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Seroprevalence of Human Papillomavirus (HPV) Type 6, 11, 16, 18, by anatomic site of HPV infection, in women aged 16-64 years living in the metropolitan area of San Juan, Puerto Rico

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Abstract

Objectives: It is unknown if human papillomavirus (HPV) serum antibody responses vary by anatomic site of infection. We aimed to assess the seroprevalence for HPV 6, 11, 16 and 18 in association with HPV DNA detection in different anatomic sites among women.

Methods: This cross-sectional population-based study analyzed data from 524 women aged 16–64 years living in the San Juan metropolitan area of Puerto Rico (PR). Questionnaires were used to assess demographic and lifestyle variables, while anogenital and blood samples were collected for HPV analysis. Logistic regression models were used to estimate the adjusted prevalence odds ratio (POR) in order to determine the association between HPV DNA infection status in the cervix and anus and serum antibody status, controlling for different potential confounders.

Results: Overall, 46.9% of women had detectable antibodies to one or more types whereas 8.7% had HPV DNA for one or more of these types detected in cervix (4.0%) or anus (6.5%). Women with cervical HPV detection tended to be more HPV seropositive than women without cervical detection (adjusted POR (95%CI): 2.41 (0.90, 6.47), p=0.078); however the type-specific association between cervical DNA and serum antibodies was only significant for HPV 18 (adjusted POR (95% CI): 5.9 (1.03, 33.98)). No significant association was detected between anal HPV and seropositivity (p>0.10).

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Conclusion: Differences in the anatomic site of infection could influence seroconversion, however, longitudinal studies will be required for further evaluation. This information will be instrumental in advancing knowledge of immune mechanisms involved in anatomic site response.

Spanish Abstract

Se desconoce si la respuesta inmunológica del virus del papiloma humano (VPH) en suero varía por lugar anatómico de infección. El objetivo de este estudio fue evaluar la seroprevalencia de HPV 6, 11, 16 y 18 en asociación con detección de DNA de VPH en diferentes lugares anatómicos en mujeres.

Este estudio poblacional transversal analizó datos de 524 mujeres entre las edades de 16–64 años residentes del área metropolitana de San Juan, Puerto Rico (PR). Se utilizaron cuestionarios para recopilar variables sociodemográficas y de estilos de vida, y se tomaron muestras anogenitales y de sangre para análisis de VPH. Modelos de regresión logística fueron utilizados para estimar la magnitud de la asociación entre la detección de HPV en cérvix y ano, y la seropositividad, controlando por variables potenciales de confusión.

Un 46.9% de las mujeres tuvo anticuerpos detectables para VPH y 8.7% tuvo presencia de VPH en cervix (4.0%) y en ano (6.5%). Las mujeres con detección cervical de VPH mostraron una tendencia a tener mayor posibilidad de seropositivad de VPH en comparación con las mujeres sin detección cervical (POR ajustado (95%CI): 2.41(0.90, 6.47)); mayor fuerza de asociación se observó para HPV 18 (POR ajustado (95% CI): 5.9 (1.03, 33.98)). La asociación específica con VPH 6/11 fue similar, aunque no para VPH 16. La detección anal de VPH no estuvo asociada con seropositividad (p>0.10).

Diferencias en el lugar anatómico de infección podrían influenciar seroconversión, aunque estudios longitudinales son necesarios. Esta información es instrumental para avanzar el conocimiento de los mecanismos inmunes envueltos en la respuesta por lugar anatómico.

Keywords

HPV; Seroprevalence; Women

Introduction

Worldwide, sexually transmitted infections (STIs) have a profound impact and continue to take an enormous toll on health, particularly on women's reproductive health, ranking among the top five disease categories for which adults seek health care (1). Detection of HPV infection at multiple anatomic sites in healthy adults has been reported in several epidemiological studies (2–5), as have with differences in HPV seroprevalence by gender (6,7). The studies that have reported type specific differences in HPV seroconversion by anatomical site of infection are limited (2,3,4). These studies have found higher detection of both HPV DNA and HPV antibodies in women than in heterosexual men, however, the association between detection of type specific DNA and serum antibody was similar across genders (3). Anatomic site-specific immune response has been observed, which suggests that HPV infection at keratinized epithelium is less likely to induce immune responses than infection of mucosal epithelium (3,7). If variations are found in HPV antibody

seroprevalence by status of infection in different anatomic sites, this could imply key differences in the natural course of persistent HPV infection that may be linked to sex and immunological factors. The purpose of this study was to assess HPV seroprevalence in association with HPV DNA detection at different anogenital anatomic sites (cervix and anus), to determine whether HPV serum antibody status varies by anatomic site of infection in women aged 16–64 years living in the San Juan Metropolitan area of Puerto Rico. To our knowledge, this is the first study to examine the likelihood of seropositivity in association with HPV DNA detection at multiple anatomic sites in women. Our results could further our understanding of differences in population seroprevalence related to anatomical site of infection.

