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Urine human papillomavirus prevalence in women with high-grade cervical lesions

P. Nicolau [a,*], G. Mancebo [a,c], S. Agramunt [a,e], J.M. Solé-Sedeño [a], B. Bellosillo [d,e], M.M. Muset [d], B. Lloveras [b], F. Alameda [b,c], R. Carreras [a,c]

[a] Obstetrics & Gynecology Department, Hospital del Mar de Barcelona, Parc Salut Mar. Passeig Marítim 25-29, 08003 Barcelona, Spain
[b] Pathology Department, Hospital del Mar de Barcelona, Parc Salut Mar. Passeig Marítim 25-29, 08003 Barcelona, Spain
[c] Universitat Autònoma de Barcelona, Hospital del Mar de Barcelona, Parc Salut Mar. Passeig Marítim 25-29, 08003 Barcelona, Spain
[d] Unit of Molecular Biology, Pathology Department, Hospital del Mar de Barcelona, Parc Salut Mar. Passeig Marítim 25-29, 08003 Barcelona, Spain
[e] IMIM Instituto de Investigación Sanitaria, Hospital del Mar de Barcelona, Parc Salut Mar. Passeig Marítim 25-29, 08003 Barcelona, Spain

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ABSTRACT

Objective: To determine the prevalence of human papillomavirus (HPV) in urine samples from women with high-grade cervical lesions. Secondary objectives are to identify the influence of socio-demographic factors and the different genotypes with urinary HPV positivity.
Study design: 75 women with a positive biopsy for CIN2+ were included in the study from October 2010 to July 2011. A sample of urine was collected immediately before conization at the outpatient clinic. We analyzed the presence of HPV using a PCR technique.
Results: The mean age of the patients was 34.8 years (range 24 to 61). All patients had histological CIN2+, of whom 54.67% had CIN3. The prevalence of HPV in urine test was 58.82% in CIN2 population versus 78.05% in CIN3 patients (p 0.072). 31 different genotypes were found. The most frequent HPV genotype was 16-HPV, which was identified in 58% of women with positive HPV-DNA in urine samples. No demographic characteristics were significantly associated to urinary HPV prevalence.
Conclusion: Most of the patients with CIN2+ showed positive results for urine HPV test. The prevalence of positive urinary HPV test was higher for patients with CIN3. HPV urine detection could be considered as an acceptable option for high-risk population who skip regular screening programs.

Introduction

Cervical cancer is one of the most frequent gynecological cancers in women around the world and represents an important medical and social problem, especially in developing countries. The screening of cervical cancer has shown to reduce drastically its incidence and mortality. Human papillomavirus (HPV) infection is a necessary cofactor of invasive cervical cancer, being Cervical Intraepithelial Neoplasia (CIN) its precursor. It has been reported that 80% of cervical cancer cases occur in population without a correct cervical screening program [1], and that only 75% of women remain in this adequate gynecologic screening in our country [2].

Previous studies have suggested an association between high-grade cervical lesions and positive HPV detection in urine samples [3,4,5]. This association has been shown to increase among HIV infected patients. In contrast, the prevalence of HPV-DNA in urine samples and HPV high-risk genotypes in low risk population, such as sexually unexposed girls and married healthy women with a normal cytology, is very low (4 to 10%) [6,7].

The reason of this association is not well established. One possible explanation could be a urine contamination with exfoliated HPV-infected cells from the cervix. Another reason could be the presence of a concurrent infection of the urinary and the lower genital tract. In all cases, the frequency of HPV detection in urine samples appears to increase proportionally to the grade of the cervical lesion.

Urine samples have shown lower sensitivity and specificity than those from cervical swabs, or other methods like self-collected vaginal or vulvar samples [8,9]. However, urine HPV detection has been proposed as an alternative method for patients who skip regular controls, or patients who decline to use traditional screening methods, being the self-collected methods more commonly accepted than those collected by a physician [9].

* Corresponding author. Tel.: +34 932483129; fax: +34 932483129.
E-mail addresses: paubatalla@hotmail.com, pnicolau@parcdesalutmar.cat (P. Nicolau).

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The aim of our study is to describe the prevalence of urine HPV in women with high-grade cervical lesions from our population [10]. Additionally, we aim to assess the predictive value of the HPV genotype of the lesions as well as socio-demographic factors, and to compare our results to the previously reported in the literature.

