DETERMINANTS OF HIGH-RISK HPV SEROPREVALENCE AND DNA PREVALENCE IN MID-ADULT WOMEN

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Abstract

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Objective: To estimate high-risk (hr) HPV seroprevalence and DNA prevalence then identify determinants of seropositivity and DNA-positivity in mid-adult women.

Methods: We conducted a cross-sectional analysis of 378 females at the University of Washington who were between 30 and 50 years of age and self-reported no history of prophylactic HPV vaccination. Vaginal samples were tested for HPV genomic DNA using polymerase chain reaction for type-specific detection of HPV-16/18/31/33/35/39/45/51/52/56/58/59/68. Blood samples were tested for type-specific antibodies to the same HPV types using a Luminex-based assay. Health and sexual history were obtained via self-reported questionnaires. Risk factors for seropositivity to hr HPV, and hr HPV DNA-positivity were assessed using Poisson regression models to obtain prevalence ratios (PRs).

Results: The mean (SD) age of participants was 38.7 (6.1) years, and the median lifetime number of male sex partners was 7. Over two-thirds (68.0%) of participants were seropositive for any of the 13 high-risk HPV types, and 14.8% were DNA positive for any hr HPV type. In multivariable analyses, married women or those living with a partner were less likely to be seropositive compared to single or separated women (adjusted prevalence ratio [aPR] = 0.84, 95% CI: 0.74-0.96). In addition, compared to women who had never used hormonal contraceptives, those who were current (aPR = 1.54, 95% CI: 1.03-2.32) or former users (aPR = 1.66, 95% CI: 1.12-2.48) were more likely to be seropositive. Women with lifetime numbers of sex partners equal to or more than 15 were borderline statistically significantly more likely to be seropositive compared to those with 0-2 partners (aPR=1.22, 95%CI: 0.98-1.53). Similar associations

were seen with DNA positivity. In addition, there was a strong association between smoking status and DNA positivity to any hr HPV type with increased likelihood in women who were current smokers compared to women who had never smoked (aPR =2.30 95% CI: 1.26-4.22).

Conclusion: Our results suggest that the majority of mid-adult women had evidence of current or prior hr HPV infection. Measures of probable increased exposure to HPV infection (single or separated marital status, increased lifetime number of sex partners and hormonal contraceptive use) were associated with both seropositivity and DNA positivity to hr HPV whereas being a current smoker was positively associated with hr HPV DNA positivity only.

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BACKGROUND

Human papilloma virus (HPV) is a common sexually transmitted infection (STI) worldwide[1] and the most commonly transmitted STI in the United States.[2] In 90% of infected women, HPV infection clears spontaneously within 12-18 months, however in some instances the infection persists.[3,4] Persistent HPV infection with high-risk (hr) HPV types can lead to cervical cancer in women.[5] Nearly all cervical cancers are due to HPV infections[6] and 70% of all cervical cancer cases are attributable to HPV 16 or HPV18 infections. [7] If administered before HPV is contracted, two HPV vaccines have proven efficacious against HPV16 and HPV18 infections as well as precancerous lesions associated with both HPV types.[8,9] A nine-valent HPV vaccine, currently under FDA review, which encompasses 7 hr HPV types (hr HPV 16,18,31,33,45,52,58 in addition to low-risk HPV 6 and 11), would elevate the coverage against HPV types associated with invasive cervical cancer to 90%.[10]

Extensive work has documented the natural history of HPV infection in adolescent girls and young women. HPV infections peak soon after sexual debut during adolescence and in the early twenties[11]. This knowledge has paved the way to facilitate the strategy for vaccination in this age group. However, only a handful of similar studies have focused on mid-adult women.[2,12–14]

Not much is known about the role of antibody response in relationship to HPV re-acquisition or reactivation that has been speculated to occur in mid-adult women[15]. The median time to seroconversion after HPV 16 infection is 8 months[16], yet not all women will seroconvert, with only less than 70% of all infected women mounting a detectable antibody response.[16,17] In addition, it has been shown that with aging there is immunosenescence as well as antibody waning that can preclude antibody detection in older women.[10,16,17]

Even though a certain proportion of mid-adult women could benefit from HPV vaccines, there is limited comprehensive epidemiologic data to evaluate HPV infection among women in this age group, and hence not enough information available to make recommendations for prophylactic HPV vaccine for these women. To better assess present infection and past cumulative exposure to HPV among mid-adult women, we have described both HPV DNA prevalence and HPV seroprevalence baseline data from a cohort of 409 women aged 30-50 years, all students, faculty or staff affiliated with the University of Washington. In addition, we have determined risk factors associated with: seropositivity to hr HPV, DNA-positivity to hr

HPV and seropositivity to multiple hr HPV vs single hr HPV. Our study is more comprehensive than a previous report for a similar age group in a USA population[2] in that we have analyzed 13 different hr HPV types including those evaluated in the nine-valent vaccine under FDA review. Epidemiological studies such as the one we carried out will contribute to understanding HPV natural history among mid-adult women.