Materials and Methods

Study design and population.

This study was approved by the Institutional Review Board (IRB) of the Medical Sciences Campus, University of Puerto Rico. We analyzed data from a population-based study of HPV infection in women in Puerto Rico, conducted between 2010–2013. Study procedures have been described in detail elsewhere (8). Participants in the parent study were identified through a complex sampling design of households and included non-institutionalized, sexually active (i.e. ever had sex) women age 16–64 years, living in the San Juan Metropolitan area of PR. Forty-two women from the 566 in the parent study were excluded [2 who did not complete the study procedures adequately, 28 who had unsatisfactory anal samples, 5 women who did not provide a serum sample and 7 women who had already been vaccinated against HPV (as antibodies present may be a marker of vaccination and not of natural exposure) (9)], resulting in a final sample set of 524 eligible women. Sociodemographic characteristics of participants (n=524) and non-participants (n=42) showed no statistically significant differences; however, a higher proportion of female participants received more than a high school diploma in comparison to non-participants (16% vs 6%) (p=0.08) (data not shown).

Data collection procedures.

A face-to-face interview was used to collect data on demographic and lifestyle variables. Information on sexual and drug use practices was collected through a self-administered questionnaire using an Audio Computer Assisted Self-Interview (ACASI) system implemented with the Questionnaire Development System (QDS) (Nova Research Co., Washington D.C.) and Audio CASI system computer-based interviews. Participants used Dracon swabs to collect cervical and anal samples which were placed in STM vials (DIGENE Corporation, Gaithersburg, MD). A blood sample was collected by venipuncture, and serum fraction isolated. All samples were stored at −20°C until shipment (8).

HPV analyses.

HPV serology (HPV types 6, 11, 16, and 18) was performed at the Centers for Disease Control and Prevention in Atlanta, using multiplex virus-like particle (VLP) based-IgG ELISA (M4ELISA) (10). Cut-off values were set at ≥99%RLU limits of children's sera that fit a Johnson-Su distribution. HPV DNA testing of anogenital samples was performed using

L1 consensus primer PCR with MY09/MY11 primers at the University of California - San Francisco. After thawing, the samples were digested with Proteinase K (PK, Invitrogen) at a final concentration of 250 μg/ml at 56°C overnight. The PK was heat inactivated, and 200 μl was precipitated and suspended in 25 μl Tris-EDTA. Five microliters of sample were used for PCR amplification using the standard 40 cycle protocol (11). Positive PCR products were typed by dot-blot hybridization using type-specific probes. Only HPV 6,11,16 and 18 were considered for this analysis.

Study variables.

The main study variables were anti-HPV 6, 11, 16, 18 serum antibody status (outcome variable) and HPV 6, 11, 16, 18 DNA status in cervix and anus (independent variables). The following variables were evaluated as possible confounders and effect modifiers: age, number of lifetime sexual partners and HPV infection at the other anatomical site (HPV 6,11,16,18 DNA infection cervix+/−; HPV 6,11,16,18 DNA infection anus+/−). Other variables of interest included educational attainment, marital status, health insurance, annual family income, alcohol use, age at first sex, ever had oral sex, ever had anal sex, physical activity, parity and history of STIs.

Statistical Analyses

Descriptive statistics summarized the demographic, sexual practices and lifestyle characteristics of the study group. Geometric mean levels of antibodies, by anatomical site, were compared with the t-test (data not shown). Logistic regression models were used to estimate the adjusted prevalence odds ratio (POR) with 95% confidence intervals in order to determine the association between 1) cervical and 2) anal HPV DNA status and anti-HPV serum antibody, controlling for different demographics, sexual practices, lifestyle characteristics, and HPV infection status at the other anatomical site. Analyses were done for grouped (Any 6, 11, 16, 18) as well as type-specific HPV results. Interaction terms in the models were assessed using the likelihood ratio test.

Results

The mean age of the study participants was 42.4 ±13.2 years. Almost 17.0% of the women had achieved more than a high school education, and 40.4% had an annual family income of $20,000 or less. More than half of the participants reported three or more lifetime sex partners (68.7%) and the median was 4 lifetime sexual partners. More than two-thirds of women indicated having had anal sex (69.3%) and 89.7% reported having had oral sex during their lifetime (Table 1).

