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CLINICAL ARTICLE

Comparison of urine and self-collected vaginal samples for detecting human papillomavirus DNA in pregnant women

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ABSTRACT

Objective: To investigate the utility of urine sampling for detecting human papillomavirus (HPV) DNA among pregnant women and to compare HPV DNA detection in urine with detection in vaginal samples. *Methods:* In a cross-sectional study, urine and vaginal samples were self-collected from pregnant women attending prenatal care at Hospital Divina Providencia, Frederico Westphalen, Brazil, between October 2006 and August 2007. Part of the L1 region of the HPV genome was amplified via $GP5^+$/$bioGP6^+$ primers. Positive urine was genotyped for high-risk HPV genotypes (HPV16, HPV18, HPV31, HPV33, HPV39, HPV45, and HPV59). *Results:* During the study period, urine samples were obtained from 133 pregnant women, 63 of whom also self-collected vaginal samples. HPV DNA was detected in 54.0% (34/63) and 61.9% (39/63) of urine and vaginal samples, respectively. HPV infection was significantly associated with first intercourse at younger than 20 years of age ($P = 0.008$). There was substantial agreement in HPV DNA test results between the urine and vaginal samples (κ value, 77.3%; $P < 0.0001$). HPV31 and HPV16 accounted for 80.7% of the oncogenic types identified. *Conclusion:* Detection of HPV DNA in urine showed good agreement with detection in self-collected vaginal samples, indicating that urine might be a reliable sample for HPV testing among pregnant women.

1. Introduction

Human papillomavirus (HPV) infection is considered to be the main cause of cervical cancer, which is the third most common cancer among women. There is an estimated incidence of 500 000 new cases annually worldwide, of which more than 80% are in low-income countries [1]. In Brazil, the National Institute for Cancer Research estimated that there were 17 540 new cases of cervical cancer in 2012, with a total risk of 17 cases per 100 000 women [2]. More than 100 different HPV genotypes, segregated into low- and high-risk oncogenic types, have been identified [3].

There is no consensus among researchers about the relationship between pregnancy and HPV infections. Some studies have reported a significant difference in HPV prevalence between pregnant and non-pregnant women [4–6], whereas others have not [7,8]. A systematic review found that the prevalence of HPV among pregnant women ranged from 5.5% to 65% [9].

HPV DNA is usually detected via cervical and vaginal samples, but several studies have reported good performance for detecting HPV DNA in urine samples ranging from 63.2% [10] to 81.5% [11]. Using urine samples to detect HPV DNA might be advantageous during pregnancy because self-collection of urine is a well-accepted, non-invasive sampling method. A urine-based HPV test might increase the compliance of women to undergo HPV screening [12].

The primary aim of the study was to investigate the utility of urine sampling as a method for detecting HPV DNA among pregnant women living in a poor area in southern Brazil. The study location, Frederico Westphalen, is a small city (28 843 inhabitants in 2010) in south Brazil with a public hospital that serves low-income individuals. A secondary aim was to investigate the prevalence of high-risk HPV genotypes in Brazil.

2. Materials and methods

In a cross-sectional study conducted between October 1, 2006, and August 31, 2007, all pregnant women who attended Hospital Divina Providencia, Frederico Westphalen, Rio Grande do Sul, southern Brazil, for prenatal care were invited to participate. The Ethics Committee of the Lutheran University of Brazil approved the study (protocol number 287H), and all participants provided written informed consent.

Healthy normal pregnant women were included in the study; women who had serious obstetrics complications or had any morbidity were excluded. None of the study participants reported cervical dysplasia or cervicitis.

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A nurse practitioner explained the study and how to self-collect urine and vaginal samples. Consenting women were instructed to collect the urine sample before the vaginal sample. The participants also answered an epidemiologic questionnaire to record social and behavioral factors.

First-stream urine (15–20 mL) was collected in 50-mL tubes and stored at 4 °C for 2–3 h before processing. After centrifugation, the cell sediment was washed twice with sterile 1× tris-ethylenediaminetetraacetic acid (TE) buffer, and stored at −20 °C until DNA extraction. Vaginal samples were collected by inserting a sterile swab into the vagina and rotating it 5 times, before placing it in a HPV DNA specimen collection tube containing 1× TE buffer. The sample was obtained while the woman squatted in the examination room.

A non-organic method was used to extract DNA from urine and vaginal samples [13]. In brief, 500 μL of each sample was concentrated by centrifugation. The resulting sediment was resuspended in 50 μL of 1× TE and incubated at 99 °C for 10 min. The DNA was then purified by using 5 μL of a glass matrix (Concert Extraction Systems; Life Technologies, Rockville, MD, USA).