METHODS

Study subjects

A cross-sectional study was conducted using baseline data from a cohort of healthy mid-adult women aged 30-50 years old, all staff, students or faculty affiliated with the University of Washington (UW). To recruit participants, flyers, advertisement and letters were distributed to potential female participants at the UW. Interested women were screened over the telephone to determine eligibility. Subjects who met eligibility criteria were invited to enroll for the study at the UW Hall Health Primary Care Center (UWHHPCC) from March 2011 to January 2012. Women who were currently pregnant, had undergone hysterectomy, or had any serious medical conditions that would preclude participation were not eligible for the study. The study protocol was reviewed and approved by the UW Institutional Review Board, and all subjects consented to the protocol onsite during the enrollment visit.

Data collection and processing

Baseline study procedures took place at the UWHHPCC. At enrollment, the study coordinator administered a face-to-face interview to all participants about their history of HPV screening and vaccination, then each subject self-collected a vaginal sample for HPV DNA testing, and a nurse obtained the subject's blood for HPV serology. Lastly, each participant self-administered an online survey pertaining to their socio-demographic, health, contraceptive use, smoking habits and sexual behaviors. The study coordinator verbally instructed all participants about vaginal sample self-collection and gave each subject written illustrated instructions about the procedure. Participants were given a self-collection kit that contained two sterile Dacron swabs, a covered tube containing 1.5mL of specimen transport medium (STM), a plastic cup and nitrile gloves. Samples were collected in a clinic restroom, then handed to the study coordinator.[18]

Genomic DNA was extracted from participants' self-collected vaginal specimens and used for HPV genotyping. Briefly, vaginal samples were digested with 20 µg/mL protease K at 37°C for one hour, and

DNA isolated from 200 µL of the digested sample using QIAmp DNA blood mini column according to the manufacturer's protocol (Qiagen, Cat. No.51104, Valencia, CA). DNA samples were then directly genotyped by polymerase chain reaction (PCR) for detection of 37 alpha genus types using the Roche Linear Array HPV genotyping test as directed by the manufacturer (Roche Molecular Systems, Inc., Alameda, CA). For our analysis, we have considered 13 high-risk (hr) HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) for which serology data were also available.[19]

Serum was obtained from each subject's blood and HPV antibody testing was done using a Luminex Enzyme-linked Immunosorbent assay (ELISA) of L1 Viral-like particle to detect 13 distinct antibodies to high-risk HPV types (16/18/31/33/35/39/45/51/52/56/58/59/68). Briefly, each HPV L1 or polyomavirus VP1 protein was fused at its N-terminus to GST and fused to its C-terminus to an 11 amino acid epitope tag, expressed and bound to microspheres (beads) containing a unique combination of fluorescent dyes purchased from MiraiBio . These beads were covalently coupled with glutathione-linked casein, and each protein preparation was bound to a different bead set. Human sera were diluted 1:50 in blocking buffer in 96 well polypropylene plates. After blocking for 1hr at room temperature with shaking, 50 µl of diluted sera was mixed with an equal volume of bead mixture containing L1 protein for all HPV types tested. Sera were incubated for 1 to 20 hours at room temperature with shaking. After washing, antibodies were detected by reaction with biotinylated anti-human IgG and streptavadin phychoerythrin. Appropriate positive and negative controls were included, all procedures took place in a 96-well filter plate. Plates were read on a luminex BioPlex 200 instrument following the manufacturer's protocol (BioRad Laboratories).

