Research Article

Cancer Prevention Research

Comparison between Urine and Cervical Samples for HPV DNA Detection and Typing in Young Women in Colombia

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Abstract

Urine sampling for HPV DNA detection has been proposed as an effective method for monitoring the impact of HPV vaccination programs; however, conflicting results have been reported. The goal of this study was to evaluate the performance of optimized urine HPV DNA testing in women aged 19 to 25 years. Optimization process included the use of first void urine, immediate mixing of urine with DNA preservative, and the concentration of all HPV DNA, including cell-free DNA fragments. Urine and cervical samples were collected from 535 young women attending cervical screening at health centers from two Colombian cities. HPV DNA detection and genotyping was performed using an HPV type-specific multiplex genotyping assay, which combines multiplex polymerase chain reaction with bead-based Luminex technology. Concordance between HPV DNA detection in urine and cervical samples was determined using kappa statistics and McNemar tests. The accuracy of HPV DNA testing in urine samples was evaluated measuring sensitivity and specificity using as reference the results obtained from cervical samples. Statistical analysis was performed using STATA11.2 software. The findings revealed an overall HPV prevalence of 60.00% in cervical samples and 64.72% in urine samples, HPV-16 being the most frequent HPV type detected in both specimens. Moreover, our results indicate that detection of HPV DNA in first void urine provides similar results to those obtained with cervical samples and can be used to monitor HPV vaccination trials and programs as evidenced by the substantial concordance found for the detection of the four vaccine types. *Cancer Prev Res; 9(9); 766–71. ©2016 AACR.*

Introduction

Three prophylactic HPV vaccines based on L1 virus like particles (VLP) have been commercially developed: Cervarix, a bivalent vaccine by GlaxoSmithKline against HPV-16 and -18 (1); Gardasil, a quadrivalent vaccine against HPV-6, -11, -16, -18 (2); and Gardasil9, a nonavalent vaccine against HPV-6, -11, -16, -18, -31, -33,- 45, -52, and 58; these two latter vaccines by Merck (3). A large number of clinical trials have proven that these vaccines are safe, well tolerated, highly immunogenic, and effective in preventing persistent infections by HPV vaccine types as well as cervical intraepithelial lesions associated with them (4–18).

In Colombia, HPV vaccination was introduced in the National Immunization Programme in 2012 as a primary prevention strategy for HPV-16/-18-related preneoplastic and neoplastic cervical lesions. The school-based program was initially launched targeting a single-year age cohort (4th graders), but since 2013 any girl between 14 and 17 years old was included. In this context, the Colombian government is designing a surveillance system, and the screening based on DNA testing for HPV types could offer the opportunity to measure the impact of a vaccination program in an early stage. Nevertheless, an efficient and feasible method for detecting and genotyping HPV with high analytical sensitivity is necessary (19, 20).

The use of a noninvasive and easy self-collection sampling method, like a urine sample to detect HPV DNA, could offer a more accessible and acceptable method to simplify HPV vaccine monitoring (19, 20). This approach could allow sampling of large cohorts to measure the impact of HPV vaccination programs in sentinel municipalities or within cohort studies (20, 21). Several studies focused on cervical cancer screening have reported a good performance to detect any HPV DNA in urine samples with sensitivities ranging from 71 % to 88% and

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specificities from 89% to 100% (22); however, only a few studies have focused on HPV surveillance in asymptomatic adolescents or young women using this method, and the data are not conclusive. Additional studies are required to define the optimal method for detection of HPV DNA in urine for its implementation in large populations considering not only the sample but also all possible variables involved, including characteristics of the targeted population in relation to HPV exposure, collection method for urine samples, urine sampling procedure, DNA isolation, and HPV DNA test sensitivity. In this context, the objective of this study was to determine the sensitivity and specificity of HPV-DNA detection in urine samples compared with concomitant HPV cervical detection as reference, by using a highly efficient DNA extraction procedure and an ultrasensitive HPV DNA genotyping assay (23, 24).

Materials and Methods

Ethics statement

This study was approved by Medical Ethics Committee at Instituto Nacional de Cancerología in Bogota, Colombia. All participants were informed about the purposes of this study by a specialist nurse and informed consent was obtained.

Study population

This study was conducted in the Colombian cities of Manizales and Soacha, from May to September 2014. Through different communication strategies developed by local health centers and local higher education institutions, a total of 540 non-vaccinated women in the age range of 18 to 25 years old were enrolled. From the 540 participants attending for cervical cancer screening, paired first voided urine and cervical samples were collected from 535 women. The participants were asked not to urinate at least 2 to 3 hours before collecting the urine sample.

