPV seems to be able to transcribe in infected sperm cells [6, 7], emphasizing the potential role of these cells in transmission of HPV.

The purpose of the present study was to evaluate whether HPV DNA can be detected in the vas deferens of clinically healthy men. After some HPV-positive vas deferens samples were found, additional samples, of semen and urine, were collected.

Subjects and Methods

Subjects. The present series comprised 27 healthy men who underwent vasectomies (“vasectomized men”) at the Department of Obstetrics and Gynecology in Turku University Central Hospital (Turku, Finland) in 1998–1999. Their mean age was 40.3 years (range, 33–49 years). Because we found some samples that were positive for HPV DNA, the men were recalled for follow-up after 6 months, to give postvasectomy semen and pre- and postejaculation urine samples for additional HPV DNA testing. Eighteen of the 27 vasectomized men participated in the follow-up study. Medical history with regard to their past and present warts was recorded.

Samples. Two 1.5-cm-long vas deferens samples were obtained at vasectomy and were stored at −70°C immediately after the procedure. At the follow-up visit, the first urine sample (∼300–700 mL, collected at home before ejaculation), was transferred to the laboratory, as was the semen plasma sample, which was obtained at home, by masturbation, after ≥2 days of abstinence from sex (range, 2–10 days), without using a condom. The pre-ejaculation urine sample and the semen sample were frozen within 2 h after ejaculation. The second, postejaculation urine sample was taken in the laboratory, within 1 h after ejaculation. The exfoliated cells of the urine samples were collected after centrifugation and were stored at −20°C.

DNA isolation. From the frozen samples, HPV DNA was extracted with the high-salt method [8]. In brief, the sample was lysed with proteinase K at 37°C overnight. The proteins were then precipitated with saturated 6 M NaCl, followed by centrifugation. DNA was precipitated with absolute ethanol. DNA from the semen was extracted with the high-pure polymerase chain reaction (PCR) template preparation kit (Boeringer Mannheim), according to the procedure out-
lined by the manufacturer. DNA was simultaneously extracted from cultured fibroblasts, to serve as a negative control for PCR.

 Nested PCR. In all samples, the human β-globin gene was first amplified, to confirm the adequacy of the extracted DNA. HPV DNA was detected by nested PCR, using MY09/MY11 as external primers and GP05+/GP06+ as internal primers targeting the L1 open-reading frame of the HPV genome. PCR was done in a 50-μL reaction containing PCR buffer, 20 pmol of each primer, 200 μM of each dNTP, 1.25 U of AmpliTaq Gold DNA polymerase (Perkin Elmer), and 300 ng of DNA. Amplification was started by an initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 55 s, and elongation at 72°C for 60 s. One microliter of the PCR product was taken for the second PCR, with the internal primer pair of GP05+/GP06+. Forty cycles were done, and one cycle comprised denaturation at 95°C for 60 s, annealing at 40°C for 60 s, and extension at 72°C for 90 s. PCR mixture without DNA was pipetted in a room reserved only for PCR. DNA was extracted under a laminar hood in a separate laboratory. DNA was added to the PCR mixture in a third laboratory room.

 Controls for PCR. DNAs extracted from fibroblasts simultaneously with the patient sample served as negative controls. Additionally, every eighth sample for PCR contained no DNA. DNA from SiHa cells was used as a positive control for HPV DNA detection.

 Confirmation of the specificity of PCR products. After PCR, the samples were run on 3% agarose gel (NuSieve; FMC BioProducts) and were transferred to a nylon membrane (GeneScreen Plus; Biotechnology Systems). The prehybridization was done at 55°C for 2 h, and the background was blocked with 2% standard saline citrate and 1% SDS salmon sperm DNA (2 mg/mL). The membranes were hybridized with high-risk (using types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, and 58) HPV oligoprobe mixtures. The probes were labeled with digoxigenin according to the manufacturer’s instructions (DIG Oligonucleotide 3’-End Labeling Kit; Boehringer Mannheim). The hybridization was carried out at 55°C overnight. The detection was done with the DIG Luminescent Detection Kit (Boehringer Mannheim) at 37°C for 5 min in darkness, according to the manufacturer’s instructions. Chemiluminescence was recorded on X-ray films (Cronex 4; DuPont), with an exposure time of 10–60 min.

 The sensitivity of the PCR method used is ~20 copies of HPV, because 20 SiHa cells mixed with 300 ng of human fibroblast DNA become strongly positive with this method. All PCR-positive samples from vas deferens samples were cloned in the Smal-digested pUC18 vector, sequenced by an ABI PRISM Big Dye Terminator Cycle Sequencing Kit, and automated on an ABI PRISM 377 DNA Sequencer (both from Applied Biosystems). A direct sequence of other PCR-positive samples was performed (except for samples 22, 23, and 27). The detected HPV sequences had a 93%–99% homology to the prototype sequences.

