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Predictors of Seropositivity to Human Papillomavirus Type 53: One of the Most Prevalent High Risk–Related Cervical Human Papillomaviruses

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Abstract

Persistent cervicovaginal infection with high-risk types of HPV is the major risk factor for subsequent cervical neoplasia. HPV53, part of the α6 species group along with HPV types 30, 56, and 66, is one of the most prevalent high risk–related HPV types, yet little is known about the molecular basis of its benign behavior. We generated and utilized HPV53 virus-like particles (VLPs) to investigate risk factors for its seroprevalence in a population of young college women. Seropositivity to HPV53 VLPs was determined using a polymer-based ELISA to measure IgG reactive antibodies. Cervicovaginal cells were collected for HPV DNA detection and typing by MY09/11 PCR. A questionnaire queried for HPV risk factors to estimate odds ratios (ORs). Prevalence of cervicovaginal HPV DNA was 26% (n = 148); 3% of women (n = 17) had HPV53 DNA and 7% (n = 40) were seropositive to HPV53. Seroprevalence of IgG to HPV53 VLPs in women with cervicovaginal HPV53, HPV53-related types (HPV30, 55, and 66), other HPV types, and no HPV was 41%, 11%, 7%, and 6%, respectively ($p_{trend} < 0.001$). Risk factors independently associated with HPV53 VLP seropositivity included use of oral contraceptive pills (OCPs) (OR: 4; 95% CI: 1.8, 9), having ≥2 regular partners in the last 6 months (OR: 2.5; 95% CI: 1.1, 5.8), having a regular male partner with ≥4 lifetime sex partners (OR: 2.6; 95% CI: 1.1 6), seropositivity to HPV16 (OR: 6.7; 95% CI: 3.1, 14.5), and isolation of HPV53 DNA from cervicovaginal lavage (OR: 17.3; 95% CI: 5.3, 55.9). In conclusion, host serological responses to HPV53 VLPs are strongly type-specific, and subjects' risk for HPV53 seropositivity is independently associated with sexual behavior and OCP use.

Background

HUMAN PAPILLOMAVIRUS (HPV) INFECTION IS THE MOST COMMON SEXUALLY-TRANSMITTED DISEASE (STD), with a reported prevalence of 28–60% among sexually active adolescent women worldwide (6). Classification of papillomaviruses is based on their genome similarity, with over 100 HPV types described to date (5). More than forty types of HPV are responsible for cervicovaginal infections; most of which clear spontaneously within a year (10). Persistent infection with high-risk types of HPV has been identified as the major risk factor for subsequent cervical neoplasia. The host immune response plays an important role in the clearance of cervicovaginal HPV infection, and women with high titers of persistent immunoglobulin G (IgG) antibodies to HPV16 virus-like particles (VLPs) have a significantly lower risk of subsequent infection with HPV16 and HPV16-related types (11). Early age at first intercourse and increasing numbers of lifetime sexual partners have been identified as risk factors for seropositivity to high-risk types of HPV (21,22).

Studies have mainly focused on the determinants of seroprevalence to high-risk types of HPV that are strongly associated with cervical cancer. HPV53 is a high risk–related type (17), and is a member of the α-papillomavirus (α-PV) species group 6, along with HPV types 30, 56, and 66 (19). HPV53 has a high prevalence among sexually-active women worldwide (20,26); it was among the top five most prevalent HPV types in the largest population-based cohort study of HPV to date (9). HPV53 was also the third most common genital type of HPV among women in the United States, as reported in the 2003–2004 National Health and Nutrition Examination Survey (NHANES) (7). Despite its very high prevalence and

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grouping with cancer-associated HPV types, little is known about the molecular basis resulting in HPV53's benign behavior. Moreover, the body of knowledge surrounding differences in host and viral risk factors associated with seropositivity to high-risk and probable high-risk types of HPV is limited. Specifically, the risk factors associated with seroprevalence to HPV53 have not been well studied.

The objective of this study was to generate and utilize HPV53 VLPs to investigate its seroprevalence in a population of young college women in whom HPV53 was exceedingly common. In addition, risk factors associated with humoral response to HPV53 VLPs were determined. We also compared risk factors for HPV53 seropositivity to those of the most oncogenic type, HPV16.