The prevalence of antibodies was highest for HPV 6 (27.4%), followed by HPV 16 (22.1%), HPV 11 (16.4%) and HPV 18 (15.6%), with 46.9% seroprevalence for any of these HPV. The prevalence of DNA for any of these types in the cervix and anus was 4.0% and 6.5%, respectively, with a combined prevalence in the anogenital area of 8.7%. HPV 16 DNA was most prevalent in the cervix (2.1%), followed by HPV 18 (1.1%) and HPV 6/11 (1.0%). Whereas HPV 6/11 DNA was most prevalent in the anus (2.7%), followed by HPV 16 (2.1%) and HPV 18 (1.7%) (Table 1). Among women who were HPV DNA positive in the

cervix, 42.8% (9/21) were HPV DNA positive in the anus, while for those HPV DNA negative in the cervix only 5.0% (25/503) were HPV DNA positive in the anus. Women with HPV DNA detected in the anus were more likely than those without to have HPV DNA detected in the cervix (data not shown, Chi-square *p*-value <0.01).

Figure 1 presents HPV seroprevalence by anogenital HPV DNA detection status. The seroprevalence of HPV types evaluated was high (>40%) in women with or without anogenital HPV DNA, but in most cases was higher for HPV DNA positive women. Only the association between cervical HPV 18 detection and HPV 18 serology was statistically significant (p<0.05). The association between any cervical HPV 6, 11, 16, 18 DNA and any HPV serology showed a trend toward statistical significance (*p*=0.07). While not reaching statistical significance, women with cervical HPV DNA detection were more likely to be seropositive than women without cervical detection (adjusted POR (95% CI): 2.4 (0.90, 6.47)). The interaction terms in the multivariate logistic regression model were not significant (*p*-value >0.05). The type-specific association between cervical HPV DNA and antibodies was only significant for HPV 18 (adjusted POR (95% CI): 5.9 (1.03, 33.98)). While results for HPV 6/11 were not statistically significant, the magnitude of the association was strong. No type-specific association was observed for HPV 16. No overall or type-specific association between anal HPV and seropositivity was observed (*p*-value >0.10) (Table 2).

Discussion

To our knowledge, this study is the first to examine the likelihood of HPV seropositivity in relation to concurrent detection of HPV DNA at multiple anatomic sites in women (cervix and anus). We found that women with cervical HPV DNA were more than 2-fold more likely to be HPV seropositive (HPV-6, 11, 16 or 18) than women without cervical HPV. This association was not found for anal HPV, despite the higher prevalence of anal HPV DNA compared with cervical HPV. Recent studies in men (2,4) have demonstrated anatomic differences in the association between HPV DNA detection and seroconversion. However in these studies, seropositivity was higher for those with anal HPV than genital HPV (2,4). Given that previous studies (3,4) also report type-specific differences in the association between genital HPV and seroconversion, future studies should further explore these results.

The explanation for anatomic differences in the association between HPV detection and seropositivity is not clear. Studies have hypothesized that differences between mucosal and keratinized epithelium in lymphatic access may impact the immune response to HPV, with HPV infection in keratinizing epithelium (skin, external genital) eliciting less seroconversion and lower titers than mucosal infections (anal canal, cervix, oral cavity). This may explain the differences between infection of the external genital surface and anus in males, but in women, both the anal canal and cervix are mucosal surfaces and both have a transitional zone, where columnar and squamous epithelium meet, so histologic differences are unlikely to contribute to differences in seroconversion (12). However, the anal squamous mucosa does quickly merge in perianal region with keratinized epithelium. Hernandez et al (2005) proposed that this higher concentration of keratinized cells in the anus could hinder HPV

persistence (13), contributing to differences between the natural history of disease and immune response between cervix and anus.

Our results demonstrate that women residing in the San Juan metropolitan area of PR are highly exposed to these four vaccine-targeted HPV types. In fact, HPV 6,11,16,18 antibodies were present in more than 40% of subjects, irrespective of the presence or absence of current genital infection. This finding highlights the burden of current, as well as of past lifetime exposure to HPV in the study group. In addition, serological data confirmed lifetime exposure to at least one HPV vaccine type in almost half of the study subjects. This estimate is higher than that for women aged 14–59 years (31.8% seropositivity) in the 2003–2006 US National Health and Nutrition Examination Survey (NHANES) (14). Although our small sample size may impact the precision of this estimate. Assay differences and a smaller sample size limiting the precision of the estimate could both contribute to the differences. Direct comparison of the M4ELISA used in the current study has shown higher detection in unvaccinated samples than the competitive luminex assay used in the NHANES survey (10). Public health intervention to vaccinate before sexual debut is needed to have an impact on HPV related morbidities. Information on seroprevalence of specific HPV types in this population can be used for monitoring HPV vaccination strategies in the future, including the inclusion of the new nanovalent HPV vaccine.