 Results

 Table 1 summarizes the detection of HPV DNA in all samples obtained. Altogether, 18.5% (5/27) of the vas deferens samples were HPV DNA positive. Three of them contained HPV 16 (a high-risk type) and 2 contained HPV 6/11 (a low-risk type). Three of the 5 men with HPV DNA–positive vas deferens samples participated in the follow-up, but their seminal plasma samples proved to be HPV DNA negative, suggesting that the HPV-infected part of their reproductive tract had remained proximal to the vas closure site. Two of the men who had HPV 16 detectable in the vas deferens also had high-risk HPV types in their pre-ejaculation urine samples, indicating a multifocal HPV infection. Altogether, 12 (44.4%) of the 27 vasectomized men had ≥1 HPV DNA–positive sample.

 HPV DNA was found in 5 (27.8%) of 18 seminal plasma samples. Four samples contained the high-risk HPV types 16, 18, 66, or 83, and 1 contained the low-risk HPV type 42. The pre-ejaculation urine sample was HPV DNA positive in 4 men (22.2%). One sample was high-risk type HPV 35, and one was low-risk type HPV 6. Two HPV types could not be confirmed by sequencing because of lack of samples. HPV DNA was detectable in 3 postejaculation urine samples, with the HPV types being 16, 57, and unknown. As shown in table 1, multiple HPV types at different sites were found in 4 men (subjects 2, 6, 22, and 23). All samples of 8 men (44.4%) remained totally HPV DNA negative.

 Seven (38.9%) men reported previous warts; on the hands (4 men), in the mouth (1 man), and on the penis (2 men). One man (subject 17) had penile warts at the examination, but all samples were HPV DNA negative. Four (22.2%) men regularly had oral sex, and 2 (11.1%) men occasionally had anal sex. Only 1 of the 2 men with previous penile warts had low-risk HPV 42 DNA detectable in his seminal plasma sample. Additionally, 2 of the 4 men with previous warts on the hands also had a high-risk HPV 16 or 18 DNA–positive seminal plasma or postejaculation urine sample.

 Discussion

 As far as we know, this is the first study to show the presence of HPV DNA in the vas deferens. This is also one of the few follow-up studies to analyze samples from different loci. HPV DNA found in vas deferens samples may originate from the proximal excurrent ducts (including efferent ductules, the vas epididymis, and the proximal vas deferens) or even from the testis [9]. Our results support the view that the human excurrent duct (vas epididymis) may harbor HPV DNA. Since HPV DNA was detected by PCR, the localization of HPV DNA was not possible. Performance of tyramine-amplified in situ hybridization was hampered by very high background.

 The detection rates of HPV DNA in semen samples have been reported to vary from 0% [10, 11] to 100% [6, 12]. HPV DNA has been detected in 4%–100% [5, 6, 13, 14] of the seminal cells, whereas the detection rate in seminal plasma has been 41.7% [6]. HPV E6 and E7 genes have been reported to be expressed strongly in infected seminal cells [6]. HPV has also been suggested to affect sperm cell motility, and the frequency of asthenozoospermia has been reported to be higher in men with HPV infection [7]. In the present study of vasectomized men, semen samples did not contain spermatozoa or immature germ cells. Thus, access of HPV to the distal parts of the male reproductive tract via these cell types was excluded.
In seminal plasma samples, we found HPV 16, 18, 42, 66, or 83 in 5 men. The origin of the HPV was difficult to estimate, because multiple HPV types were found at different sites. In 3 men, the most likely site may have been the prostate or the seminal vesicles, because semen of vasectomized men consists mostly of secretions from the prostate and seminal vesicles. The detection rate of HPV DNA in the prostate is 24.1%, based on a recent review of 20 studies with 968 subjects [15, p. 275]. We found HPV DNA in 22.2% of the pre-ejaculation and 16.7% of the postejaculation urine samples. Only 1 man had all 3 samples positive for HPV DNA, but different HPV types were found. Of interest, this man reported previous hand warts, and thus the HPV 57 in his postejaculation urine sample might have originated from his hands. Two postejaculation urine samples were positive, indicating that the site of HPV infection might be the urethra or even the bladder. HPV could stay so tightly on the basal cell layer of the urethra that the first urine flow or even the force of ejaculation does not exfoliate enough HPV infected cells [12]. This suggests that, when HPV infection of the male urinary tract is suspected, it is necessary to test not only urine and semen samples, but also a postejaculation urine sample. However, not even this guarantees the absence of the virus in the male reproductive tract.

To conclude, HPV DNA was found in the vas deferens for the first time. Our results also suggest that multiple HPV types are frequently found at different anatomical sites of the male reproductive tract. This could explain the different HPV types found in sex partners. HPV DNA could not be detected in 8 men. The role of men as transmitters remains open, because only the presence of HPV DNA was assessed, not the presence of viral particles. However, the presence of HPV in the vas deferens suggests that the reservoir of HPV DNA may be located more proximally in the reproductive tract than previously thought.

Acknowledgments

We thank Johanna Järvi for her enthusiastic support and for providing us with invaluable samples. We also thank Sarita Järvinen and Minna Linnavalli for their technical assistance in the human papilloma virus laboratory. Most of all, we thank the men who took part in this study.

References