Materials and Methods

Study population

A description of the study participants and eligible nonparticipants has been previously reported (1,10). Institutional Review Boards of the Albert Einstein College of Medicine and Rutgers University approved the study. Briefly, 608 female students at a state university in New Jersey participated in a longitudinal study investigating the natural history of cervicovaginal HPV infection. Complete data on HPV53 VLP IgG was available for 575 women, who form the study group.

Data collection

At the baseline visit, participants filled out a self-administered questionnaire that obtained information on their demographics, sexual history, sex partners' characteristics, recreational drug and alcohol use, smoking history, contraceptive use, and medical history. A Pap smear was obtained at baseline, and exfoliated cervicovaginal cells were collected by lavage for HPV determination and typing for 39 types of HPV by Southern blot hybridization and MY09/MY11 polymerase chain reaction (PCR), as described previously (1,2). Additionally, serum was collected from 10 mL of blood at the baseline visit.

Cloning and production of HPV53 virus-like particles

The production of HPV type 53 VLPs was achieved by cloning and over-expressing the L1 major capsid protein that assembled into VLPs, which were then isolated by physical means. We utilized the baculovirus system for production of VLPs based on the high level of expression of foreign genes recombined into the baculovirus, and the proven efficacy of the system to produce HPV VLPs (15,27).

For generation of HPV53 L1 recombinant baculovirus, the HPV53 L1 open reading frame (nucleotides [nt] 5641–7165) was amplified by PCR using cloned prototype HPV53 DNA as a template (8). Unique restriction sites (underlined) were incorporated into the oligonucleotide primers. The HPV53 L1 primer sequences were as follows: 5′-CTAAGATCTTAAATATGGCGGTGTG-3′ as the forward primer, and 5′-ACAATCTAGACACACAACCTACTAT-3′ as the reverse primer. The L1 gene was cloned as 5′ *Bgl*II-to-3′ *Xba*I fragment into the pFASTBac1 vector (Invitrogen, Grand Island, NY). The pFASTBac1-HPV53 L1 construct DNA was isolated and validated by sequence analysis. The plasmid DNA was then transformed into *E. coli* cells containing the baculovirus shuttle vector (bacmid). A helper plasmid containing the required functions was used to allow site-specific recombination and generation of L1-containing recombinant bacmid DNA (16). Recombinant baculovirus stocks were generated when Sf9 cells (ATCC, #CRL-1711), maintained in Sf-900 III Serum Free Medium (SFM) (Invitrogen) were transfected with recombinant bacmid DNA using cellfectin (Invitrogen).

For VLP production, Sf9 cells were grown as 200-mL suspension cultures at 27°C, using Sf-900 III SFM. Then 200 mL of cells at a density of 2×10^6 to 2.5×10^6/mL were infected with HPV53 L1 recombinant baculovirus at a multiplicity of infection (MOI) of 10. After 72 h, the cells were harvested, centrifuged at $1100 \times g$ for 5 min, and washed with ice-cold phosphate-buffered saline (PBS). The cell pellet was resuspended in 1 volume of PBS and sonicated on ice twice, for 2 min each. The total cell lysate was loaded on six 40% (wt./vol.) sucrose-PBS cushions and centrifuged in a SW-28 rotor at 25,000 rpm ($110{,}000 \times g$) for 2.5 h. Each pellet was resuspended in 2 mL of 27% (wt./wt.) CsCl-PBS by short-pulse sonication, pooled into two quick-seal tubes, and centrifuged to equilibrium in 27% (wt./wt.) CsCl-PBS for 20 h at 28,000 rpm ($141{,}000 \times g$). The visible band (density of 1, 29–1.30 g/mL) was harvested by puncturing the tubes with a 16-gauge needle. Fraction densities were calculated from the refractive index, as determined by an Abbe-3L refractometer (Bausch & Lomb, Rochester, NY). The band was centrifuged again under identical conditions and dialyzed extensively against PBS at 4°C prior to use. Proteins were analyzed in 10% gels by SDS-PAGE, and either stained with 0.25% Coomassie blue, or analyzed by Western blotting with the polyclonal rabbit anti-bovine papillomavirus type 1 (BPV-1) antibody (DAKO, Carpinteria, CA). Formation of HPV53 VLPs was confirmed by electron microscopy.