Materials and methods

Study population

Patients with high-grade cervical disease were recruited from October 2010 until July 2011. Patients referred to the Cervical Pathology Unit at Obstetrics and Gynecology Department at Hospital del Mar in Barcelona, with a positive biopsy for CIN2 or CIN3, who were eligible for a conization procedure, were asked to participate in the study. After giving informed consent, a sample of urine was obtained. The collection was performed immediately before the conization at the outpatient clinic, previous to any pelvic examination, and without any specific collection recommendation, in a direct voided way. No preservative technique was used. Socio-demographic characteristics and relevant clinical information were also collected from all patients. CIN2+ was defined as lesions of CIN2 and CIN3.

HPV DNA detection

Detection and typing of HPV was performed by PCR using the Linear Array HPV Genotyping Test (LA) (Roche Diagnostics GmbH, Manneheim, Germany). We analyzed the presence of HPV using a PCR-based technique, defined as the gold standard because it has the highest sensitivity [11]. This assay consists in amplification of a DNA sample by PCR and an hybridization using a reverse line blot system for simultaneous detection of 37 genotypes of HPV. Detected HPV genotypes were 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, −82 variant (82v or IS39) and −89 (CP6108). This test was based on the amplification of a small region of 450 pb inside the L1 gene using a pool of primers. Moreover, a region inside beta-globin gene was also amplified as an internal control. After the hybridization reaction, the strip was interpreted with a reference guide. Excess DNA of Cobas 4800 HPV test was used, and 10 μl of Tris hydrochloride solution 1 M with pH 7.5 were added for the PCR in the master mix. Similarly, we compared the HPV genotype detected in urine samples and in the biopsy in the concurrent loop excision specimen with the same technique.

Statistical analysis

Descriptive statistics were calculated for the entire population, as well as for the urinary HPV positive and negative groups, respectively. Regarding these results, Chi-Square was used to compare proportions for dichotomous variables, and Student t test for quantitative variables. For the correlation analysis, kappa correlation coefficient was calculated. All the statistical analysis was performed using STATA SE v10 software (Texas, USA).

Results

A number of 75 women were included in the study. The mean age was 34.8 (+/− 8.6 years), with 47% of nulliparous, 33% of smokers (being 56% of them active smokers of above 10 cig/day). Regarding the contraceptive method, 84% of women who report the information used some method (56% of them used a non-hormonal contraceptive in comparison to 44% that used an hormonal method), as shown in Table 1.

All patients had histological CIN2+, of whom 55% had CIN3. No differences were found among demographic characteristics when comparing patients with positive urine HPV to those with a negative result.

The prevalence of positive urine HPV test was 69.3% in our study population (CIN2+). For the subpopulation of CIN3 patients, the prevalence rose up to 78.1%, but there was no statistically significant difference although it had a tendency (p 0.072) comparing CIN2 versus CIN3 (Table 2).

Regarding the viral subtype, different 31 genotypes were identified. The most frequent HPV genotype was 16-HPV. This genotype was positive in 57.7% of urine samples and 53.9% of cervical biopsies with a good level of correlation (kappa coefficient of 0.69). In contrast, 18-HPV was the second most frequently detected genotype in cervix but with a lower prevalence in urine samples (Tables 3 and 4).

The other high-risk genotypes were homogeneously distributed in both types of samples, with an especially important presence of 33-HPV, 53-HPV, 59-HPV, 61-HPV, 62-HPV, 72-HPV in urine (Fig. 1).

Comments

Most of the patients with CIN2+ showed positive urine HPV test. The prevalence in our study was of 69.3%, concordant to the previously reported in the literature for cases of high-grade

Table 1
Demographic characteristics of the study population.

Factor	Study population			*p* Value
	Total (*n* = 75)	Urine HPV (−) (*n* = 23)	Urine HPV (+) (*n* = 52)	
Age; mean (s.d. years)	34.8 (8.6)	32.6 (7.4)	35.8 (8.9)	0.136
Parity; *n* (%)				
Nulliparous	35 (46.67%)	10 (43.49%)	25 (%)	0.383
Multiparous	31 (41.33%)	12 (52.17%)	19 (%)	
Missing	9 (12.0%)	1 (4.35%)	8 (15.38%)	
Smoking status; *n* (%)				
Non-smoker	43(57.33%)	14 (60.87%)	29 (55.77%)	0.962
Active smoker	25 (33.33%)	8 (34.78%)	17 (32.69%)	
Missing	7 (9.33%)	1 (4.35%)	6 (11.54%)	
Contraceptive method; *n* (%)				
No method	8 (10.67%)	3 (13.04%)	5 (9.62%)	0.646
Non-hormonal method	23 (30.67%)	7 (30.43%)	16 (30.77%)	
Hormonal method	18 (24.0%)	5 (21.74%)	13 (25.0%)	
Missing	26 (34.67%)	8 (34.78%)	18 (34.62%)	