A 150-bp fragment of the L1 region of the HPV genome was amplified by using $GP5^+$/$bioGP6^+$ consensus primers [14]. A negative control (no DNA) was included in each PCR run to ensure that no cross-contamination had occurred. Samples were amplified under the following conditions: initial denaturation at 95 °C for 5 min; 40 cycles of 95 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min; and final elongation at 72 °C for 10 min. DNA from CaSki cells (HPV16-positive) was used as a positive control in all PCR reactions. All samples were prescreened with β-globin primers PCO3 (5′-ACACAACTGTGTTCACTAGC-3′) and PCO4 (5′-CAACTTCATCCACGTTCACC-3′) (110-bp fragment) to assess sample integrity [15]. The final PCR product was visualized in an electrophoresis agarose gel (2.5%) under ultraviolet light.

HPV DNA genotyping was carried out only for DNA extracted and amplified from urine samples by using a microplate colorimetric hybridization assay that detects some of the most prevalent high-risk HPV types in Brazil (HPV16, HPV18, HPV31, HPV33, HPV39, HPV45, and HPV59) [16].

Statistical analysis was performed with SPSS version 16.0 (IBM, Armonk, NY, USA). The χ^2 test was used to study differences in categorical parameters. A P value of less than 0.05 was considered statistically significant. The κ statistic was used to assess the agreement between urine and vaginal samples for HPV DNA detection. The sensitivity, specificity, and predictive values (positive and negative) of HPV DNA detection in urine were calculated by using data from the vaginal samples as the standard. Negative and positive predictive values were calculated with 95% confidence interval (CI) using Epi Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

3. Results

During the study period, 133 women were eligible for the study and agreed to self-collect urine samples for HPV DNA testing. Table 1 shows the sociodemographics characteristics of the 133 pregnant women. Of these women, 70 (52.6%) refused to self-collect vaginal sample.

Among the 63 women who provided both samples, HPV DNA was detected in 54.0% (34/63) and 61.9% (39/63) of urine and vaginal samples, respectively. All samples were positive for β-globin amplification. HPV infection was significantly associated with having first intercourse at younger than 20 years of age ($P = 0.008$). No significant association was found between HPV prevalence and age, education, marital status, smoke, use of oral contraceptive, gestational age, or age of menarche. No significant difference was found in genotype distribution between early and late pregnancy.

Twenty-six of the 34 HPV-positive urine samples were analyzed for the oncogenic types HPV16, HPV18, HPV31, HPV33, HPV39, HPV45, and HPV59. Of these 26 samples, 9 were positive for HPV16 (34.6%), 2 for HPV18 (7.7%), 12 for HPV31 (46.1%), 4 for HPV33 (15.4%), 1 for HPV 39 (3.8%), and 3 for HPV59 (11.5%). None of the samples was positive for HPV45. Overall, 9 samples (34.6%) were negative for all of the 7 types analyzed. Simple infection was found in 23.1% (6/26) of women, co-infection with 2 oncogenic types of HPV in 34.6% (9/26), co-infection with 3 types in 3.8% (1/26), and co-infection with 4 types in 3.8% (1/26) of women. HPV31 and HPV16 accounted for 80.7% of the HPV types identified.

Table 1
Sociodemographic characteristics of pregnant women from southern Brazil.

Characteristic	Number (%) of women (n = 133)
Age	
≤34 y	116 (87.2)
>35 y	17 (12.8)
Education	
Elementary school	98 (73.7)
Junior college	32 (24.0)
University	03 (2.3)
Marital status	
Married	99 (74.4)
Unmarried	34 (25.6)
Smoker	
Yes	29 (21.8)
No	104 (78.2)
Use of oral contraceptive	
Yes	117 (88.0)
No	16 (12.0)
Gestational age	
4–20 wk	54 (40.6)
21–40 wk	79 (59.4)
Age at first intercourse	
<20 y	122 (91.7)
≥20 y	11 (8.3)
Age of menarche	
≤12 y	60 (45.2)
≥13 y	73 (54.8)

Concordance between the 2 biologic (urine and vaginal) specimens was estimated by considering only those samples for which the results were available for both specimens. Vaginal samples were used as a reference. There was substantial agreement between urine and vaginal samples for HPV DNA detection (κ statistic, 77.3% [95% CI, 66.1%–88.4%]; $P < 0.0001$). The urine sample's diagnostic performance showed 84.6% sensitivity (95% CI, 74.8%–94.3%), 95.8% specificity (95% CI, 90.0%–101%), 97.0% positive predictive value (95% CI, 91.9%–1.02%), and 79.3% negative predictive value (95% CI, 68.5%–90.0%). Table 2 compares the results obtained from both urine and vaginal samples for the 63 women who provided both samples.

4. Discussion

The present study evaluated the utility of urine sampling as a method for detecting HPV among pregnant women living in southern Brazil. Although several studies have evaluated HPV DNA in urine samples [12], none has evaluated samples from pregnant women. Moreover, only a few studies have described the prevalence of HPV DNA among a population of pregnant women [4–6,8,17].

Table 2
Concordance of HPV DNA detection between 63 paired urine and vaginal samples from pregnant women.[a]

Urine sample	Vaginal sample		
	HPV +	HPV −	Total
HPV +	33	1	34
HPV −	6	23	29
Total	39	24	63

[a] κ value, 0.773 (95% confidence interval, 0.661–0.884), $P < 0.0001$.