Sample collection

Prior to undergoing pelvic examination, each participant collected about 20 mL of first void urine using a standard urine collection vial; 9.0 mL of urine were immediately transferred to another vial containing 4.5 mL of Urine Conservation Medium (UCM) provided by the Vaccine and Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Belgium (23–25). After collecting urine samples, cervical samples were obtained from both, the endocervix and the exocervix, by using a Cervex-Brush (Rovers Medical Devices) that was introduced into the cervical canal and rotated 360° 3 times. The obtained sample was spread on the slide using conventional technique and then the brush head was detached and placed in a vial with 20 mL of PreservCyt transport medium (Roche Diagnostics, GmbH) for HPV testing. Urine and cervical samples were stored at −20° C until delivered to the Centre for the Evaluation of Vaccination (VAXINFECTIO, University of Antwerp, Belgium) and to the Infections and Cancer Biology Group at the International Agency for Research on Cancer (IARC) in Lyon, France, respectively, for DNA extraction and HPV genotyping, respectively. The cytological slides were then referred to COLCAN, a clinical laboratory offering Pap smear reading services. The slides were stained with Papanicolaou stain, evaluated for a pathologist-supervised cytotechnologist, and classified according to The Bethesda System 2001. The positive cervical smears and 10% of negative cervical smears were again evaluated by the pathologist.

DNA extraction from urine samples

Frozen tubes were thawed in a water bath at room temperature. Tubes were vortexed for 10 seconds at 1,550 rpm and 4 mL of the urine/buffer mixture were immediately transferred to an Amicon Ultra-4 50K filter device (Merck Millipore). In order to concentrate all DNA, including cell-free DNA fragments, the filter device was then centrifuged at 4,500 rpm for 20 minutes. Centrifugation was repeated for 10 minutes if the remaining volume on the filter was greater than 1 mL. After filtration, 2 mL of NucliSENS Lysis Buffer (BioMérieux) were added to the concentrate retained on the filter, and incubated for 10 minutes at room temperature. All material was subsequently transferred to the NucliSENS Lysis Buffer vial, and DNA extraction of the complete content of the vial was performed using the generic easyMAG off-board lysis protocol. DNA was eluted in 55 μL elution buffer (24, 26), and the DNA extracts were then shipped to IARC on dry ice.

DNA extraction from cervical samples

One aliquot of 1 mL PreservCyt media was centrifuged at 6,000 rpm for 10 minutes to pellet the cervical exfoliated cells. After removing the supernatant, DNA extraction was performed using the Qiagen BioRobot EZ1 with the EZ1 DNA tissue kit according to the manufacturer's instructions (Qiagen). DNA was eluted in 100 μL of elution buffer (23).

HPV type-specific E7 PCR bead-based multiplex genotyping

The presence of HPV DNA was detected using a type-specific E7 PCR bead-based multiplex genotyping assay (E7-MPG, IARC, Lyon, France) as described previously (23, 27, 28). The E7-MPG assay utilizes HPV type-specific primer pairs targeting the E7 region of 19 probable/possible high-risk (pHR) or high-risk (HR) HPV types (HPV-16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68a and b, -70, -73, and -82) and 2 low-risk HPV types (HPV-6 and -11), plus primers for the amplification of a b-globin sequence (23, 28, 29). Ten microliters of DNA extracted from urine or cervical samples have been used to perform the PCR. The PCR generates a fragment of approximately 100 bp for HPV types HPV16, 18, 31, 33, 35, 52, 56, 66, 6, and 11, and a fragment of 117 bp for β-globin.

Following PCR amplification, 10 μL of each reaction mixture were analyzed by MPG using the Luminex technology as described previously (Luminex Corporation). Briefly, the reporter fluorescence was quantified using Luminex reader 200 (Luminex Corporation), and cutoffs were computed by adding 5 to 1.1 multiplied by the median background value expressed as median fluorescence intensity (23, 27).

Statistical analysis

Overall and type-specific HPV prevalence was estimated as the proportion of patients who tested HPV DNA positive for a given HPV DNA type. The agreement rate, kappa coefficient with 95% confidence interval, and McNemar *P* value were calculated to estimate the concordance between the results from urine and cervical samples. K value was interpreted as poor < 0, slight = 0.01–0.20, fair = 0.21–0.40, moderate = 0.41–0.60, substantial = 0.61–0.80, and almost perfect = 0.81–1.00. McNemar *P* value was used to calculate differences between paired proportions. The accuracy of urine HPV DNA testing, as measured by sensitivity and specificity, was evaluated using the results obtained from cervical samples as reference.

