Research Article

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Behavioral/Lifestyle and Immunologic Factors Associated with HPV Infection among Women Older Than 45 Years

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Abstract

Background: Cervical human papilloma virus (HPV) detection increases after menopause, but its determinants need clarification.

Methods: In a case–control study nested within a 10,049 women cohort, we evaluated women 45 to 75 years old who acquired HPV infection and were HPV positive 5 to 6 years after enrollment ($N = 252$), and HPV-negative women as matched controls ($N = 265$). Detailed sexual behavior and cellular immune response were investigated. Odds ratios (OR) and attributable fractions were estimated.

Results: Women with 2+ lifetime partners had 1.7-fold (95% CI = 1.1–2.7) higher risk than monogamous women, with similar findings if their partners had other partners. Women with 2+ partners after last HPV-negative result had the highest risk (OR = 3.9; 95% CI = 1.2–12.4 compared with 0–1 partners). Weaker immune response to HPV-16 virus-like particles increased risk (OR = 1.7; 95% CI = 1.1–2.7 comparing lowest to highest tertile). Among women with no sexual activity in the period before HPV appearance, reduced immune response to phytohemagglutinin was the only determinant (OR = 2.9; 95% CI = 0.94–8.8). Twenty-one percent of infections were explained by recent sexual behavior, 21% by past sexual behavior, and 12% by reduced immune response.

Conclusions: New infections among older women may result from sexual activity of women and/or their partners or reappearance of past (latent) infections possibly related to weakened immune response.

Impact: HPV infections among older women are associated with current and past sexual exposures and possibly with immune senescence. The risk of cancer from these infections is likely to be low but could not be fully evaluated in the context of this study. *Cancer Epidemiol Biomarkers Prev; 19(12); 3044–54. ©2010 AACR.*

Introduction

Age-specific human papilloma virus (HPV) incidence peaks in late adolescence and early adult life and in most countries declines with age thereafter (1, 2). It is well understood that this early peak closely parallels initiation of sexual activity and that sexual behavior of women and their partners is the primary determinant of HPV acquisition at these ages (3–5).

In recent years, a second peak in HPV prevalence has been observed among older, postmenopausal women in several populations (2, 6–12). Less is known about the determinants of this second peak. Factors that could explain the second peak in HPV prevalence at older ages include increased incidence due to new infections through sexual contact of the women or their partners with new partners later in life, increased detectability of persistent vaginal infections among older women due to physiologic changes at the cervix associated with aging (such as localization of the transformation zone deep into the canal that enriches the samples with vaginal cells instead of endocervical cells), reactivation of latent infections associated with age-related immunologic and/or hormonal changes, longer persistence of HPV infections among older women, and/or trends of HPV prevalence in succeeding birth cohorts (8, 9, 13, 14).

We have previously reported a second peak in age-specific HPV prevalence in a population-based cohort study in Guanacaste, Costa Rica. In this population, overall HPV prevalence was observed to peak at ages 18 to 25 (36.9%), to decline thereafter (nadir observed at ages 35–54; 20%), and to increase again after the age of 55 (31.4% prevalence among women ≥65 years). The second peak in HPV prevalence among older women was observed for both oncogenic and nononcogenic HPV types, with a

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more pronounced effect observed for nononcogenic HPV types. (6, 7)

In an attempt to better understand the second peak in HPV observed among older women in our study population, we conducted several evaluations. We showed, for example, that women with detectable HPV infections that persist at older ages have reduced immune responsiveness, as measured by the ability of peripheral blood mononuclear cells (PBMC) to respond to mitogenic/antigenic stimuli (15). This suggests that immunologic changes with aging might partially explain the high prevalence of HPV at older ages.

We also showed that nononcogenic HPV types have a greater tropism for the vaginal epithelia than for cells of the cervix (16, 17). Nononcogenic HPV types from the α-2/α-3/α-15 species tend to be detected more often than oncogenic types in cervical tissues of older women, which are composed predominantly of mature squamous epithelial cells (18). The second peak in nononcogenic HPV types, which have a predilection to infect the vagina, is at least partially linked to increased vaginal sampling relative to cervical sampling among older women.

Most recently, we compared the likelihood of persistence among newly detected HPV infection in women by age and showed that rates of persistence of such infections do not increase with age (19). Therefore, the second peak in HPV prevalence observed among older women is unlikely to be explained by an increased likelihood of infections at older ages to persist.