Data Analysis

Subjects without serology results and those who self-reported a history of prophylactic HPV vaccination were excluded from all analyses. To examine socio-demographic and health and behavioral risk factors associated with 1) seroprevalence and 2) DNA prevalence to any of the 13 hr HPV types tested (16/18/31/33/35/39/45/51/52/56/58/59/68), we determined unadjusted prevalence ratios (PRs) and 95% confidence intervals (CI) using Poisson regression for each of the following possible determinants: race, age, marital status, number of pregnancies, cigarette usage, hormonal contraception use, lifetime number of male sex partners, age at sexual debut, male sex partners within the past 6 months, history of STDs, history of an abnormal Pap test, and history of genital warts. Risk factors that were statistically significant

($p<0.10$) in univariate analyses were entered into final multivariate models, and Poisson regression was carried out to estimate the adjusted PRs and 95% CIs for the associations sought.

In addition, all risk factors were separately evaluated to determine if they were associated with seropositivity to multiple hr HPV types vs a single hr HPV type among all seropositive participants. Risk factors that were statistically significant ($p<0.10$) in univariate analyses were entered into a final multivariate model, and Poisson regression was carried out to calculate adjusted PRs and 95% CIs for the associations.

All analyses were performed using STATA 13.0 software.

RESULTS

409 women enrolled in the study. 30 subjects who self-reported a history of prophylactic HPV vaccination, and one subject without a serology results due to an insufficient sample were excluded, leaving 378 subjects for our analyses. Demographic, health and sexual behavior characteristics are described in Table 1. Briefly, the majority of subjects were white (77.5%), married or living with a partner (61.0%) and had never smoked cigarettes (73.3%). 60.8% of subjects reported they had previously been pregnant, 41.7% reported a history of an abnormal Pap smear, and 27.3% reported a history of STD. The study participants' mean age was 38.7 (SD 6.1) years and their median lifetime number of male sex partners was 7 (interquartile range 3-15).

Overall, 68.0% of all women were seropositive for any of the 13 high-risk (hr) HPV types tested whereas 14.8% were DNA positive to any hr HPV type, and 70.6% were positive by either serology or PCR. We also observed that 45.2% of all subjects tested positive for antibodies to multiple hr HPV types, while 4.5% were DNA positive to multiple hr HPV types, and 46.6 % were either DNA positive or seropositive for multiple hr HPV types. When evaluating seroprevalence and DNA prevalence for hr HPV vaccine types, we found that 50.5% of women were seropositive or DNA positive to any of the 7 hr HPV types included in the nine-valent vaccine (HPV 16, 18, 31, 33, 45, 52, 58),. Moreover, 31.5% were seropositive or DNA positive to HPV 16 or HPV 18. We also observed that 9.5% of all participants were seropositive for both HPV16 and HPV 18 (none were DNA positive for both HPV16 and HPV 18). The most commonly detected HPV serotypes included HPV 59 (26.2%), HPV 31 (25.9%), HPV 51 (25.7), and HPV 16 (25.1%) whereas HPV 16 (3.4%) and HPV51 (3.4%) were the most commonly detected types by PCR. (Table 2)

On the infection-level, a total of 903 type-specific infections were detected by either serology or PCR. These included 825 type-specific infections detected by serology only, 46 detected by PCR only, and 32 jointly detected by both assays. Whereas only 3.7% (32 of 857) of type-specific seropositives were jointly DNA positive, 41.0% (32 of 78) of type-specific DNA positives were jointly seropositive.

Seroprevalence to any of the 13 hr HPV types was similar across age groups, whereas DNA prevalence differed across age groups with the lowest prevalence in the 45-50 year age group (7.8%) and the highest prevalence in the 30-34 year age group (13.5%) (Although the difference was not statistically significant) (Table 3).

In multivariable analyses, marital status, hormonal contraceptive use and lifetime number of sex partners were each associated with likelihood of hr HPV seropositivity. Married women or those living with a partner were less likely to be seropositive compared to single or separated women (adjusted prevalence ratio [aPR]=0.84, 95% CI: 0.74-0.96). In addition, compared to women who had never used hormonal contraceptives, those who were current (aPR =1.54, 95% CI: 1.03-2.32) or former (aPR =1.66, 95% CI: 1.12-2.48) users were more likely to be seropositive. Women with lifetime numbers of male sex partners equal to or more than 15 were borderline statistically significantly more likely to be seropositive compared to those with 0-2 partners (aPR=1.22, 95%CI: 0.98-1.53).

