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Urine versus brushed samples in human papillomavirus screening: study in both genders

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Abstract

Aim: To investigate whether urine is a good medium for screening and whether there is a correlation between the amount of extracted DNA and human papillomavirus (HPV)-positivity. **Methods:** In the present study, 30 first-voided urine (FVU) specimens and 20 urethroglandular swabs using cervix-brushes from male partners of HPV-positive patients, and 31 FVU specimens and 100 liquid-based cervix cytology leftovers sampled with cervix-brushes from HPV-positive women were examined for the presence of β -globin. Oncogenic HPV were detected using type-specific PCR. **Results:** β -globin was found in all the brushed samples, whereas it was found in only 68.9% of the FVU specimens. HPV-PCR was positive in 60.0% of the male brushes, in 29% of the female brushes and in 0% of the male FVU specimens. DNA concentration was, respectively, 0.9998 ng/ μ L, 37.0598 ng/ μ L and 0.0207 ng/ μ L. **Conclusion:** Urine is not a good tool for HPV detection, probably because the low DNA concentration reflects a low amount of collected cells. β -globin is measurable in FVU by real time quantitative PCR, but the DNA concentration is lower compared to brush sampling for both genders. β -globin-positivity of urethral and cervical swabs is 100%, showing a higher mean concentration of DNA, leading to a higher detection rate of HPV. This is the first article linking DNA-concentration to the presence of HPV. (*Asian J Androl* 2007 Sep; 9: 705–710)

Keywords: DNA; human papillomavirus; men; polymerase chain reaction; screening; urine

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1 Introduction

The human papillomavirus (HPV) causes the most common sexual transmitted infection (STI) of viral aetiology. At least 70 types of HPV are known to infect the anogenital region. Low-risk types (6, 11) are responsible for rather benign conditions like genital warts,

whereas high-risk types (16, 18, 31, 33, 45) are related with anogenital cancer [1]. The most common members of the low-risk group and the high-risk group are HPV 6 and HPV 16, respectively. Cervical cytology can detect (pre)malignant lesions in asymptomatic women. Polymerase chain reaction (PCR) can be used to detect oncogenic HPV types that are responsible for the detected lesions. There is no standard screening method for asymptomatic infected men. Detection of HPV DNA in urine could be a screening possibility because screening kits for other STI are available using PCR-testing of first-voided urine (FVU) specimens [2]. To evaluate if HPV-testing on urine is a good technique, β -globin concentration and HPV positivity for oncogenic HPV types are measured using real-time PCR in FVU specimens of men and women and compared with the findings in swabs of the glandular urethra and of the glans penis for men and with swabs of the cervix for women. Samples are always checked for β -globin first: the amount of β -globin is important because it correlates with the amount of DNA found in the sample. Furthermore, we looked for a connection between the amount of DNA and HPV-positivity.

2 Patients and methods

2.1 HPV testing on FVU

After obtaining informed consent, FVU-specimens of 30 men and 31 women were processed according to the Roche Amplicor protocol [3]. All samples tested negative for *Chlamydia trachomatis* and *Neisseria gonorrhoea* (Roche Amplicor, San Diego, CA, USA). The rest of the extracted material was used for HPV-testing by PCR. DNA isolation from FVU specimen was performed according to the Roche Amplicor protocol. The DNA extracts were stored at -20°C until PCR was performed.

2.2 HPV on glandular scrape and urethra sampling

Urethro-glandular sampling for men ($n = 20$) and cervical sampling for women ($n = 100$) were performed using the Cervex-Brush (Rovers, Oss, the Netherlands). All brush-heads were placed in SurePath vials (Tripath Imaging, Burlington, NC, USA). Enriched cell suspensions were obtained by using the fully robotic AutoCyte PREP System (AutoCyte, Tripath Imaging, Burlington, NC, USA) [4]. Briefly, vials containing the brush-heads were vigorously vortexed before transferring on top of a density gradient. After centrifugation and washing, a 1-mL

cell suspension was obtained. From this cell suspension, 400 μL was used to extract DNA, as previously described [5].

2.3 PCR analysis of HPV DNA

First, each sample was subjected to a quantitative real-time PCR amplification for the detection of β -globin. This was done to confirm that the DNA quality was still suitable for PCR analysis. Only samples that tested positive for β -globin were tested with the MY 9/11 consensus PCR, and only samples that tested positive for the consensus PCR were typed for oncogenic HPV types using type-specific PCR [5].