Finally, we evaluated the suitability of urine and cervical samples for HPV DNA detection according to cytological reports. Results were considered statistically significant at $P < 0.05$. Statistical analysis was performed using STATA11.2 software.

Results

HPV prevalence in urine and cervical samples

A total of 535 paired urine and cervical samples were collected. Beta-globin DNA was not detected in two cervical specimens and in three urine samples; these samples were excluded from the study and a total of 530 paired samples were used for analysis. Overall HPV prevalence was 60.00% in cervical samples (95% CI: 57.85%–62.15%) and 64.72% in urine samples (95% CI: 62.63%–66.81%). pHR/HR-HPV DNA (HPV-16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68a and b, -70, -73, and -82) was detected in 58.61% (309/530) of cervical samples and 65.54% (348/530) of urine samples, HPV-6 or -11 was detected in 5.06% (27/530) of cervical samples and in 6.93% (36/530) of urine samples. HPV-16 was the most common type detected in both, urine and cervical samples, 14.50% (77/530) and 13.80% (73/530), respectively. The second most frequent HPV type detected was HPV-58 (51/530) in cervical samples and HPV-52 (47/530) in urine samples. The distribution of the other HPV types detected is comparable (Fig. 1). Single HPV infections were detected in 27.16% (144/530) of cervical specimens and in 23.20% (123/530) of urine samples while multiple HPV infections were detected in 32.83% (174/530) of cervical samples and in 41.50% (220/530) of urine samples. In general, urine samples contained a higher number of HPV types (up to nine) in comparison to cervical samples (Supplementary Fig. S1).

Table 1. Sensitivity and specificity of urine HPV DNA test for HPV types included in bi- and quadrivalent vaccines (results of cervical samples used as gold standard)

	Cervical sample		Sensitivity	Specificity
Urine sample	Positive	Negative	(95% CI)	(95% CI)
Any HPV				
Positive (343)	288	55	90.57% (87–94)	74.06% (68–80)
Negative (187)	30	157		
HPV 6				
Positive (29)	21	8	95% (87–100)	98% (97–100)
Negative (501)	1	500		
HPV 11				
Positive (8)	5	3	100% (48–100)	99% (99–100)
Negative (522)	0	522		
HPV 16				
Positive (77)	58	19	79% (68–88)	96% (94–97)
Negative (453)	15	438		
HPV 18				
Positive (25)	13	12	62% (38–82)	98% (96–99)
Negative (505)	8	497		

Validity of urine HPV detection compared with cervical HPV detection as gold standard

When sensitivity and specificity were evaluated, urine HPV DNA testing had a sensitivity of 95.00% (95% CI, 87.00–100.00) for HPV 6; 100% (95% CI, 48–100) for HPV 11; 79% (95% CI, 68–88) for HPV-16 and 62% (95% CI, 38–82) for HPV-18. While the specificity was 98% (95% CI, 97–100) for HPV-6; 99% (95% CI, 99–100) for HPV-11; 96% (95% CI, 94–98) for HPV-16, and 98% (95% CI, 96–99) for HPV-18 (Table 1). Similar results were observed for the other pHR/HR-HPV types (Supplementary Table S1).

