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Lack of HPV 16 and 18 Detection in Serum of Colposcopy Clinic Patients

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Abstract

Background—Persistent infection with high-risk human papillomavirus (HPV) types is necessary for the development of high-grade cervical dysplasia and cervical carcinoma. The presence of HPV DNA in the blood of cervical cancer patients has been reported; however, whether HPV DNA is detectable in the blood of patients with pre-invasive cervical disease is unclear.

Objectives—The objectives of this study were to determine if HPV 16 and HPV 18 DNA could be detected in the serum of colposcopy clinic patients, and if serum HPV detection was associated with grade of cervical disease and HPV cofactors.

Study Design—Samples were selected from a biorepository collected from non-pregnant, HIV-negative women ages 18–69 attending colposcopy clinics at two urban public hospitals. Cervical disease status was based on review of colposcopy, biopsy and cytology findings. Serum HPV DNA detection was conducted using a novel PCR and mass spectroscopy-based assay.

Results—Of the 116 adequate serum samples, all (100%) were negative for HPV 16 and HPV 18. Over half (51.7%) of participants had cervical HPV 16 and/or HPV 18 infection. Nearly one-third (31.1%) had high grade, 10.3% had low grade, and 50.9% had no cervical disease. Nearly

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Conflict of Interest:

Competing interests: None declared. *Ethical approval:* This study protocol was deemed “not regulated” by the Institutional Review Board of the University of Michigan Medical School as the data could not be linked directly or indirectly to a specific individual.

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one-third (28.5%) had ever regularly smoked cigarettes, 70.7% had early onset of sexual intercourse, and 75% had ever used oral contraceptives.

Conclusions—In this colposcopy clinic population with a range of clinical characteristics and established HPV cofactors, HPV DNA was undetectable in their serum. Our findings suggest that serum HPV DNA detection is not a cervical cancer screening tool.

Keywords

Human papillomavirus; Cervical neoplasias; Screening; Serum; Quantitative polymerase chain reaction; Mass spectroscopy

Background

Previous studies have demonstrated HPV DNA detection using PCR-based methods in serum,^{1–3} plasma,^{4–7} and peripheral blood^{1,8} of patients with invasive cervical cancer, suggesting potential diagnostic and prognostic applications. If circulating HPV DNA was reliably detected in women with *pre-invasive* cervical disease, the method could have promise for cervical cancer screening.

Objectives

The objectives of this study were to determine if HPV 16 and HPV 18 DNA could be detected in the serum of patients attending colposcopy clinics using a novel, highly sensitive assay, and if serum HPV detection was associated with grade of cervical disease and presence of HPV cofactors.

Study Design

Biorepository

Samples were selected from a well-characterized biorepository collected from non-pregnant, HIV-negative women ages 18–69 attending colposcopy clinics at urban public hospitals in Atlanta, Georgia, and Detroit, Michigan, between December 2000 and December 2002, as previously described.⁹ Consenting women were interviewed by clinic coordinators using a standardized questionnaire covering demographics; health history; sexually transmitted disease risk factors; cancer screening; personal and familial cancer history; smoking history; and hormone use.

Cervical disease status was based on review of colposcopy, biopsy and cytology findings. Cervical intraepithelial neoplasia (CIN) was grouped into three grades (CIN I, II and III), and subjects with no evidence of dysplasia were designated “No CIN.” HPV status was determined on total nucleic acid extracts of exfoliated cervical cells collected at the time of colposcopy using Roche prototype line blot assay.⁹

Serum was obtained from peripheral blood collected by venipuncture at the colposcopy visit, either before or after colposcopy. Blood was allowed to clot at room temperature, and 1 ml aliquots of serum were stored at –70°C. For this study, 135 serum samples were selected to include approximately equal numbers of women positive for HPV 16 or 18 (with or without other HPV types; n=69) or negative for all high-risk HPV types (n=66) at the cervix. This study protocol was deemed “not regulated” by the Institutional Review Board of the University of Michigan Medical School as the data could not be linked directly or indirectly to a specific individual.

Serum HPV DNA detection

Serum HPV testing was conducted using a PCR and mass spectroscopy (PCR-MS)-based assay at Sequenom Center for Molecular Medicine (Ann Arbor, MI). Investigators conducting the serum HPV testing were blinded to corresponding cervical HPV status. DNA was extracted from 1 ml serum using the QIAamp MinElute Virus Spin Kit, eluted in 50 μ L elution buffer (Qiagen Inc., Germantown, MD). Extracted DNA samples were initially screened by a PCR-MS assay which included primer pairs designed to amplify regions of the E6 gene in 15 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 73) plus human β -globin. The sensitivity of this assay was 25 aM. Samples were deemed positive if 3 of the 4 valid replicates gave an HPV peak probability of 0.97 or greater. Assays were determined to be valid if 3 of the 4 replicates gave a β -globin peak probability of 0.90 or greater. Samples found to be positive for HPV 16 or 18 were then quantitated in a 3-plex PCR-MS assay with HPV 16, HPV 18 and human β -globin as an adequacy control (detection threshold: 250 copies HPV genome/ml serum). The sensitivity of this test was 5 aM. Details of the PCR-MS methodology for quantitative, type-specific detection of HPV have been previously described.¹⁰ Briefly, PCR-MS involves a 3-step process composed of competitive PCR (cPCR), primer extension, and matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) separation of products on a matrix-loaded silicon chip array to detect HPV. A competitive template is used for quantitation.