Herein, we report results from a nested case–control study within a 10,049 women population-based cohort study in Guanacaste, Costa Rica, developed to addresses whether sexual, other behavioral/lifestyle, or immunologic factors of women and their partners are associated with acquisition of HPV infections at older ages.

Methods

Population and study design

Between June 1993 and December 1994, a population-based cohort was established in Guanacaste, Costa Rica, to study the natural history of HPV infection and cervical neoplasia. After providing informed consent, 10,049 women were enrolled (93.6% of eligible women). At enrollment into the cohort, all women were administered a questionnaire that obtained information on their sexual and reproductive behaviors and other potential risk factors for HPV and cervical neoplasia. Among women who were sexually active, a pelvic examination was conducted from which exfoliated cells were collected using a cytobrush for conventional and liquid-based cytology and for HPV DNA detection and typing. Women with cervical abnormalities were referred to colposcopy and treated as necessary. After exclusion of hysterectomized women and those with prevalent cervical precancer or cancer, the remaining women were followed for up to 7 years at varying intervals (every 6 months, 1 year, or 5–6 years) according to cervical disease risk categories defined at enrollment as described elsewhere (21). Briefly, women with low-grade cytologic abnormalities were followed every 6 months; women with atypical squamous cells of uncertain significance (ASCUS), HPV-positive women, women with more than 4 sexual partners, and a random sample of the rest of the women were followed every year; virgins were followed every year and when they started having sex were followed every 6 months; and all other women were followed at years 5–6. At each follow-up visit, women were reinterviewed to obtain information on sexual, reproductive, and other risk factors since the preceding visit, and biological specimens were collected as described for the enrollment visit. Women with evidence of cervical precancer or cancer during follow-up were referred to colposcopy for evaluation and treatment and were censored from further follow-up within the cohort. Additional details of the study design and methods have been described elsewhere (20, 21).

Details of the methods employed for our case–control study among older women (OW study) nested within the cohort described earlier have previously been published (15). In brief, individuals for the nested study were selected from the 7,008 women enrolled in our cohort and for whom PCR-based HPV DNA results from their year 5–6 follow-up were available at the time when the OW study was designed. The year 5–6 follow-up was chosen as the reference visit to define cases and controls because all women in the cohort, irrespective of risk category, were scheduled for a study visit during this period. Of the 2,992 women who were between 45 and 75 years of age at their year 5–6, 324 were HPV DNA positive and were selected as cases. A subset of women with a negative HPV DNA result at that same visit, independent of previous results, was selected as controls ($N = 310$), with frequency matched to the HPV-positive cases on age and time in study. Of note, participants were chosen on the basis of their year 5–6 HPV results, but designing and conducting the nested case–control study took some years to complete, as follows. All women selected were invited to participate in the OW study and received an appointment for a visit (OW study visit) that occurred on average 3.0 years after the time of the HPV DNA test that led to their selection into the nested study (year 5–6 visit; range = 1.0–4.5 years) and 8.5 years after enrollment in the cohort (range = 7.8–9.3 years).

At the time of the OW study visit, women signed a new informed consent and an interview was conducted including more detailed information on sexual behavior than had been obtained by the routine questionnaires administered during the regular enrollment and follow-up study visits within the cohort. Specifically, we collected information from the women on their sexual behavior, that of each of their sexual partners, and information on the timing of each relationship.

A pelvic examination was conducted including collection of exfoliated cells by cytobrush for liquid-based cytology and PCR-based HPV DNA testing. An assessment of cervical atrophy was conducted by the

cytopathologist when reviewing the cytology. Finally, 40 mL of blood were collected in heparinized tubes from which PBMCs were isolated and cryopreserved to conduct cell-mediated immunity studies.

The participation rate in our nested case–control study was more than 92% (591/634 women). For this analysis, we were interested in evaluating women with appearance of an HPV infection between enrollment and years 5–6. Therefore we excluded 40 cases who were HPV positive at enrollment for the same HPV types they had at the year 5–6visit (these represent long-term persistent infections rather than new appearance of an infection) and 3 women with missing HPV DNA results at enrollment. We also excluded 29 controls with evidence of HPV infection at the time of the OW study visit (these are in fact appearance of HPV infection at older ages but were also excluded from the case group to prevent bias related to the timing of specimen collection/exposure measurements) and 2 controls with missing HPV DNA results at the OW study visit. After these exclusions were applied, 252 cases and 265 controls remained. There were 5 women included in the overall analyses who were excluded from the analyses related to sexual behavior because they had missing information regarding their number of sexual partners. Three of these women were also excluded from analyses that reported on variables within the specific time window of interest (see the following text) because the window start date could not be defined.