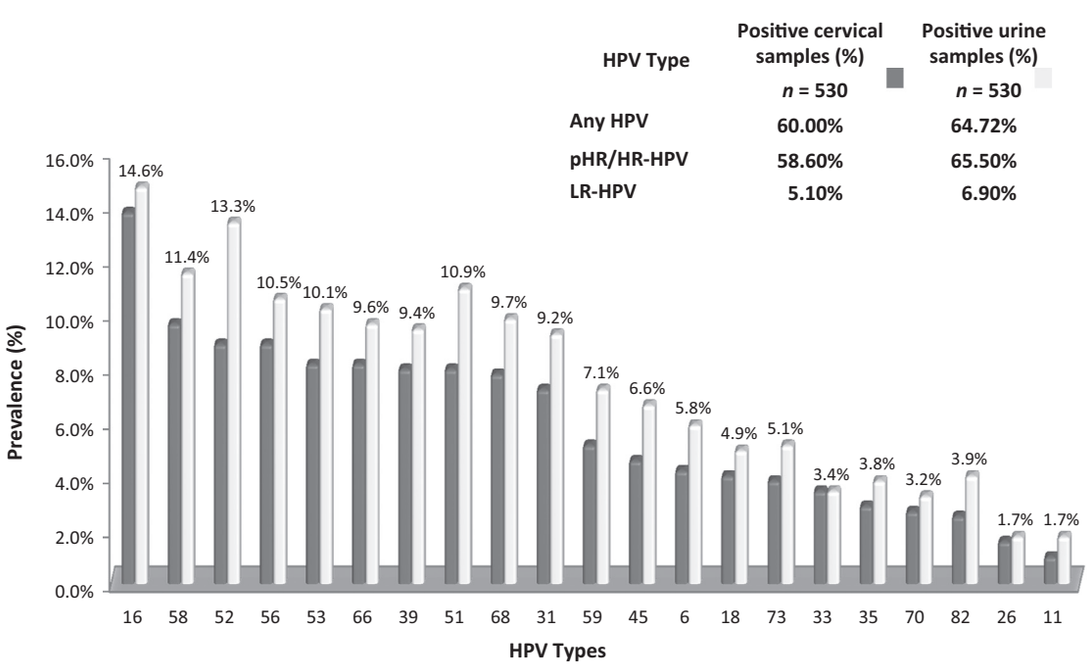

Figure 1.
Prevalence of HPV types in cervical and urine samples in young women aged 18–25 years from Soacha and Manizales, Colombia.

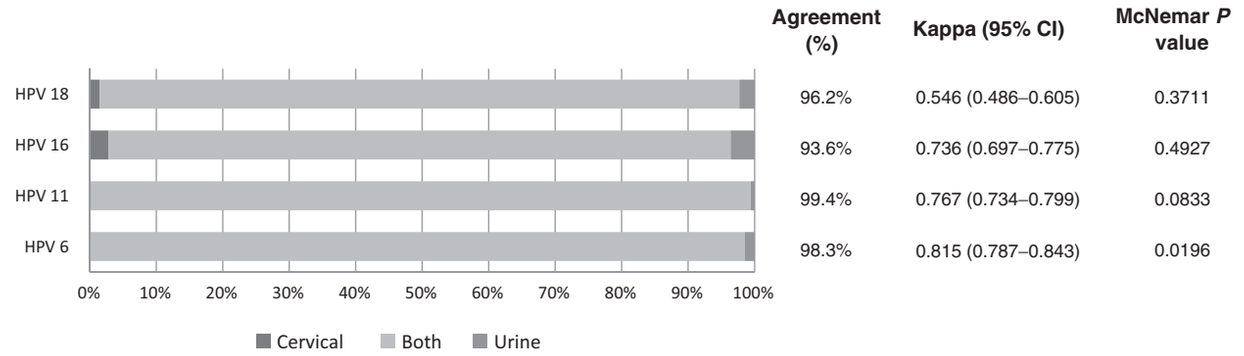

Figure 2.
Agreement among cervix and urine samples for the detection of four types included in quadrivalent vaccine.

Concordance of HPV DNA detection in cervical and urine samples

Agreement between HPV detection in urine and cervical samples for pHR/HR-HPV (19 HPV types) and LR-HPV (-6 and -11) was 78.90% (k = 0.81; 95% CI, 0.78–0.84) and 97.90% (k = 0.55; 95% CI, 0.49–0.61), respectively. When we analyzed the agreement between HPV types included in the quadrivalent vaccine in both samples, the overall agreement rate for all HPV types analyzed was higher than 93%. A moderate to almost perfect concordance of HPV detection between cervical and urine samples was observed for HPV-6, HPV-11, HPV-16, and HPV-18 as shown in Fig. 2. No statistically significant differences were found in overall positivity between the paired samples, except for HPV-6. Similar results were observed for other pHR/HR-HPV types (Supplementary Fig. S2). Even though the aim of this study was HPV vaccine monitoring, we also observed a good correlation of HPV detection between urine and cervical samples from women with ASCUS and low-grade cytological lesions (Supplementary Tables S2 and S3).

Discussion

Several studies have established the use of urine sampling as a useful, noninvasive alternative for HPV detection with screening purposes. Similarly, urine sampling has been proposed as an adequate alternative for monitoring HPV prevalence in female adolescents in order to determinate the early effect of HPV vaccination in the targeted age cohorts (19, 30–33). However, the results have not been conclusive due to a number of variables that could negatively affect HPV detection. In this study, we aimed to evaluate the usefulness of urine as a specimen for detecting HPV among young women, taking the results obtained from cervical samples as reference.