Results

Of the 135 serum samples analyzed, 116 (85.9%) were β -globin positive by PCR-MS and thus considered adequate for interpretation. All 116 (100%) serum samples were negative for HPV 16 and HPV 18. Most participants were Black (73.3%) and non-Hispanic (89.6%), with median age of 27 years (Table 1). Regarding established HPV cofactors, 28.5% ever regularly smoked cigarettes, 70.7% had early onset of sexual intercourse (≤ 17 years), and 75% ever used oral contraceptives. Over half (51.7%) had cervical HPV 16 and/or HPV 18 infection (43.3% single and 56.7% multiple infections; Table 2). A large proportion of those with multiple HPV infections at the cervix also had other high-risk HPV types. Nearly one-third (31.1%) had high grade (CIN II/III) cervical disease, 10.3% had low grade (CIN I), and 50.9% had no disease.

Discussion

In this population of women enriched for cervical HPV infection and representing the range of clinical disease and established HPV cofactors, we did not detect HPV 16 or HPV 18 DNA in the serum using a highly sensitive PCR-MS assay. Previous PCR-based studies detecting HPV DNA in blood used different material (serum, plasma or blood), DNA extraction methods, and primers for amplifying HPV DNA.^{1-8,11} Some of these studies also included patients with pre-invasive cervical disease, but the findings were inconsistent. Yang et al. detected HPV 16 DNA in the serum and peripheral blood fraction of four out of five patients with untreated high-grade cervical dysplasia, but not in any of the individuals who had been treated and had no active lesions at the time of study.¹ Ho et al. failed to detect HPV 16, HPV 18, or HPV 52 DNA in the peripheral blood of 10 CIN I patients, 10 CIN II patients, and 20 CIN III patients.⁸ Kay et al. detected HPV 16 and HPV 18 DNA in the blood of 24.4% of patients with advanced cervical cancer, but not in any of the patients with cervical HPV infection or pre-invasive cervical lesions.¹¹ Interpretation of these findings is generally limited by small sample sizes and sparse description of the study populations. Given the distribution of clinical characteristics and established HPV cofactors in our study population, along with cervical HPV 16 and/or HPV 18 positivity in over half (51.7%) of participants, we expected to find HPV DNA in the corresponding serum if this

were a truly viable site for HPV detection. Extracting a larger volume of serum could be attempted to increase the chance of detecting HPV in the free DNA in the blood. However, using the newly described,¹⁰ highly sensitive assay makes it unlikely that HPV detection would be increased. Our findings are consistent with the lack of a viremic state for HPV infection and suggest that *serum* HPV DNA detection is not a cervical cancer screening tool.

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Table 1

Demographics, health history, and HPV cofactors (n=116)

	n	%
<i>Demographics</i>		
Age at enrollment (years)		
18–20	15	12.9
21–30	53	45.7
31–40	24	20.7
41–50	14	12.1
>50	6	5.2
Missing (Median: 27; SD: 10.8)	4	3.5
Race		
Black/African American	85	73.3
White	18	15.5
American Indian/Alaskan	1	0.9
Other	1	0.9
Missing	11	9.5
Hispanic ethnicity	14	12.1
<i>Health history</i>		
Ever prescribed female hormones	11	9.5
First degree relative with cancer	24	20.7
<i>HPV cofactors</i>		
Smoked at least 100 cigarettes in lifetime	42	36.2
Ever regularly smoked cigarettes	33	28.5
Age at first sexual intercourse		
≤ 17 years	82	70.7
>17 years	30	25.9
Missing	4	3.5
Ever used oral contraceptives		
Yes	87	75.0
No	28	24.1
Missing	1	0.9

Table 2

Cervical HPV and disease status (n=116)

	n	%
HR HPV status		
Positive	60	51.7
Negative	56	48.3
Number of HR HPV infections, among HR HPV- positive women (n=60)		
Single	26	43.3
Multiple	34	56.7
Number of HR HPV types, among women with multiple HR HPV infections (n=34)		
2	14	41.2
3	11	32.4
≥4	9	26.5
Low-risk HPV status		
Positive	12	10.3
Negative	104	89.7
Degree of CIN ¹		
No disease	59	50.9
CIN I (low grade)	12	10.3
CIN II/III (high grade)	36	31.1
Other	4	3.4
Undetermined/Missing	5	4.3

HR, high-risk; CIN, cervical intraepithelial neoplasia

¹Based on review by multiple study pathologists