The study protocol was approved by the National Cancer Institute and the Costa Rican INCIENSA Institutional Review Boards.

HPV DNA detection and typing

PCR-based HPV DNA detection and typing were conducted on exfoliated cervical cells, using the L1 MY09/MY11 degenerate primer system with AmpliTaq Gold polymerase as described previously (22), followed by dot-blot genotyping of the amplification product using type-specific oligonucleotide probes for HPV types 2, 6, 11, 13, 16, 18, 26, 31 to 35, 39, 40, 42 to 45, 51 to 59, 61, 62, 64, 66 to 74, 81 to 85, 82, and 89, as reported elsewhere (6–23). Probes for HPV types 2, 13, 34, 42 to 44, 57, 64, 69, 74, 82, and 54 were combined in dot-blot hybridization for detection of rare types (dot-blot mix). Specimens that were HPV positive based on a radiolabeled generic probe mix but were not positive for any type-specific probe were considered to be positive for uncharacterized HPV types.

Lymphoproliferation assays

Lymphoproliferation responses were assessed as previously described (15). In brief, PBMCs were resuspended and cultured in triplicate for 3 or 5 days in the presence of HPV-16 L1 virus-like particles (VLP) and influenza A virus (infectious virus, H3N2, A/Hong Kong/8/68) as recall antigens; phytohemagglutinin (PHA) as mitogen; and AIM-V media alone as the negative control. Cultures were pulsed for 18 hours with 1 μCi of [^{3}H]thymidine and then harvested and counted in an automated scintillation counter. On the basis of findings from a previous publication from this study (15) suggesting that PBMCs from women with persistent HPV infections have a generalized (rather than antigen-specific) reduced ability to respond to exogenous stimuli, the response to PHA and HPV-16 VLPs was interpreted in the present study as an indicator of the level of general immune activity. The results were expressed as mean counts per minute. Responses to PHA and HPV-16 VLPs were categorized into approximate tertiles representing low, medium, and high responses.

Statistical analysis

Because a main focus of this study was to evaluate the sexual behavior of women and their partners during the period of time immediately preceding the appearance of HPV infection, we defined retrospectively a "window" of exposure for the cases and controls by reviewing all HPV DNA results each woman had since enrollment into our cohort. Among cases, women who were HPV positive at the year 5–6 follow-up in the cohort, we defined as the exposure "window" the time between the last HPV-negative result and the first HPV-positive result for the HPV type detected at the year 5–6 visit (Fig. 1). Of the 67 cases who had more than 1 HPV types detectable at the year 5–6 visit, 60 had the appearance of all the new HPV types at the same time and therefore the window was the same for all the types. For the remaining 7 women (2.8% of cases), the window was defined as the time from the last HPV-negative result to the first HPV-positive result for the HPV type that appeared last. Exclusion of these 7 women did not alter findings (data not shown). Of note, cases with multiple infections and those with single infection had similar window length (median length of window 47.7 for cases with multiple infections and 47.6 months for women with single infections; $P = 0.98$)

Comparable windows for controls were defined by setting the period of interest as the time between the year 5–6 follow-up when they were HPV negative and the preceding follow-up visit (see Fig. 1). Using this approach led to time windows of similar length for cases and controls [median length of windows = 47.6 months, interquartile range (IQR) = 11.9–73.4 months, for cases and 52.9 months, IQR = 13.0–79.4 months, for controls; $P = 0.06$].