HPV53 VLP ELISA

Serum samples were tested for IgG antibodies to HPV53 by a modified enzyme-linked immunosorbent assay (ELISA), as described previously (11,23). Seropositivity cutpoints were determined by receiver operating characteristic (ROC) analyses to maximize the specificity among women who were negative for HPV53 DNA. The log absorbance cutpoint of ≥ -0.79 gave a sensitivity of 47% and specificity of 100% for the HPV53 IgG assay. Serum samples were assayed in duplicate, and the median log absorbance was used to determine the subject's serostatus.

Statistical analysis

The association between HPV53 seropositivity and each of the potential categorical risk factors was estimated using odds ratios (ORs). Parsimonious logistic regression models were fit using a $p < 0.05$ as the criterion for inclusion following model-building strategies suggested by Hosmer and Lemeshow (13). Variables that were statistically significant in the model were retained; other variables were excluded unless there was evidence of confounding (≥20% change in the parameter estimate). Interaction was assessed by creating product terms that were separately tested in the multivariable model containing the main effects terms. No significant interactions were found. Models were tested for lack of fit using the Hosmer and Lemeshow goodness-of-fit test statistic and the area under ROC curve.

TABLE 1. RISK FACTORS ASSOCIATED WITH HPV53 SEROPOSITIVITY IN YOUNG WOMEN

Subject's risk factor	*No. of HPV53 VLP-positive samples* n = *40 (%)*	*No. of HPV53 VLP-positive samples* n = *535 (%)*	*Univariate odds ratio (95% CI)*	*Multivariate odds ratio (95% CI)*
Demographic factors				
Age (y)				
≤20	25 (62.5)	436 (81.5)	1	
>20	15 (37.5)	99 (18.5)	2.6 (1.3, 5.2)	N/S
Ethnicity				
White/Asian/other	28 (70)	399 (75)	1	
Hispanic/Black	12 (30)	136 (25)	1.3 (0.6, 2.5)	N/S
Annual famiy income				
<$40,000	20 (50)	191 (36)	1	
≥$40,000	11 (28)	242 (45)	0.4 (0.2, 0.9)	N/S
Sexual behavior factors				
Age at first vaginal sex				
>16 y	16 (40)	323 (60)	1	
≤16 y	24 (60)	212 (40)	2.3 (1.2, 4.4)	N/S
No. of male vaginal sex partners in lifetime				
None or one	5 (12.5)	202 (38)	1	
≥2	35 (87.5)	333 (62)	4.2 (1.6, 11.0)	N/S
No. of male vaginal sex partners in last 6 mo.				
None or one	22 (55)	397 (74)	1	
≥2	18 (45)	138 (26)	2.3 (1.2, 4.5)	N/S
No. of regular partners in last 6 mo.				
None or 1	28 (70)	469 (88)	1	1
≥2	12 (30)	66 (12)	3.0 (1.5, 6.3)	2.5 (1.1, 5.8)
Ever lived with a partner for >6 months				
No	31 (77.5)	498 (93)	1	
Yes	9 (22.5)	36 (7)	4.0 (1.8, 9.1)	N/S
Before age 16 y, had sex with men ≥5 y older				
No	31 (77.5)	489 (91)	1	
Yes	9 (22.5)	46 (9)	3.1 (1.4, 6.9)	N/S
Had non HPV-related STD				
No	26 (65)	470 (88)	1	
Yes	14 (35)	65 (12)	3.9 (1.9, 7.8)	N/S
Ever had a Pap smear				
No	5 (12.5)	217 (41)	1	N/S
Yes	35 (87.5)	317 (59)	4.8 (1.8, 12.4)	
Ever used OCPs				
No	11 (27.5)	324 (61)	1	1
Yes	29 (72.5)	210 (39)	4.1 (2.0, 8.3)	4.0 (1.8, 9.0)
Currently using OCPs				
No	23 (59)	419 (78)	1	
Yes	16 (41)	115 (21.5)	2.5 (1.3, 4.9)	N/S
Ever been pregnant				
No	27 (67.5)	485 (90.6)	1	
Yes	13 (32.5)	50 (9.3)	4.7 (2.3, 9.6)	N/S
Ever had an abortion				
No	28 (70)	488 (91.2)	1	
Yes	12 (30)	47 (8.8)	4.4 (2.1, 9.3)	N/S
Laboratory features				
HPV16 serostatus				
Seronegative	20 (50)	474 (88.5)	1	1
Seropositive	20 (50)	61 (11.4)	7.8 (3.9, 15.2)	6.7 (3.1, 14.5)
HPV DNA positivity				
No	21 (53)	394 (74)	1	
Yes	16 (40)	132 (25)	2.3 (1.1, 4.5)	N/S
HPV53 DNA positivity				
No	30 (75)	509 (95)	1	1
Yes	7 (17.5)	10 (2)	11.9 (4.2, 33.4)	17.3 (5.3, 55.9)
Lifestyle factors				
Frequency of attending religious service				
Up to 5 times/y	29 (72.5)	296 (55)	1	
≥6 times/y	11 (27.5)	238 (44.5)	0.5 (0.2, 0.9)	N/S