2.4 Real-time PCR and probe

Oligonucleotide primers were designed with the Primer Express software (version 1.0; Applied Biosystems, Foster City, CA, USA). The probe was designed to ensure a higher T_m than that for the primers and was manufactured by Applied Biosystems (Cheshire, UK). The size of the β -globin PCR product was 167 bp (GenBank Accession Number U01317). B-globine-143F 5'-TGCATTTGACTCCTGAGGAGAA-3', B-globine-223R 5'-GGGCCTCACCACCAACTTC-3', B-globine-167T 5'-CTGCCGTTACTGCCCT-3'. β -globin probe was labeled with 6-carboxyfluorescein at the 5' end and minor groove binding at the 3' end.

The PCR amplification was performed in a 25 μL volume containing $2 \times$ TaqMan Universal PCR master mix (Applied Biosystems, Cheshire, UK), 200 nmol/L concentration of each primer and probe and 10 μL of extracted DNA. Amplification and detection were performed using an ABI Prism 7000 sequence detection system (Applied Biosystems, Cheshire, UK). The amplification conditions were 2 min at 50°C to activate the uracil N'-glycosylase, followed by 10 min at 95°C to inactivate the uracil N'-glycosylase and release the activity of the DNA polymerase, and by a two-step cycle of 95°C for 15 s and 60°C for 60 s for a total of 45 cycles.

2.5 Positive and negative controls for PCR

A tube that contained all PCR components but no template DNA was used in all runs to ensure that the reagents were free of contamination. For the β -globin real-time PCR a standard curve was obtained by amplification of a dilution series of 150 ng to 0.015 ng of female human DNA (Promega, San Luis Obispo, CA, USA).

2.6 Calculations

The threshold cycle number (C_t) was calculated with Sequence Detection System software (Applied Biosystems, Cheshire, UK) and an automatic setting of the baseline (10 standard deviations above the background in the first 3–15 cycles). A standard curve was generated by plotting the C_t values against the log of the amount of β -globin DNA (in nanograms) and the amount of β -globin DNA for unknown samples inferred from the regression line.

2.7 Statistical analysis

Comparisons of means were studied by analysis of variance (ANOVA), followed by the Student–Newman–Keuls test for all pairwise comparisons. The χ^2 -statistics were used to verify the existence of a trend across ordered groups, such as an increase in HPV positivity. Statistical tests were considered significant at $P < 0.05$. Statistical analysis was performed using the MedCalc program (MedCalc Software, Mariakerke, Belgium) [6].

3 Results

An overview of β -globin concentrations and HPV positivity for the different groups is given in Table 1. The percentage of FVU specimens with detectable β -globin concentration did not differ between men (66.7%) and women (71.0%). In the group where the Cervex-brush was used to collect urethro-glandular or cervical cells, β -globin was always detected. β -globin concentration rises as follows: FVU specimens in men, FVU specimens in women, Cervex-brush sampling in men, Cervex-brush sampling in women. Ultimately, β -globin concentration in women was 100 times more elevated than that in FVU specimens of men. Although the β -globin is measurable in FVU specimens by real time quantitative PCR, the DNA concentration is lower compared to Cervex-brush sampling for both men (urethro-

glandular, 100-fold) and women (cervical, 10 000-fold; Figure 1). The DNA concentration (ng/ μ L) is significantly higher in the brushes in both men (0.9998 ng/ μ L) and women (37.0598 ng/ μ L) than that in FVU specimens of men (0.0207 ng/ μ L) and of women (0.0916 ng/ μ L) (ANOVA: $P < 0.05$). Oncogenic HPV were detected in 60.0% of the brushes from men and in 28.0% from women, and in only one sample (3.2%) of the FVU specimens in women and was not detected in FVU specimens of men. HPV prevalence is given in Table 2 for each of the sampling methods. For men, HPV type 16 was the most frequently detected HPV, whereas in women HPV 31 was more detected.