Moreover, we evaluated risk factors associated with seropositivity to multiple hr HPV types vs a single hr HPV type. Multivariable analysis revealed that compared to women who had never been pregnant, those who reported more than 4 pregnancies had an increased likelihood of multiple hr HPV vs single hr HPV seropositivity (aPR =1.28, 95% CI: 1.01-1.62). Likewise, women who reported a lifetime number of male sex partners of 7-14 (aPR =1.54, 95% CI: 1.02-2.32), or more than 15 (aPR =1.57, 95% CI: 1.03-2.39) were more likely to be multiple hr HPV seropositive compared to women who reported 0-2 sex partners (Table 3).

Multivariable analyses showed that there was an increased likelihood of DNA-positivity to any hr HPV type in women who were current smokers compared to women who had never smoked (aPR =2.30, 95% CI: 1.26-4.22). There was also a borderline statistically significantly increased likelihood of DNA-positivity to any hr HPV in women who had a previous abnormal Pap smear (aPR: 1.65, 95% CI: 0.92-2.98) compared to women who had never had an abnormal Pap smear. Women who had previously been

pregnant 1-3 times were borderline statistically significantly less likely to be DNA positive to any hr HPV type compared to women who had never been pregnant (aPR =0.60, 95% CI: 0.34-1.06). Women who were married or living with a partner were less likely to be DNA-positive to any hr HPV type when compared to single or separated women (aPR =0.47, 95% CI: 0.29-0.77). Moreover, compare to women with 0-2 partners, those with 7-14 partners had an increased likelihood of DNA positivity to hr HPV (aPR: 3.11, 95% CI: 0.90-1073). Similarly, women with equal to or more than 15 lifetime numbers of male sex partners were also more likely to be DNA positive compared to those with 0-2 partners (aPR=3.34, 95%CI: 0.97-11.49) (Table 3).

DISCUSSION

In order to assess past cumulative and present HPV infection, we have described seroprevalence and DNA prevalence within the same population. There is paucity of such comprehensive epidemiological data to evaluate HPV infection among mid-adult women as only a handful of studies have focused on both seroprevalence and DNA prevalence of HPV in women within this age group.[2,12,14,20,21] We have determined risk factors associated with either seroprevalence or DNA prevalence to any of 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) among 30-50 year old women. Compared to the few others, our study is unique in that we have assessed a more comprehensive number of hr HPV types, including hr HPV types in the nine-valent HPV vaccine currently under FDA review. In our study population, the majority of women (68.0%) were seropositive for at least one of the hr HPV types whereas 45.2% where seropositive for more than one hr HPV tested, and 48.2% were seropositive to all 7 hr HPV types included in the nine-valent vaccine. 29.6% of all subjects tested positive to antibody against either HPV 16 or HPV 18, whereas 9.5% were seropositive for both vaccine types HPV16 and HPV 18. The proportion of women seropositive for HPV16 or HPV 18 was higher than the proportion observed (18.5%) in a clinic-based study among Korean women of similar ages.[14] This difference with the Korean group might reflect differences in populations as well as methods used for testing antibody. Kim et al had used a competitive assay with monoclonal antibodies whereas we had tested L1 using beads.

The most commonly detected HPV serotypes included HPV 59, HPV 31, HPV 51, and HPV 16. HPV 59 and HPV 31 were among the most prevalent serotypes, however they were not detected among

the most prevalent DNA types, suggesting that these types possibly cleared faster than HPV 16 and HPV 51, two of the highly seroprevalent and DNA prevalent HPV types in our study population.

Antibody prevalence was higher than DNA prevalence for all HPV types tested, indicating that the women in our study showed more markers of past cumulative HPV exposure than evidence of current HPV infections. These serology results might slightly underestimate the true proportion of women ever seropositive to any of hr HPV tested, as detectable antibody might have waned in some of the participants in these middle-age groups. It is also possible that serology results reflected HPV infection in non-genital sites that were not accounted for in the vaginal DNA testing.