In contrast to the prevalence of HPV infection around 20% reported in cervical samples of young women (34–36), we found prevalence around 60%. Our results concur with the 59% HPV prevalence reported by Cuschieri and colleagues, in women ages 16 to 25 years old in Glasgow, United Kingdom (37). Similar outcomes were reported by Ramqvist and colleagues, who found a 62% of HPV prevalence involving sexually active women from Stockholm, Sweden, ages 15 to 23 years and by Wheeler, who found a maximum HPV prevalence of 52% at age 20 years in the general New Mexico population (38, 39). These results are consistent with previous observations that indicate that HPV infection rates are higher after sexual initiation and are shown to peak around the age of 25 (31, 40).

This study also provided evidence of a slightly higher HPV prevalence in urine samples (65%, 95% CI, 63–67) compared with cervical samples (60%, 95% CI, 58–62). Comparing these results with other studies is challenging. As described previously, there is a great inconsistency of the results across studies. This variability could be related to the variation of target age and population characteristics as well as the different methods used from sample collection, sample storage, DNA extraction, and DNA detection. However, comparable results have been described by Cuschieri and colleagues (67% vs. 59%; ref. 31), Bernal and colleagues (53% vs. 50%; ref. 41), and Ducancelle and colleagues (59% vs. 54%; ref. 31), among women between 18 and 25 years old. In addition to a higher HPV prevalence in urine, the absolute number of HPV genotypes that were detected in urine samples was higher (up to 9) than the number of genotypes identified in cervical samples. The higher prevalence and higher amount of identified genotypes may be explained by the fact that in urine samples there is presence of exfoliated cells containing HPV DNA or free virions that are continuously being shed not only from cervical epithelium but also from urethral and vulvar epithelium, which are also tissues susceptible to HPV infection (42).

As aforementioned, the procedures involved in sample processing are very important. In this study, we used strategies described by Vorsters and colleagues (25) to optimize HPV DNA detection in urine. These strategies included the use of a UCM right after sample collection, which contains a nuclease inhibitor that allows stabilization of cell free HPV DNA; the use of the first void of urine flow; concentration of all DNA, including cell-free DNA fragments, improving DNA extraction and the use of an ultrasensitive HPV genotyping assay (23, 28), which definitely had an important contribution for the very good concordance observed for HPV prevalence in urine and cervical specimens. These results confirm that HPV testing in urine samples can provide robust results for surveillance of HPV vaccination programs. However, the highly sensitive HPV detection method we used has not been demonstrated to be applicable for screening programs and further studies are necessary.

In this study, sensitivity and specificity for any HPV were similar to those reported by Cuschieri and colleagues, whose

population and goals were similar to ours. Nevertheless, the methods used for urine collection and preservation, HPV DNA extraction, detection, and genotypification were different (SPF10 primers and genotyping with INNO-LiPA detecting 27 HPV genotypes; ref. 37).

Regarding concordance, Kappa values reported by previous studies have remained in the range of 0.60 to 0.96 (substantial to almost perfect). In our study, the agreement between any HPV type detection in urine and cervical samples was 84.00% with a Cohen's kappa coefficient of 0.60, similar to that reported by Cuschieri and colleagues. In relation to concordance for LR-HPV and HR-HPV types included in tetravalent vaccine, in our study, concordance was substantial for both, k = 0.70 (95% CI, 0.66–0.74) for HPV-6 and -11 and k = 0.76 (95% CI, 0.78–0.84) for HPV-16 and -18 (31, 37, 41, 43–45).

Our findings provide strong evidence that detection of HPV DNA in urine and cervical specimens provides comparable results. This is particularly interesting in the context of HPV vaccination program monitoring, as urine could be used as a simple, noninvasive sampling method for HPV DNA testing that can be self-collected. However, further research is imperative to standardize the procedures involved in urine HPV DNA testing.

Disclosure of Potential Conflicts of Interest

P. Van Damme and A. Vorsters have ownership interest (including patents) in Novosanis, a University of Antwerp spin off company. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Gheit, P. González, D. Puerto, L. Montoya, P. Van Damme, M. Tommasino, G. Hernández-Suárez, L. Sánchez, C. Wiesner

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