4 Discussion

Persistent HPV-infection is a risk factor for anogenital dysplasia and carcinoma. Normally, the body clears the infection in 6–12 months [7], but persistent HPV-load is maintained in patients with a weakened immunity system and by the presence of HPV in the partners' anogenital region. *In vitro*, the role of sperm as a vector for HPV has clearly been suggested [8]. HPV prevalence in a younger population (18–29 years) varies around 33% [9]; it rises with age and with promiscuity. Cytological examination of endocervical smears can detect abnormal cellular changes in asymptomatic women. PCR on cervical scrapes is the golden standard to confirm the presence of HPV. A satisfactory counterpart does not exist for asymptomatic men. Cytological screening of urine for abnormal cells is not a good diagnostic tool; it cannot confirm or rule out the presence of visible intraurethral disease: there is no correlation between cytological abnormalities and the presence of HPV-DNA [10]. Urinary HPV-DNA reflects the presence of infected cells shed from the epithelium of the urethral meatus. The amount of β -globin is an indirect method for measuring these

Table 1. DNA and human papillomavirus (HPV) content in different sampling methods. HPV⁺, positive for oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 66 and 68); FVU, first-voided urine; Mean conc., mean human DNA concentration. β -globin⁺, β -globin-positivity proves adequacy of the DNA-sample for PCR analysis.

Sampling method	Sex	<i>n</i>	β -globin ⁺	%	Mean conc. (ng/ μ L)	HPV ⁺	%
FVU specimens	Female	31	22	71.0	0.0916	1	3.2
	Male	30	20	66.7	0.0207	0	0
Cervex-brush [®]	Female	100	100	100	37.0598	29	29
	Male	20	20	100	0.9998	12	60

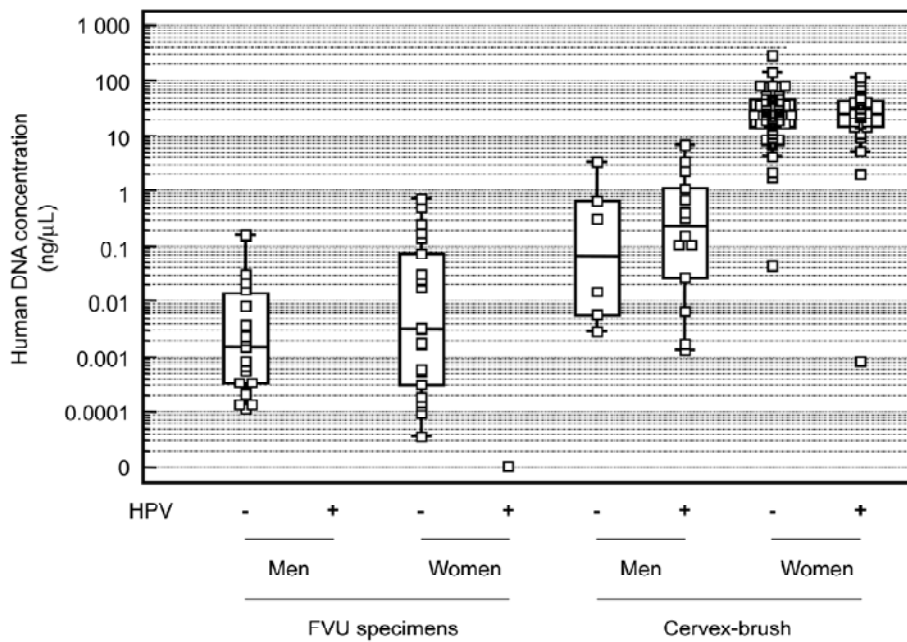


Figure 1. DNA concentration and human papillomavirus (HPV) status in different sampling methods. In the Y-axis the concentration of DNA is given (ng/μL). In the X-axis the HPV status is given for the different sampling methods. In all the urine samples the concentration of DNA is low and no HPV is found. Only one female urine sample was positive without the finding of DNA. In the brushed samples the amount of DNA is higher; also is the number of HPV-positive samples. FVU, first-voided urine.