Finally, our findings showed an association between cervical HPV 18 detection and HPV 18 serology that was statistically significant ($p<0.05$). The literature suggests that the pattern of HPV-18 seroconversion is more similar to that of HPV-16, than any other HPV type (15). However, our results showed no significant difference between HPV 6/11 and 16 and anogenital HPV DNA infection, as observed in other studies, whose results did show significant associations of HPV 6/11 and 16 antibodies and anogenital HPV DNA infection (3,4). HPV DNA infection does not always induce an immune response that results in HPV-specific antibodies (15–17).

The low detection rates of HPV DNA in cervix and anus limited the power of this study to evaluate associations with HPV serology, particularly for specific HPV types. Further, the cross-sectional data do not give insights into differences in seroconversion rates and anatomic site infection. While our study showed that in women, HPV seropositivity was more strongly associated with cervical than anal HPV and seropositivity, this result needs to be interpreted with caution. Longitudinal studies will give a better understanding of the natural history of HPV infections at various anatomic sites and of the immune response to these natural infections.

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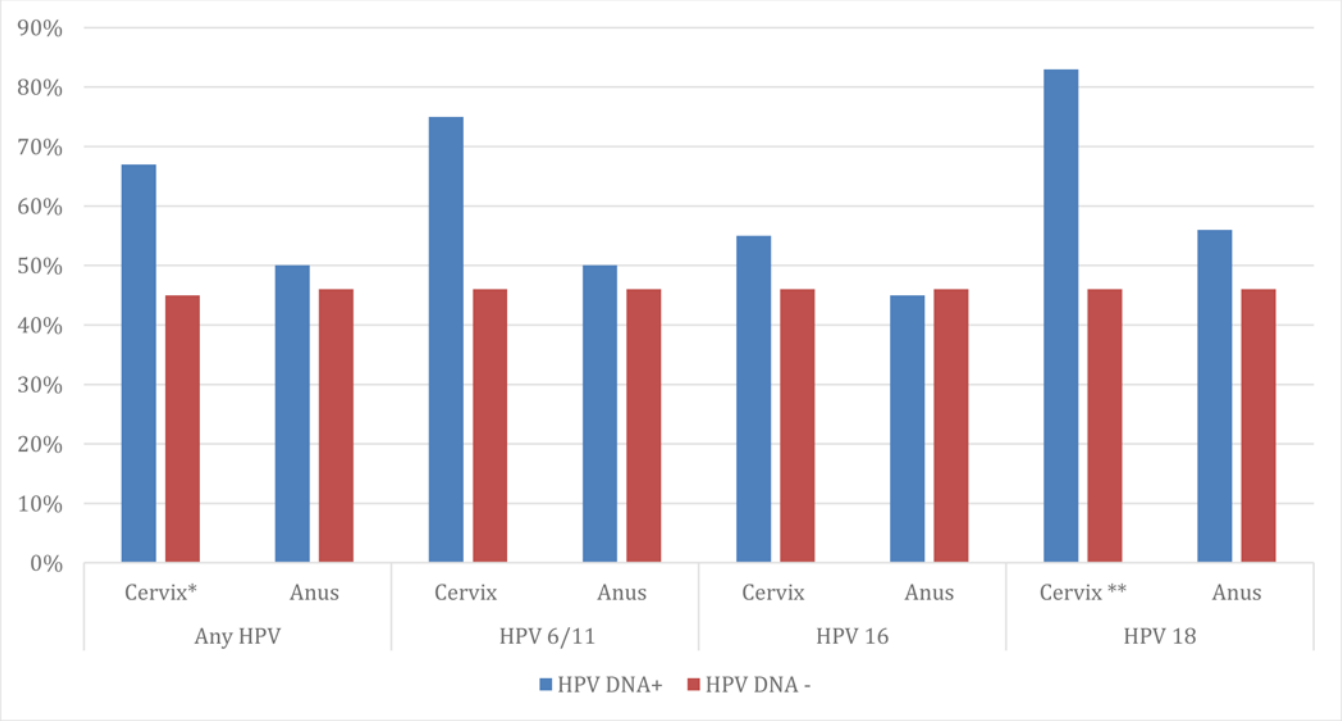

Figure 1.

HPV seroprevalence by anogenital HPV infection status.