Note: This table represents women with complete data for HPV53 VLPs ($n = 575$). Risk factors that were significant on univariate analysis are presented in this table. The percentages may not total 100% due to missing data for some variables. Only variables with $p < 0.05$ were retained in the final multiple logistic regression model.

Abbreviation: N/S, not significant.

Two models are presented. The first model includes all subjects regardless of their sexual experience. This model aimed to identify demographic and behavioral risk factors associated with HPV53 seropositivity. Certain sexual behavior variables had meaningful values only for subjects who had a history of sexual intercourse. Subjects who denied having ever been sexually active were included using a statistical analytical approach previously described (24). The second model includes only those women who had had vaginal sex, and reported at least one regular partner in the last 6 mo. This model examined characteristics of the male partner that were potential risk factors for HPV53 seropositivity in women. In addition to other risk factors, only the subjects' age and ethnicity were entered into the model, rather than age and ethnicity of both subject and partner, due to collinearity.

All statistical analyses were performed using Stata software, version 9.2 (StataCorp, College Station, TX). *P*-values are two-tailed, with an alpha of 0.05 considered statistically significant for all analyses.

Table 2. Association between HPV DNA and HPV53 VLP ELISA IgG Seropositivity in Young Women

Patient status	*No. of HPV53 VLP IgG-positive samples/total number (%)*
HPV DNA negative	21/415
Sexually active	21/343 (6)
No vaginal sex	0/72 (0)
HPV DNA positive[a]	16/141
HPV53	7/17 (41)
HPV53-related types[a]	1/9 (11)
Other types[c]	8/115 (7)

[a] $p_{trend} < 0.001$.
[b] "HPV53-related type" include HPV 30, 55, and 66.
[c] "Other types" include HPV types exclusive of HPV53 and HPV53-related types.
Note: This table represents only women ($n = 556$) who had complete data for both HPV53 DNA and HPV53 VLP IgG tests.

Results

Characteristics of the 575 women who constitute the study cohort are given in Table 1. Subjects had a mean age (± standard deviation) of 20 ± 3 y and came from diverse ethnic backgrounds: 57% white, 13% Hispanic, 12% African-American, 10% Asian, and 8% other ethnicities. Only 13% of subjects denied being sexually active with a male partner at enrollment, and none had HPV DNA detected in their cervicovaginal lavage specimens.

Association between HPV53 VLP IgG seropositivity and HPV53 infection

HPV DNA prevalence at baseline was 26% (n = 148). The prevalence of HPV53 DNA was 3% (n = 17), and that of HPV16 DNA was 4% (n = 24). Forty subjects (7%) had IgG to HPV53, whereas 79 (14%) demonstrated seropositivity to HPV16, and all of them reported being sexually active with male partners. The risk of seropositivity to HPV53 was more than twice as high in women who had any HPV DNA identified in cervicovaginal lavage samples compared to women without HPV DNA (OR: 2.27; 95% CI: 1.15, 4.48). The rates of HPV53 co-infection with high-risk types of HPV in our cohort were low. Of the 17 women with HPV53 DNA isolated on cervicovaginal lavage specimens, two were co-infected with HPV16 and only one was co-infected with HPV33.

The association between HPV DNA detection and HPV53 seropositivity is presented for 556 women who had data for both HPV DNA and HPV53 VLP IgG tests in Table 2. Seroprevalence of HPV53 IgG was highest among subjects with concurrent cervicovaginal HPV53 infection, followed by infection with HPV53-related types (i.e., HPV types 30, 55, and 66), and "other" HPV types (i.e., neither HPV53, nor HPV53-related types) ($p_{trend} < 0.001$) (Table 1). Twenty-one women (5%) were seropositive to HPV53 in the absence of concurrent cervicovaginal infection with any HPV type.