Using data collected from the questionnaires applied during the follow-up visits in the cohort ending at the year 5–6 visit, we defined the following exposure variables within the time windows of interest for the analysis: age at the end of the window, time since last menstrual period at the end of the window, smoking status through the end of the window, number of pregnancies reported through the end of the window, and hormonal contraceptive use [oral contraceptives (OC) and injectables] through the end of the window. From the detailed sexual behavior questionnaire administered to women at the OW study visit we defined the following: total number of

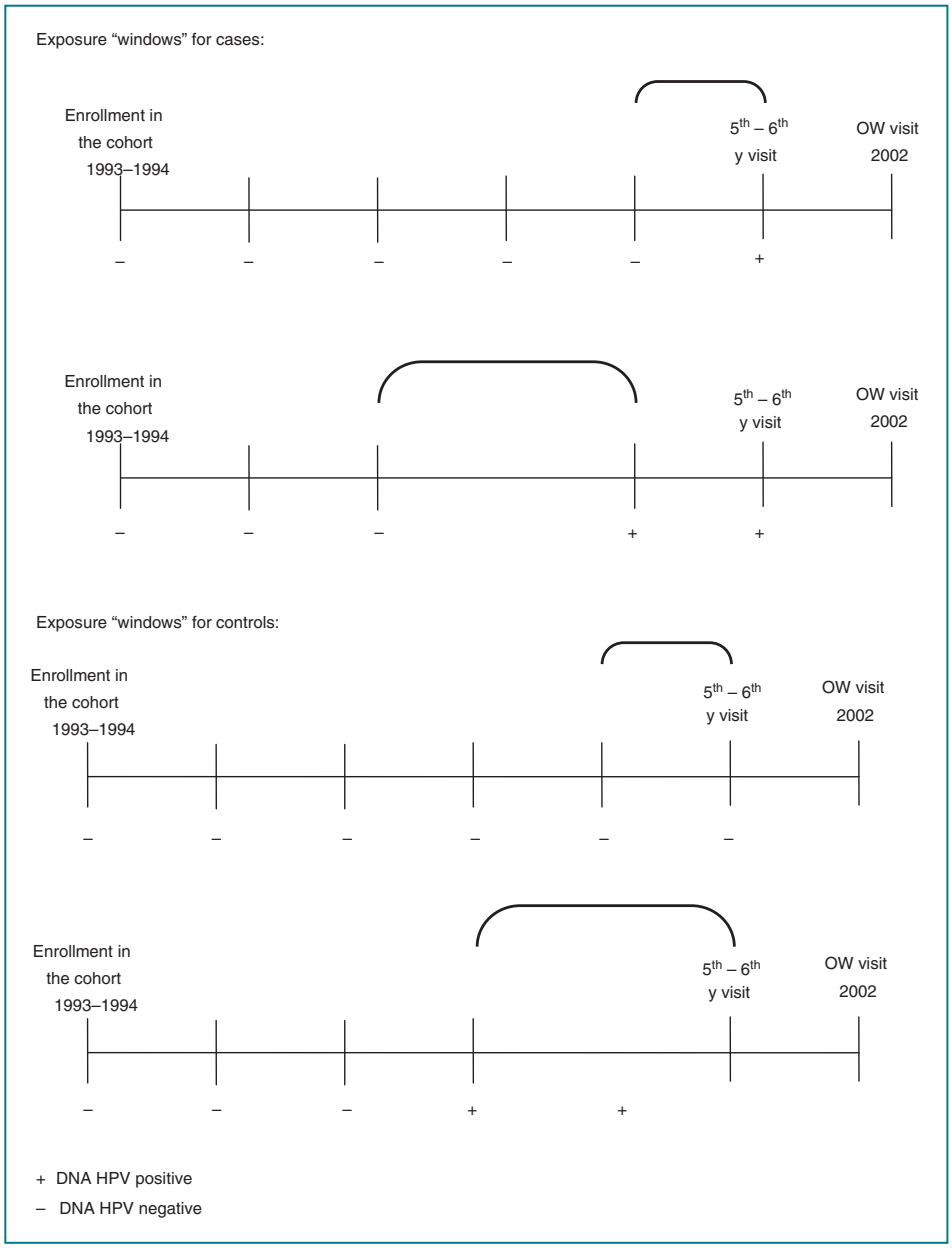

Figure 1. Definition of exposure "windows" for cases and controls.

partners within the window, number of past sexual partners (defined as the cumulative number of partners up to the end of the window minus the number of partners within the window), age of partners reported within the window, condom use with partners reported within the window, whether the partners reported within the window had other partners during their relationship, report of sex with prostitutes for partners reported during the window, duration of relationship with partners reported within the window, and frequency of sex with partners reported within the window. We also evaluated the following information at the time of the OW study visit: marital status, presence and degree of atrophy in the cytology evaluation, and lymphocyte proliferation in response to antigenic/mitogenic stimulation as previously described (15). These latter data were designed to reflect the correlated measurements we expect to have obtained had we been able to take direct measurements at the year 5–6 visit.