Consistent with previous HPV DNA studies conducted in North America,[22] HPV 16 (3.4%) and HPV51 (3.4%) were the most prevalent infections of the 13 HPV types tested among our study subjects. 14.8% of our participants harbored DNA to any hr HPV type tested, comparable to previously observed prevalences ranging from 11-13% in similar age groups within the general US population.[23]

Even though age did not appear to be significantly associated in a final multivariate model, DNA prevalence to hr HPV types declined with age within our study population, a finding similar to that reported by other studies within the USA population.[2,11,22]

We evaluated risk factors associated with either seropositivity or DNA positivity to hr HPV among mid-adult women. Our data demonstrated that seropositivity to hr HPV types was associated with common markers of increased sexual activity, hence high exposure to HPV, such as being single or separated, increased lifetime number of sex partners and usage of hormonal contraception. These same risk factors have previously been reported as determinants of seropositivity to hr HPV by others.[24,25] Married women or those living with a partner were less likely to be seropositive to hr HPV compared to single or separated women, suggesting that stable relationships are associated with reduced past cumulative exposure to HPV consistent with other reports. Likewise, women with lifetime numbers of male sex partners equal or more than 15 were more likely to be seropositive compare to those with 0-2 lifetime number of sex partners, which corroborated with previous findings.[26] Additionally in our study, similarly to what has been reported elsewhere,[27] associations with seropositivity were significant with cumulative lifetime exposure to HPV as opposed to recent infections as demonstrated by associations with risk factors such as lifetime number of sex partners instead of recent sexual activity with new partner.

Our findings that among our study participants, past and current usage of hormonal contraceptive increased the likelihood of seropositivity to hr HPV is consistent with previous reports that found similar association with seropositivity to HPV 16[26,28] and to HPV 18.[26] Even after we adjusted for sexual behavior, there was still a significant association with seropositivity, an indication that this association was not merely due to sexual behavior. Moreover, studies by Safaeian describe hormonal contraception among factors that impact production of immunoglobulin in the cervix,[29] suggesting a plausible biological mechanism of hormonal contraception specific to the immune response against HPV. It is possible that hormonal contraception might modulate the production of antibody within the cervix thereby altering immune response locally. Our study did not adjust for non-condom use, which could be a confounder in the association of hormonal contraception usage and seropositivity to hr HPV. Seropositivity to multiple hr HPV as opposed to a single hr HPV was positively associated with increased parity, and having a lifetime number of sex partners greater than two, suggesting that infection with multiple hr HPV reflects increased cumulative past exposure to HPV.

Even though it was not statistically significant, the magnitude of association of hormonal contraception with DNA-positivity to hr HPV was similar to that of seropositivity against HPV. However, it is noteworthy that previous reports on the association between hormonal contraception and DNA positivity to hr HPV have been inconsistent.[24,30,31] There were some clear differences in determinants associated with seropositivity compared to those associated with DNA-positivity to hr HPV. The association between DNA positivity to any hr HPV type and indicators of sexual activity was stronger than that with seropositivity, possibly reflecting that present sexual activity translated to present new infections and that some subjects had not yet mounted an immune response.

In addition, DNA-positivity to any hr HPV type in mid-adult women was strongly associated with smoking status where current smokers were at a greater risk than non-smokers. It is known that byproducts of cigarettes cause damage to hosts' DNA,[32] and a similar mechanism might occur in the cervix thereby enhancing HPV DNA integration into the host genome. It has also been reported that smoking suppresses immunity, specifically hampering suppressor T lymphocytes that fight viruses.[33] The aforementioned mechanisms are plausible explanation of why current smokers have increased likelihood of HPV infection.

However, smoking history as a determinant of DNA positivity has not been unanimous across studies as some have reported it as a risk factor[24] while others have not found it as such.[30]

There were some limitations to our study. We conducted a cross-sectional study, which does not capture the full spectrum of the participants' HPV exposure status overtime. Additionally, not all women who were HPV infected will seroconvert,[16,17] and hence HPV antibodies will not get detected in their serum and therefore were not accounted for into our study. Likewise, some women may have been infected, but mounted a weak antibody response below detectable levels by ELISA. Likewise, in some women antibody levels could have waned over time and hence were no longer detected by the time of enrollment into our study. Moreover, our study did not have power to evaluate risk factors associated with seropositivity to hr HPV when stratified by DNA infection, results from which could inform about determinants of immune response during HPV infection among mid-adult women. Another limitation to our study is that it took place in a university setting, and hence participants were highly educated women, more than 80% of whom had a college education, who might not reflect the general population. In this study, sexual, behavior and health characteristics were self-reported by participants who might not have disclosed their history accurately, possibly introducing bias into our risk factor evaluations. In addition, we did not have enough power to evaluate DNA positivity to multiple hr HPV types vs a single hr HPV type.

