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Short communication

Human papillomavirus DNA detection and typing in male urine samples from a high-risk population from Argentina

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Abstract

The aim of the present study was to evaluate the first void urine (FVU) as a non-invasive sampling method for HPV detection and genotyping in a high-risk population. Men presenting with HPV associated penile lesions or HPV positive partners attending a urological department in La Plata, Argentina were enrolled for HPV detection and genotyping. DNA from 185 first-void urine samples was evaluated for the presence of HPV by nested polymerase chain reaction using MY09/11 and GP05/06 primers. The viral genotype was analyzed by means of the single-stranded conformation polymorphisms (SSCP) method.

Seventy-three percent (135/185) of the FVU specimens were positive for HPV-DNA. The viral prevalence in patients with HPV-DNA positive partners was 68.8% (77/112), and 79.5% (58/73) of patients with penile lesions were found to be HPV positive. The most frequent viral type was HPV-11 (26.7%), followed by HPV-6 (23%), HPV-16 (21.5%), HPV-18 (6%), and HPV-31 (4.4%). In this study, 11.1% (15/135) of the HPV positive specimens were double infections.

These results indicate that high-risk HPVs can be found in clinical lesions in a high percentage (43.8%), as simple or double infections. In this sense, the male population represents an important reservoir for the virus and may play a role in the transmission and perpetuation of the infection in the general population. The method described below provides a tool for detection and typing of HPV-DNA using samples obtained by non-invasive techniques and thus easy to obtain.
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Human papillomavirus (HPV) is one of the most prevalent sexually transmitted infections (Koutsky, 1997) and is the cause of both cervical cancer in women and penile cancer in men (zur Hausen, 1996; Ferlay et al., 1998). Although several studies have investigated the prevalence of HPV infection in both asymptomatic and symptomatic women (Bosch et al., 1995; Franco et al., 1999), only a few recent studies are available in men (Iwasawa et al., 1997; Ferlay et al., 1998; Fife et al., 2003; Weaver et al., 2004).

More data are needed currently on the prevalence and natural history of genital HPV infections in men, and on the transmission dynamics of HPV infection between sexual partners. Men are an important reservoir of HPV infection and may play an important role in the transmission of HPV infection to women, and thus in the perpetuation of the HPV-associated disease in the general population (Barrasso et al., 1987; Burk et al., 1996).

Only a few studies have been conducted on HPV positivity in men because there is a general lack of agreement in regard to the anatomical sites that should be sampled from men and the optimal method for sampling from each anatomical site (Weaver et al., 2004). Several different anatomical sites have been suggested for reliable detection of HPV in men, including first void urine (Melchers et al., 1989), sampling internal foreskin and penile shafts with dacron swabs or emery paper

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(Weaver et al., 2004), semen sampling (Olatunbosun et al., 2001) or visual inspection of clinical, penile lesions (Hillman et al., 1993).

Since the first report of using urine samples for HPV detection in men in 1989 (Melchers et al., 1989), urine samples from men have been used for HPV-DNA detection and typing in only a limited number of studies. Some studies have shown a high overall sensitivity for HPV detection in urine-based sampling (Melchers et al., 1989; Forslund et al., 1993; Iwasawa et al., 1997), while others have shown a low overall HPV-DNA detection in urine samples (Geddy et al., 1993; Zambrano et al., 2002; Rintala et al., 2002; Fife et al., 2003; Weaver et al., 2004). Considering the spectrum of sampling sites, first void urine samples appear to be one of the easiest, non-invasive methods to sample for HPV infection in men.

We describe results on the prevalence of HPV infection obtained in urine samples from men with penile lesions and from men with HPV-positive female partners in order to evaluate first void urine (FVU) as a possible sampling method for HPV detection and genotyping in men.

One hundred and eighty-five FVU from male patients attending a urological department of a General Hospital in La Plata, Argentina with HPV-DNA positive sexual partners and HPV-associated penile lesions were studied for HPV-DNA detection and typing. Patients were asked to participate in the study and informed consent was obtained for HPV testing. Group 1 were 112 men with female sexual partners who were positive for HPV-DNA, as ascertained by highly sensitive PCR methods (median age 31 years; range: 17–71). Group 2 were 73 men with penile lesions (i.e. balanitis, urethritis, condyloma, papules, lichen-like lesions) (median age 27 years; range: 20–56).