The unit of analyses reported herein is the woman, as only a minority of women in our study acquired HPV infection with more than 1 HPV type (27%). The women who had appearance of multiple HPV types were included defining the window of interest as described earlier. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI),

comparing HPV-positive cases against HPV-negative controls with respect to exposure variables of interest. Univariate ORs were adjusted for matching variables (age and time under study in the cohort), and multivariate models were developed as specified in the footnotes to the tables presented in the Results section. To test for trend, categorical variables were evaluated as continuous in the model. To ensure that the findings reported herein were not affected by our analytic approach, parallel analyses were conducted at the infection level (i.e., cases with multiple HPV types were included as multiple observations in the analysis) using general estimating equation (GEE) models that account for the lack of independence between multiple observations from the same woman (24, 25) The GEE analyses yielded comparable results (data not shown).

Analyses were conducted in different subsets of women. All women were included for characteristics and behaviors related to the women themselves. For exposures associated with the characteristics and behaviors of the sexual partners of women in our study, analysis was restricted to women who reported a single sexual partner during the time window of interest (n = 182 cases and 202 controls; 74% of all women). Separate analyses were also conducted, restricted to women who reported having no sexual activity during the time window of interest (n = 53 cases and 54 controls).

We evaluated the combination of risk factors observed among cases in our study to determine the fraction of newly detected infections among women older than 45 years that could be explained by risk factors observed in our study and the fraction of such infections that remained unexplained. For this analysis, factors that were significantly associated with acquisition in our analysis or that had an OR higher than 1.5 in the multivariate analysis were considered and grouped into 3 categories: recent sexual behavior (report that the participant had more than 1 partner during the window of interest and/or that her partner had other partners in that period of time or that she did not know), past sexual behavior (report that the participant had more than 1 past sexual partners), and immune response (weak proliferation response of PBMCs after stimulation with HPV 16 VLPs). In addition to raw percentages, ORs and 95% CIs for each of these categories were estimated. Adjusted population attributable risks were estimated for each of the 3 categories, using the method proposed by Bruzzi et al. (26) for case–control studies.

To ensure that patterns observed did not differ between oncogenic and nononcogenic HPV types, separate analyses were conducted for women who acquired only oncogenic HPV infections (n = 59) and those who acquired only nononcogenic HPV infections (n = 167). Women who acquired oncogenic infections were directly compared against those who acquired nononcogenic infections. No significant or notable differences were observed between these 2 groups (data not shown). Only 26 women acquired infections with both oncogenic and nononcogenic HPV types, precluding careful evaluation of this subgroup.

To rule out the possibility that misclassification due to false-negative HPV results affected our results (i.e., that some of the women classified as having acquired a new HPV infection were incorrectly classified as such due to a false-negative intervening HPV result between positive tests), the analyses were repeated after redefining the windows of the cases assuming that 2 or less negative results between 2 positive results were positive (e.g., for a woman $--+-+$, in this analysis the last negative was assumed to be positive and the window of interest was redefined as being between the second negative HPV test and the first positive test). This resulted in redefinition of windows for 13 (5.2%) cases. Results from this reanalysis were consistent with those reported herein (data not shown).

Additional sensitivity analyses were conducted among women who acquired more than 1 HPV types within the window of interest and stratified by age (45–54, 55–64, and 65+ years) to evaluate whether other risk factors could be identified in this group. Patterns observed were similar to those seen overall (data not shown).

Finally, we restricted the analysis to the best "pure controls" we could define, those who were HPV negative at all study visits since enrollment (53 controls excluded). We note that most of the "pure controls" were followed only at the 5–6 year visit, so we cannot rule out the possibility that some of them acquired and cleared an HPV infection during that period. We also excluded cases who had appearance of more than 1 HPV types with different exposure "windows" (7 cases) to rule out incorrect definition of the windows among cases with appearance of more than 1 HPV types. Patterns observed were the same as those presented here (data not shown).

Analyses were conducted using STATA 10.0.