In conclusion, our results indicate that the majority (70.6%) of mid-adult women in our study had either previously been exposed to or were currently infected with hr HPV. Even though a high proportion of study participants was shown to currently be infected or showed markers of prior infections with hr HPV vaccine types HPV 16 and/or HPV18 (31.6%), our data suggest that there still remains a large proportion of middle-age women that could be naïve for both of these hr HPV vaccine types and hence could potentially benefit from the HPV vaccine. Additional studies, with a higher number of participants for power, are needed to examine risk factors associated with seropositivity to hr HPV when stratified by DNA infection.

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Table 1: Demographic, Health and Sexual Behavior Characteristics of 30-50 year old Women in Seattle, Washington tested for HPV DNA and antibody to HPV (N=378).

Variable	N=378	(%)
Race		
White	293	(77.5)
Asian	42	(11.1)
Other[a]	43	(11.4)
Age (years)		
30-34	139	(36.8)
35-39	90	(23.5)
40-44	73	(19.3)
45-50	77	(20.4)
Education		
High School Diploma or GED	2	(0.5)
Some college credit but no college degree	24	(6.4)
Technical School	11	(2.9)
Associate's degree	27	(7.1)
Bachelor's degree	136	(36.0)
Master's or Doctoral degree	178	(47.1)
Marital Status		
Single or Separated	147	(38.8)
Married/Living with partner	230	(61.0)
Missing	1	(0.2)
Cigarette Usage		
Never smoked	277	(73.3)
Former smoker	81	(21.7)
Currently smoking	19	(5.0)
Missing	1	(0.0)
Number of Pregnancies		
0	148	(39.2)
3	184	(48.6)
≥ 4	46	(12.2)
Usage of hormonal birth control		
Former	211	(55.9)
Currently	127	(33.5)
Never	40	(10.6)
Age at sexual debut (years)		
≤15	65	(17.2)
16-19	178	(47.0)
≥ 20	119	(31.8)
Missing	16	(4.0)

Lifetime number of male sex partners[b]		
0-2	80	(21.2)
6	96	(25.4)
7-14	97	(25.7)
≥ 15	101	(26.6)
Missing	4	(1.1)
Male sex partners in past 6 months		
No partners	82	(21.7)
Non new partners only	206	(54.5)
≥1 new partner	84	(22.2)
Missing	6	(1.6)
History of STD[c]		
Yes	103	(27.3)
No	273	(72.2)
Missing	2	(0.5)
History of abnormal Pap test		
Yes	158	(41.7)
No	220	(58.3)
Ever had genital warts		
Yes	42	(11.1)
No	336	(88.9)

[a]Other race included: African American , American Indian/Alaska Native, Native Hawaiian/other Pacific Islander, women reporting more than one race and women reporting “other” as race.

[b]This category represents approximate quartiles, Interquartile range (25^{th}-75^{th} percentile) 3-15

[c]STD included: genital warts, chlamydia, herpes, gonorrhea, HIV, and syphilis

Table 2: Seroprevalence and DNA Prevalence by HPV type for 378 Subjects.

	Antibody positive		DNA positive		Antibody or DNA positive	
	n	(%)	n	(%)	n	(%)
HPV-16	95	(25.1)	13	(3.4)	103	(27.2)
HPV-18	53	(14.0)	4	(1.1)	55	(14.6)
HPV-31	98	(25.9)	5	(1.3)	100	(26.5)
HPV-33	43	(11.4)	1	(0.3)	44	(11.6)
HPV-35	52	(13.8)	2	(0.5)	54	(14.3)
HPV-39	78	(20.6)	7	(1.9)	83	(22.0)
HPV-45	33	(8.7)	3	(0.8)	34	(9.0)
HPV-51	97	(25.7)	13	(3.4)	103	(27.2)
HPV-52	23	(6.1)	8	(2.1)	31	(8.2)
HPV-56	82	(21.7)	8	(2.1)	86	(22.8)
HPV-58	67	(17.7)	6	(1.6)	71	(18.8)
HPV-59	99	(26.2)	5	(1.3)	100	(26.5)
HPV-68	37	(9.8)	3	(0.8)	39	(10.3)
Multiple High Risk HPV	171	(45.2)	17	(4.5)	176	(46.6)
Any High Risk HPV	257	(68.0)	56	(14.8)	267	(70.6)
7 hr HPV*	182	(48.2)	38	(38)	191	(50.5)
HPV 16 or HPV18	112	(29.6)	17	(4.5)	120	(31.7)
HPV 16 and HPV18	36	(9.5)	0	(0.0)	38	(10.1)

* HPV 16/18/31/33/45/ 52/58

Table 3: Analysis of risk factors associated with seropositivity or DNA-positivity to any high risk HPV type, N=378.