Men were asked to collect the first 20–40 ml corresponding to the first void urine in the early morning in a sterile plastic recipient. Urine samples were stored at 4 °C and processed the same day. Approximately 15 ml of FVU samples were centrifuged at 3000 rpm for 20 min, suspended and then washed three times with 1 ml of phosphate buffer saline (PBS). The obtained cell pellets were digested for 3–5 h at 56 °C in 150 μl of digestion buffer (50 mM Tris–HCl pH 8.5; 1 mM EDTA; 1% Triton X100 and 0.5% Tween 20) containing 200 μg/ml of Proteinase K (Promega, Madison, Wisconsin, USA) and boiled for 10 min for proteinase inactivation. All cell digests were stored at −20 °C until testing.

In order to test the DNA quality for PCR amplification, a fragment of the human thymidine kinase (*tk*) gene was amplified by PCR in all the samples according to the procedures described elsewhere (Golijow et al., 2001).

HPV-DNA detection was conducted using a nested PCR protocol with MY09/11 and GP05/06 primer pairs, as previously described (Evander et al., 1992). The PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide (Fig. 1). CaSki cell line DNA (containing the HPV-16 genome) served as a positive control. Strict precautions were made in order to avoid cross-contamination. Aerosol resistant tips were used in order to avoid spray contamination. All reagents were aliquoted in a clean, decontaminated, UV-light irradiated bench. All PCR reactions were performed using a negative carryover control every three samples. As a rule, a positive amplification in negative controls invalidated the results obtained.

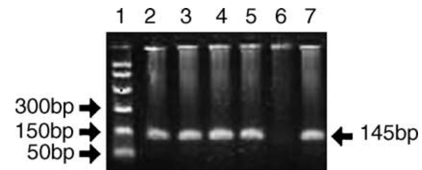

Fig. 1. Electrophoresis of PCR products on 2% agarose gel. Line 1: molecular weight marker (100 bp DNA ladder; Promega); lines 2–5: HPV-DNA positive sample (145 bp); line 6: negative control; line 7: positive control (CaSki cell line DNA).

For HPV-DNA positive samples, HPV viral genotype was determined using the 'low ionic strength-single strand conformation polymorphisms' (LIS-SSCP) technique (Fig. 2) (Abba et al., 2003a). We used the plasmids containing the entire viral genome as controls for HPV types -6, -11, -16, -18, -31, -33, -34, and -51 (kindly provided by Dr. de Villers and Dr. Ort), according to the HPV types more frequently found in this area (Abba et al., 2003b). HPV types -6, -11, and -34 were considered low-risk HPV types, whereas HPV types -16, -18, -31, -33, and -51 were considered high-risk HPV types (zur Hausen, 1996).

All DNA samples obtained from FVU were satisfactory for PCR testing, as demonstrated by the presence of the *tk* gene amplification product. Approximately 73% (135/185) of the FVU specimens tested were positive for HPV-DNA (Table 1). The prevalence of HPV-DNA in men with HPV-positive female sexual partners was 69% (77/112), whereas 80% (58/73) of the patients with penile lesions were HPV positive. These two groups of high-risk men had no significant differences in the prevalence of HPV-DNA overall, or for the prevalence of oncogenic or non-oncogenic HPV viral types ($p > 0.05$). The most prevalent single type HPV infections for the total population were HPV-11 (27%) and HPV-6 (23%), followed by HPV-16 (21.5%). In 1.5% (2/135) HPV-

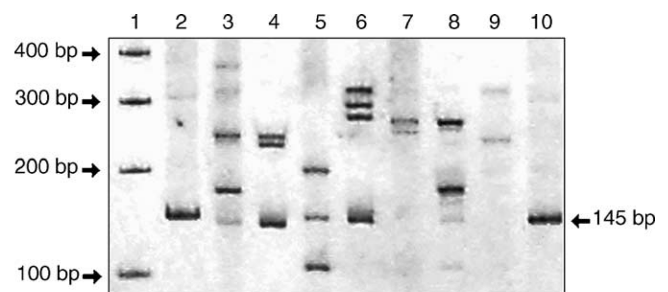

Fig. 2. PCR-LIS-SSCP analysis of HPV-DNA viral type. Line 1: molecular weight marker (pGEM® DNA Markers, Promega); lines 2 and 10: dsDNA of positive control; lines 3–9: ssDNA from cloned viral DNA corresponding to types -6, -11, -16, -18, -31, -33, -34. Electrophoresis was conducted in a 10% polyacrilamide gel (39 acrylamide:1 bis-acrylamide) at 4 °C for 4 h. SSCP patterns were detected by silver staining technique.