HPV DNA viral load was defined as high (positive Southern blot, with or without a positive PCR) or low (positive PCR with negative Southern blot), in order to evaluate the association between HPV viral load and seropositivity. Among the 17 women with HPV53 DNA isolated from their cervicovaginal specimens, one had a low viral load and demonstrated concurrent HPV53 seropositivity, whereas 6 of the 16 women (38%) with a high viral load were seropositive to HPV53. In contrast, 75% of women with a low HPV16 DNA viral load, and 50% of women with a high viral load demonstrated HPV16 IgG seropositivity.

Risk factors for HPV53 seropositivity among participants

Risk factors associated with HPV53 seropositivity are presented in Table 1. Independent risk factors associated with HPV53 seropositivity were identified by multivariate logistic regression analysis (Table 1). A subject's history of having ever used OCPs, concurrent seropositivity to HPV16, two or more regular sexual partners in the preceding 6 mo, and cervicovaginal detection of HPV53 DNA were independently associated with seropositivity to HPV53. OCP use remained statistically significant even after adjusting for condom use in the multivariate logistical regression model.

Risk factors for HPV53 seropositivity among subjects and their regular male partner

In order to identify the characteristics of male sex partners that were associated with HPV53 seropositivity in women, 422 sexually-active subjects with at least one regular partner in the previous 6 mo were included in this analysis (Table 3). Independent risk factors included a subject's past use of OCPs, concurrent seropositivity to HPV16, isolation of HPV53 DNA from cervicovaginal lavage specimens, and having a male partner with two or more lifetime sexual partners.

Discussion

The prevalence of HPV53 DNA positivity in our subjects was 3%, and HPV53 seropositivity was 7%. This suggests past exposure to HPV53 with clearance of detectable infection in most women (10,18,22). Our results demonstrate that a subject's risk for HPV53 seropositivity was independently associated with sexual behavior and contraceptive use.

Table 3. Risk Factors Associated with HPV53 Seropositivity: Young Women and Their Most Recent Regular Male Partner

Risk factors	*No. of HPV53 VLP-positive samples* n = *40 (%)*	*No. of HPV53 VLP-positive samples* n = *535 (%)*	*Univariate odds ratio (95% CI)*	*Multivariate odds ratio (95% CI)*
Subject age:				
≤20 y	23 (66)	313 (81)	1	
>20 y	12 (34)	74 (19)	2.2 (1.0, 4.6)	N/S
Subject: ethnicity				
White/Asian/other	25 (71)	280 (72)	1	
Hispanic or Black	10 (29)	107 (28)	1.0 (0.5, 2.2)	N/S
Subject: lifetime number of vaginal sex partners				
≤3 partners	10 (29)	254 (66)	1	
>3 partners	25 (71)	133 (34)	4.8 (2.2, 10.2)	N/S
Regular partner in school				
No	14 (40)	97 (25)	1	
Yes	20 (57)	286 (74)	0.5 (0.2, 0.9)	N/S
Male partner's lifetime number of sex partners				
1–3	15 (43)	277 (71.5)	1	
≥4	17 (49)	103 (27)	3.0 (1.5, 6.3)	2.6 (1.1, 6)
Subject: ever used OCPs				
No	9 (26)	214 (55)	1	
Yes	26 (74)	173 (45)	3.6 (1.6, 7.8)	3.5 (1.4, 8.7)
Subject: number of regular partners in last 6 mo				
1	23 (66)	325 (84)	1	
≥2	12 (34)	62 (16)	2.7 (1.3, 5.8)	N/S
Subject: before age 16 had sex with men ≥5 years older				
No	26 (74)	352 (91)	1	
Yes	9 (26)	35 (9)	3.5 (1.5, 8)	N/S
Subject: ever pregnant				
No	23 (66)	342 (88)	1	
Yes	12 (34)	45 (12)	3.9 (1.8, 8.5)	
Subject: attending religious service				
Up to 5 times/y	25 (71)	212 (55)	1	
More than 5 times/y	10 (29)	175 (45)	0.5 (0.2, 1.0)	N/S
Subject: HPV16 serostatus				
Seronegative	17 (49)	342 (88)	1	
Seropositive	18 (51)	45 (12)	8.0 (3.9, 16.7)	5.8 (2.4, 13.8)
Subject: HPV DNA positivity				
No	18 (51)	266 (69)	1	
Yes	15 (43)	115 (30)	1.9 (0.9, 3.9)	N/S
Subject: HPV53 DNA positivity				
No	27 (77)	367 (95)	1	1
Yes	6 (17)	8 (2)	10.2 (3.3, 31.5)	15.1 (4.1, 55.2)

Note: This table represents women who were sexually active and had at least one regular male sexual partner in the preceding 6 mo (n = 422). Risk factors that were significant on univariate analysis are presented in this table. The percentages may not total 100% due to missing data for some variables. Only variables with $p < 0.05$ were retained in the final multiple logistic regression model.