Variables	N[a]	HR HPV Antibody ab+ (%)	Univariate Analysis RR	(95% CI)	Multivariate Analysis RR	(95% CI)	HR HPV DNA DNA+ (%)	Univariate Analysis RR	(95% CI)	Multivariate Analysis RR	(95% CI)
Race											
White	293	199 (67.9)	1.00	ref			47 (16.0)	1.00	ref	1.00	ref
Asian	42	26 (61.9)	0.91	0.71 - 1.17			2 (4.8)	0.30	0.07 - 1.18	0.50	0.12 - 2.14
Other	43	32 (74.4)	1.10	0.90 - 1.33			7 (16.3)	1.01	0.49 - 2.10	1.05	0.50 - 2.22
Age											
30-34	139	98 (70.5)	1.00	ref	1.00	ref	29 (20.9)	1.00	ref	1.00	ref
35-39	89	64 (71.9)	1.02	0.86 - 1.21	0.99	0.84 - 1.17	12 (13.5)	0.65	0.35 - 1.20	0.76	0.39 - 1.49
40-44	73	50 (68.5)	0.97	0.80 - 1.17	0.91	0.76 - 1.11	9 (12.3)	0.59	0.30 - 1.18	0.63	0.33 - 1.21
45-50	77	45 (58.4)	0.83	0.67 - 1.03	0.85	0.69 - 1.05	6 (7.8)	0.37	0.16 - 0.86	0.52	0.21 - 1.29
Age at Sexual debut											
≤15	65	48 (73.9)	1.00	ref			13 (20.0)	1.00	ref		
16-19	178	121 (68.0)	0.92	0.77 - 1.10			27 (15.2)	0.76	0.42 - 1.38		
≥20	119	78 (65.6)	0.89	0.73 - 1.08			16 (13.5)	0.67	0.34 - 1.31		
Marital status											
Single/separated	147	112 (76.2)	1.00	ref	1.00	ref	36 (24.5)	1.00	ref	1.00	ref
Married*	230	144 (62.6)	0.82	0.72 - 0.94	0.84	0.74 - 0.96	20 (8.7)	0.36	0.21 - 0.59	0.47	0.29 - 0.77
Number of pregnancies											
0	148	106 (71.6)	1.00	ref			32 (21.6)	1.00	ref	1.00	ref
1-3	184	123 (66.9)	0.93	0.81 - 1.08			19 (10.3)	0.48	0.28 - 0.81	0.60	0.34 - 1.06
≥4	46	28 (60.9)	0.85	0.66 - 1.10			5 (10.9)	0.50	0.21 - 1.22	0.64	0.25 - 1.61
Abnormal Pap before enrollment	158	120 (76.0)	1.22	1.07 - 1.40	1.11	0.97 - 1.28	36 (22.8)	2.51	1.51 - 4.16	1.64	0.91 - 2.96
Ever had genital warts	42	30 (71.4)	1.06	0.86 - 1.30			5 (11.9)	0.78	0.33 - 1.86		
Ever had STD	103	79 (76.7)	1.18	1.03 - 1.35	1.06	0.92 - 1.22	10 (9.7)	0.59	0.31 - 1.13		
Hormonal birth control usage											
Never	40	16 (40.0)	1.00	ref	1.00	ref	4 (10.0)	1.00	ref		
Former	211	154 (73.0)	1.82	1.24 - 2.69	1.66	1.12 - 2.48	24 (11.4)	1.14	0.42 - 3.11		
Currently	127	87 (68.5)	1.71	1.15 - 2.55	1.54	1.03 - 2.32	28 (22.1)	2.20	0.82 - 5.91		