Table 1
HPV prevalence and type-distribution in participating men

Cases	Patients with penile lesions		Patients with HPV (+) female partners		Total	
	73		112		185	
	n	%	*n*	%	*n*	%
HPV-DNA positive	58	79.5	77	68.8	135	73
Single HPV infection[a]						
16	11	19	18	23.4	29	21.5
18	5	8.6	3	3.9	8	6
31	2	3.4	4	5.2	6	4.4
33	2	3.4	2	2.6	4	3
51	1	1.7	1	1.3	2	1.5
X	1	1.7	1	1.3	2	1.5
6	12	20.7	19	24.7	31	23
11	17	29.3	19	24.7	36	26.7
34	1	1.7	1	1.3	2	1.5
Multiple infections[a]						
16, 18	1	1.7	2	2.6	3	2.2
16, 6	1	1.7	3	3.9	4	3
16, 11	1	1.7	1	1.3	2	1.5
33, 6	0	0	1	1.3	1	0.7
6, 11	3	5.2	2	2.6	5	3.7

High-risk HPV types include HPV-16, -18, -31, -33 and -51. Low-risk HPV types include HPV-6, -11, and -34. X: undetermined.
[a] HPV prevalence for single and multiple HPV types expressed as percentage of overall HPV-DNA positivity.

DNA positive samples, the HPV genotype could not be determined. Approximately 11% (15/135) of HPV-positive men had infections with two different HPV types, and the most common multiple infections were HPV-6 and -11 (3.7%), and HPV-6 and -16 (3%).

In this study of 185 Argentine men with penile lesions or with HPV positive sexual partners, first-void urine samples were found to be satisfactory for PCR analysis, yielding a high prevalence of HPV-DNA (73%) and suggesting that urine-based sampling may be useful for HPV-DNA detection and genotyping in high-risk men populations.

Previous studies showed contradictory results about the use of urine samples for HPV detection. Melchers et al. (1989) found 88% HPV positivity urines from 17 men with condiloma acuminata in the meatus urethrae in men from The Netherlands. Forslund et al. (1993) found 5% HPV positivity in urine samples from 138 healthy military conscripts volunteers and near 50% positivity in 20 patients (eight male and 12 female) with genital warts, sexual partners with genital warts or a history of sexual transmitted diseases in patients from Sweden. Another study conducted in California, USA, in 32 prostate cancer patients found similarly high HPV positivity of 50% with urine-based samples (Zambrano et al., 2002). In contrast, two other studies have indicated that urine samples are probably not reliable samples for determining genital HPV infections in men. Fife et al. (2003), after studying 60 male individuals reported that urethral carriage of HPV-6 and -11 is unusual, finding only 2.5–10% of HPV positivity in patients with history of penile lesions and patients with penile warts, respectively. Weaver et al. (2004) in a study comprising 1323 men reported 3 and 6% of HPV positivity in urine specimens belonging to the general population and patients from an STD clinic, respectively. Also, these authors compared the efficiency of different anatomical sites as sources for HPV detection in men. Their results showed that penile shafts and foreskin had the highest frequencies of HPV positivity (24 and 28%, respectively); contrarily, urine-based samples brought the lowest rate of sensitivity for HPV detection (6%).

The results obtained in this work suggest that HPV can be transported by the urine, probably in the exfoliated HPV-infected cells. A similar mechanism may occur during ejaculation, allowing sexual transmission of HPV harbored in the sperm cells of the male genital tract. This situation was demonstrated by Olatunbosun et al. (2001), studying semen samples from patients with HPV history and sperm samples from healthy donors.

The present study has two main limitations: the small sample size and only symptomatic or high-risk men were examined. However, the results obtained showed clearly the value of first void urine samples for the study of HPV in the male population. Another minor limitation could arise from the observations addressed by Brinkman et al. (2004). These researchers stated that the presence of some kind of molecules and the method of DNA extraction influence the efficiency of PCR amplification. In this sense, the presence of nitrites or urea in urine samples decreased the rate of PCR amplification, as demonstrated by the rate of amplification of the *β-globin* housekeeping gene. Also, refrigeration of urine samples overnight at 4 °C improves the detection of HPV in the samples tested. The control of these aspects is required prior to the clinical application of a urine-based HPV-DNA detection and typing system. Considering these issues, the method described here provides a tool to detect and type the

HPV-DNA using a non-invasive sample technique, easily to obtain. As stated by others, the study of urine samples would allow for increased numbers of individuals to be tested, and help in the understanding of HPV dynamics in men population (Vossler et al., 1995).

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