cells. A low amount of β-globin could result in a lower HPV detection rate, although approximately 6% is positive for HPV-DNA while negative for β-Globin [11]. In these cases HPV-DNA is transferred by desquamated, anucleated cells, packed with HPV virions. In the present study, one of the β-globin-negative-FVU specimens in the female group was HPV positive. The number of HPV copies per infected cell can be as low as one, so a minimum of infected cells is required to obtain a positive PCR signal. The first part of voided urine contains most of the exfoliated cells; delayed catching of the FVU specimens or collecting a larger amount of urine will adversely affect the amount of collected cells. Urine samples can be stored for 1 week at room temperature without reduction of β-globin. Urinary HPV-detection can be impaired by white urea at a concentration of higher than 50 mmol/L, by proteins, or by iron and heparin, all present in the urine sample. These inhibitory components can be removed by boiling the specimen, by phenol-chloroform extraction, by diluting or by dialysis, or by pre-treating the samples with glass beads using GeneClean (Bio 101, Inc., La Jolla, CA, USA) [12]: all are time consuming and generate extra costs. Logically, the chance

of detecting HPV in urine is higher in cases of intrameatal warts and lower in patients with genital dermatoses or warts located outside the urethra [12–14]. Some authors found additional value in urine testing: Weaver *et al.* [11] identified an additional 7% of HPV-DNA positive men. Others have failed to find HPV in urine. Laczano *et al.* [15] compared urethral swabs and urine samples of 120 sexual active Mexican men, finding detectable β-globin in respectively 95% and 14% of the samples. In 43% of the sample, HPV-DNA was found in penile-urethral swabs, never in urine. For women, the results are somewhat better, probably because of the close proximity of the urinary tract to the vagina, vulva and cervix [16–21]. We found only one positive HPV 18 sample in FVU of women. The women in our study do not have invasive cervical cancer, so we are unlikely to find many positive cases. In Zimbabwe, cervical and urine samples of 43 women with invasive cervical cancer were investigated for the presence of HPV DNA [18]. HPV was identified and typed in 98% of cervical swabs and 72% of urine samples. Type-specific concordance between cervical and urine samples was only 79%. The distance from the cervix to the sampling spot has an impact on the detec-

Table 2. Human papillomavirus (HPV) prevalence in first-voided urine (FVU) specimens and scrapes with Cervex-brush®. X, HPV positive with MY 9/11 consensus PCR but negative for oncogenic HPV types; Total onc HPV+, the total number of oncogenic HPV-types found in that group.

HPV type	Cervex-brush®		FVU specimens	
	Men	Women	Men	Women
16	2	1		
16, 51, 52, 59		1		
16, 51, 56	1			
16, 51, 56, 58		1		
18		1		1
18, 31, 51, 56, 66, 67		1		
18, 51		1		
31	1	3		
31, 33, 52		1		
31, 35, (52)		1(1)		
31, 53		1		
31, 67		1		
33		1		
33, 56		1		
35	1	1		
35, 51		1		
35, 52, 59		1		
35, 59		1		
39		1		
45, 56, 66	1			
51	1	4		
52	1	1		
53	2	1		
58	1			
66	1	1		
X	2	11		
Σ 16	3	3		
Σ 18		3		
Σ 31	1	9		
Σ 33		3		
Σ 51	1	8		
Total Onc HPV+	12	28	0	1
Total tested groups	20	100	30	31
%	60.0	28.0	0	3.2

tion rate: Sellors *et al.* [21] noted that the concordance between cervical specimens and the vaginal, vulva and urine specimens for the presence of HPV was 0.76, 0.55 and 0.41, respectively.

We would like to evaluate which medium was appropriate for HPV screening in men. We know from the published literature that urine is not suitable for cytological examination. Therefore, urine (of both genders) and male urethral scrapes were tested for β -globin-concentration and HPV-positivity. We found β -globin in 69% of the urine samples and in 100% of the brushed samples, whereas HPV-PCR was positive in 3.2% of the female FVU specimens, in 0% of the male FVU specimens and in 60% of the male brushes. Our results show that for both men and women, the percentages as well as the absolute concentration of DNA is significantly lower in urine (68%) than in swabs taken with the Cervex-brush (100%). Despite the encouraging amount of β -globin in urine, the amount of HPV-DNA is disappointingly low. We conclude that urine is not the first choice for HPV screening. Testing on urine might only be beneficial in high-risk populations with a high HPV viral load (e.g. cervical cancer and HIV positive women). It is not only crucial to be able to amplify β -globin but also the absolute amount of DNA present is important. We have shown for the first time that the amount of human DNA measured by real-time quantitative PCR is important for HPV detection in the same sample.

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