*Chi-square p-value: 0.05<p-value <0.1; **Chi-square p-value: p-value<0.05

Table 1.

Demographics, lifestyles and HPV status of the study population (n=524)

Variable	n (%)
Age in years	
≤35	347(66.2)
>35	177(33.8)
Education in years *	
≤high school diploma	434(83.2)
>high school diploma	88(16.8)
Marital status *	
Single	120(22.9)
Married	268(51.5)
Divorced/Separated	134(25.6)
Annual family income *	
≥$20,000	191(40.4)
<$20,000	282(59.6)
Smoking status *	
Never	242(46.4)
Ever	280(53.5)
Alcohol use *	
Never	64(12.3)
Ever	458(87.7)
Physical Activity *	
Yes	100(19.2)
No	422(80.8)
Parity	
0 pregnancies	69(13.2)
1–2 pregnancies	201(38.4)
≥3 pregnancies	254(49.4)
Number of sex partners-Lifetime	
≤partners	164(31.3)
3+ partners	360(68.7)
Median number of sex partners: 4	
Age at first sexual intercourse	
≤ years old	75(14.3)
>years old	449(85.7)
Anal sex (lifetime)	
Never	161(30.7)
Ever	363(69.3)
Oral sex (lifetime)	

Variable	n (%)
Never	54(10.3)
Ever	470(89.7)
HPV Seropositivity	
HPV-6	144 (27.4)
HPV-11	86 (16.4)
HPV-16	116 (22.1)
HPV-18	82 (15.6)
Any of 4 types (6, 11, 16,18)	246 (46.9)
Cervical HPV DNA detection	
HPV-6/11	4 (0.76)
HPV-16	11 (2.10)
HPV-18	6 (1.15)
Any of 4 types (6, 11, 16, 18)	21 (4.0)
Anal HPV DNA detection	
HPV-6/11	14 (2.7)
HPV-16	11 (2.10)
HPV-18	9 (1.7)
Any of 4 types (6, 11, 16, 18)	34 (6.5)
Type-specific Concurrent HPV detection – serum and cervix	
HPV-611	3 (1.7)
HPV-16	3 (2.4)
HPV-18	3 (3.4)
Any of 4 types (6, 11, 16, 18)	14 (5.7)
Type-specific Concurrent HPV detection – serum and anus	
HPV-6/11	5 (2.9)
HPV-16	3 (2.6)
HPV-18	2 (2.4)
Any of 4 types (6, 11, 16, 18)	17 (7.4)

* Variations in number are due to missing values

Table 2.

Magnitude of the association between anogenital HPV DNA and HPV serum antibodies

Model	POR (95% CI)							
	HPV 6, 11, 16 or 18‡		HPV 6 or 11‡		HPV 16‡		HPV 18‡	
	Cervix	Anus	Cervix	Anus	Cervix	Anus	Cervix	Anus
HPV DNA infection in Site (Crude)	2.35 (0.93, 5.93) *	1.17 (0.58, 2.35)	5.71 (0.59, 55.37)	1.04 (0.34,3.16)	1.23 (0.31,4.71)	1.23 (0.32,4.71)	5.18 (1.02,26.18) **	1.45 (0.29,7.10)
+ Adjustment for DNA status of other site	2.45 (0.93,6.45) *	0.95 (0.45, 1.99)	5.81 (0.59, 57.21)	0.92 (0.29, 2.9)	1.15 (0.25,5.23)	1.15 (0.25,5.23)	5.36 (0.96,29.88) *	0.89 (0.15,5.36)
+ Above adjustments + age	2.37 (0.89,6.30) *	1.12 (0.55, 2.27)	5.17 (0.51, 51.62)	0.90 (0.28,2.84)	1.10 (0.24,5.04)	1.12 (0.24,5.11)	5.42 (0.95,30.86) *	0.68 (0.10,4.38)
+ Above adjustments + lifetime sexual partners	2.33 (0.87,6.22) *	1.10 (0.54, 2.23)	5.06 (0.50, 50.63)	0.89 (0.28,2.80)	1.11 (0.24,5.06)	1.12 (0.24, 5.09)	5.02 (0.88,28.60) *	0.68 (0.10,4.29)
+ Above adjustments + smoking	2.41 (0.90,6.47) *	1.10 (0.54, 2.25)	4.88 (048, 49.51)	0.87 (0.27,2.74)	1.08 (0.23, 4.96)	1.15 (0.25, 5.26)	5.93 (1.03,33.98) **	0.66 (0.10,4.30)

* 0.05<p-value <0.1;

** p-value<0.05;

‡ No significant interaction terms in the model (p value >0.05)