Abbreviation: N/S, not significant.

The prevalence of seropositivity to HPV53 was 41% in women with concurrent HPV53 cervicovaginal infection, compared to 6–7% in women without HPV infection or those with isolation of non HPV53-related DNA (Table 2). Serologic prevalence of HPV16 IgG antibody was 55% in women with HPV16 DNA compared to 12% in women without HPV16 DNA (14), which is higher than that observed for HPV53 in our study. However, unlike HPV16, for which infected women with high viral loads demonstrate robust IgG seropositivity, only 38% of infected women with a high HPV53 viral load were seropositive in our cohort. This suggests that common non-oncogenic types of HPV may have different immunogenicity compared to the very common and most high risk HPV16.

An association between OCP use and HPV seropositivity (12,28) may reflect sexual behavior, including increased fre-

quency of unprotected sexual intercourse. However, the association between lifetime use of OCPs and HPV53 seropositivity remained significant despite adjusting for markers of sexual activity such as condom use and the number of regular partners in the last 6 mo. This suggests a potential biological effect of OCP use, as opposed to it being a surrogate marker for sexual frequency. Glucocorticoid response elements (GREs) have been demonstrated on the HPV genome, which increase viral transcription and activation of the oncogenes E6 and E7 in high-risk types of HPV (4). This increased viral transcription has been demonstrated *in vitro* for HPV types 11, 16, and 18 (3), and may stimulate an increased humoral immune response to HPV infection. This biological mechanism suggests that humoral responses to HPV vaccine in OCP users and non-users should be similar.

The immune response to HPV53 was predominantly type-specific, with some cross-reactivity to HPV53-related types. The development of a cross-protective immune response to high-risk types of HPV is mediated by the presence of type-specific and common epitopes (25). Our results suggest a similar mechanism for the development of cross-reactive antibody responses to HPV53. An association between a subject's lifetime number of sex partners and HPV16 seropositivity has been consistently reported. In contrast to HPV16, however, the numbers of sex partners in the last 6 mo, but not lifetime, showed an independent association with HPV53 seropositivity. This suggests that serological response to HPV53 infection may develop quickly, and be more transient than host responses to HPV16 infection. However, the number of HPV53-seropositive women in our study was small, and additional data are required to test this notion.

Seropositivity to HPV16 was a significant independent predictor of HPV53 seropositivity. In a multivariate regression model lacking the variable for HPV16 seropositivity, older male sexual partners were independently associated with HPV53 seropositivity in the presence of other statistically significant variables. When the HPV16 seropositivity variable was added, partner age was no longer significant. This suggests that sex with older men may represent a key factor in transmission of HPV to women.

This study has limitations. Since the women were young, their patterns of sexual behavior and OCP use may not represent those seen in older women. In addition, since subjects provided information regarding their male sexual partners, the validity of reported information may be biased by the subjects' assumptions regarding their partners. Another limitation is the small numbers of women with HPV53 DNA or HPV53 VLP IgG in our study, which may have the potential to lead to type II error. Despite these small numbers, however, we were able to fit multivariate parsimonious logistical regression models identifying risk factors associated with HPV53 VLP IgG seropositivity. It is possible that the small numbers of observations in our study led us to type I error; however, this is the largest study evaluating the risk factors specific to HPV53 in the literature to date.

Conclusion

In conclusion, anti-HPV53 VLP antibodies are strongly type-specific. A subject's lifetime use of OCPs, HPV16 seropositivity, number of sex partners of the subject or her male partner, and detection of HPV53 DNA from cervical specimens were independent predictors of HPV53 seropositivity in sexually-active women. Older male partners having sex with younger women may represent a key reservoir for HPV, resulting in infection and seropositivity in their female sex partners.

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