Smoking status											
Never	277	187 (67.5)	1.00	ref			32 (11.6)	1.00	ref	1.00	ref
Former	81	56 (69.1)	1.02	0.87 - 1.21			17 (21.0)	1.82	1.06 - 3.10	1.56	0.94 - 2.56
Currently	19	14 (73.7)	1.09	0.82 - 1.45			7 (36.8)	3.19	1.63- 6.26	2.30	1.26 - 4.22
Number of lifetime sex partners											
0-2	80	45 (56.3)	1.00	ref	1.00	ref	3 (3.8)	1.00	ref	1.00	ref
3-6	96	59 (61.5)	1.09	0.85 - 1.40	0.99	0.77 - 1.27	10 (10.4)	2.78	0.79 - 9.77	2.19	0.62 - 7.74
7-14	97	65 (67.0)	1.19	0.94 - 1.51	0.99	0.78 - 1.26	19 (19.6)	5.22	1.60 - 17.04	3.11	0.90 - 10.73
15+	101	85 (84.2)	1.50	1.21 - 1.85	1.22	0.98 - 1.53	24 (23.8)	6.34	1.98 - 20.32	3.34	0.97 - 11.49
Sexual activity with males 6 months prior to enrollment											
No sexual partner	82	52 (63.4)	1.00	ref			9 (11.0)	1.00	ref		
Non-new sex partners only	206	137 (66.5)	1.05	0.87 - 1.27			33 (16.0)	1.46	0.73 - 2.92		
Sex with new partners	84	62 (73.8)	1.16	0.95 - 1.43			14 (16.7)	1.52	0.70 - 3.32		

[a]Numbers might not add up to the total number of subjects due to missing data.

*Married or living with a partner

Table 4: Analysis of risk factors associated with seropositivity to multiple high risk HPV types vs seropositivity to a single high-risk HPV type, N=257.

Variables	N[a]	Multi HR HPV Antibody ab+(%)	Univariate Analysis RR	(95% CI)	Multivariate Analysis RR	(95% CI)
Race						
White	199	132 (66.3)	1.00	ref	1.00	ref
Asian	26	13 (50.0)	0.75	0.51 - 1.12	0.84	0.56 - 1.27
Other	32	26 (81.3)	1.22	1.01 - 1.49	1.13	0.93 - 1.36
Age						
30-34	98	61 (62.2)	1.00	ref		
35-39	64	43 (67.2)	1.08	0.86 - 1.36		
40-44	50	36 (72.0)	1.16	0.92 - 1.46		
45-50	45	31 (68.9)	1.11	0.86 - 1.42		
Age at Sexual debut						
≤15	48	39 (81.3)	1.00	ref	1.00	ref
16-19	121	81 (66.9)	0.82	0.68 - 0.99	0.90	0.75 - 1.08
≥20	78	46 (59.0)	0.73	0.58 - 0.91	0.96	0.74 - 1.23
Marital status						
Single/separated	112	78 69.6)	1.00	ref		
Married/living with a partner	144	93 (64.6)	0.93	0.78 - 1.10		
Number of pregnancies						
0	106	64 (60.4)	1.00	ref	1.00	ref
1-3	123	83 (67.5)	1.12	0.92 - 1.36	1.04	0.85 - 1.28
≥4	28	24 (85.7)	1.42	1.14 - 1.76	1.28	1.01 - 1.62
Abnormal Pap before enrollment	120	83 (69.2)	1.08	0.91 - 1.28		
Ever had genital warts	30	23 (76.7)	1.18	0.94 - 1.46		
Ever had STD	79	60 (76.0)	1.22	1.03 - 1.44	1.07	0.90 - 1.28
Hormonal birth control usage						
Never	16	7 (43.8)	1.00	ref	1.00	ref
Former	154	110 (71.4)	1.63	0.93 - 2.87	1.15	0.67 – 1.96
Currently	87	54 (62.1)	1.42	0.79 - 2.54	1.04	0.60 - 1.79
Smoking status						
Never	187	126 (67.4)	1.00	ref		
Former	56	36 (64.3)	0.95	0.77 - 1.19		
Currently	14	9 64.3)	0.95	0.64 - 1.43		
Number of lifetime of sex partners						
0-2	45	21 (46.7)	1.00	ref	1.00	ref
3-6	59	32 (54.2)	1.16	0.79 - 1.72	1.15	0.74 - 1.77
7-14	65	48 (73.9)	1.58	1.12 - 2.23	1.54	1.02 - 2.32
15+	85	68 80.0)	1.71	1.23 - 2.39	1.57	1.03- 2.39
Sexual activity with male partner 6 months prior to enrollment						
No sexual partner	52	30 (57.7)	1.00	ref	1.00	ref
Sex with non-new partners only	137	90 (65.7)	1.14	0.88 - 1.48	1.07	0.82 - 1.39
Sex with new male partners	62	46 (74.2)	1.29	0.98 - 1.69	1.16	0.88 - 1.52

[a]Numbers might not add up to the total number of subjects due to missing data.