Results

Of the 517 women included in the analysis, 266 were 45 to 54 years, 176 were 55 to 64 years, and 75 were older than 64 years. After adjustment for matching variables (age and time in study), sexual behavior, OC use, and weak lymphocyte proliferation were significantly associated with HPV appearance among women 45 years and older (Table 1). Women with 2 or more partners during the window of interest had a higher risk of HPV appearance than women who reported no partners during the window (OR = 4.6; 95% CI = 1.4–15.0). Also, women with 2 or more past sexual partners had a 1.7-fold (95% CI = 1.1–2.5) higher risk of having an HPV appearance than lifetime monogamous women. In multivariate analysis, both factors remained significantly associated with HPV appearance. Weak lymphocyte proliferation upon stimulation with HPV-16 VLPs (assessed on average 3 years after the case–control selection visit at years 5–6) also increased the risk of HPV appearance 1.7-fold (95% CI = 1.1–2.6) and the increased risk remained after

Table 1. Distribution and risk of appearance of an HPV infection associated with sociodemographic, sexual, reproductive, lifestyle, and immune factors. Study of women aged 45 to 75 years in Guanacaste, Costa Rica

	Controls	Cases	OR[a]	P_{trend}	Multivariate OR[b]	P_{trend}
Length of the window						
≤13 mo	65	84	1			
13.1–62.5 mo	103	83	0.62 [0.40–1.0]			
>62.5 mo	97	85	0.68 [0.44–1.0]	0.10		
Marital status[c]						
Married	189	172	1			
Separated	26	29	1.2 [0.67–2.1]			
Widowed	21	27	1.4 [0.73–2.6]			
Single	29	24	0.91 [0.49–1.6]			
Time since last menstrual period						
<1 y	95	95	1			
1.1–5 y	43	26	0.55 [0.30–1.0]			
>5 y	127	130	1.0 [0.59–1.8]	0.97		
No. of past sexual partners						
0 (lifetime monogamous)	117	91	1		1	
1	66	66	1.2 [0.79–1.9]		1.3 [0.82–2.1]	
2+	77	95	**1.7 [1.1–2.5]**	**0.02**	**1.7 [1.1–2.7]**	**0.02**
No. of partners within the window [between last HPV(−) and first HPV(+) visits][d]						
0	54	53	1			
1	202	182	0.90 [0.57–1.4]		1	
2+	4	17	**4.6 [1.4–15.0]**	0.23	**3.9 [1.2–12.4]**	
No. of pregnancies						
0–2	42	32	1			
3–5	104	87	1.0 [0.60–1.8]			
6–8	49	65	1.8 [1.0–3.3]			
9+	70	67	1.2 [0.68–2.3]	0.21		
Smoking						
Never	229	219	1			
Former	26	18	0.72 [0.38–1.4]			
Current	10	14	1.5 [0.64–3.4]	0.80		
Ever OC use						
Never	126	99	1		1	
Ever	139	152	**1.4 [1.0–2.0]**		1.4 [0.92–2.0]	
Ever injectable contraceptive use						
Never	209	188	1			
Ever	56	63	1.3 [0.84–1.9]			
Cervical atrophy assessed by cytology[c]						
None	143	142	1			
Mild	25	23	0.89 [0.48–1.7]			
Moderate	49	48	0.92 [0.56–1.5]			
Severe	48	38	0.73 [0.43–1.2]	0.27		
Lymphocyte proliferation in response to HPV-16 VLPs[c]						
Strong	117	90	1		1	
Middle	86	77	1.1 [0.73–1.7]		1.1 [0.74–1.7]	
Weak	58	79	**1.7 [1.1–2.6]**	**0.03**	**1.7 [1.1–2.7]**	**0.03**
Lymphocyte proliferation in response to PHA[c]						
Strong	112	85	1			
Middle	84	84	1.3 [0.84–1.9]			
Weak	67	78	1.4 [0.92–2.2]	0.1		

[a]Adjusted for matching variables (age and time in study).
[b]Adjusted for age, time in study, number of past sexual partners, number of partners within the window, OC use, lymphocyte proliferation in response to HPV-16 VLPs, and other partners of the partner.
[c]At the time of OW study visit.
[d]For the multivariate model, women with 1 partner were grouped with women with 0 partner as the comparison group.

multivariate adjustment. OCs conferred a 1.4-fold (95% CI = 1.0–2.0) increased risk of HPV appearance compared with women who never used them, but this association was no longer significant in multivariate analysis. Other factors evaluated were not significantly associated with risk of HPV appearance (Table 1).

When the analysis was restricted to women who reported only 1 partner during the window of interest, the effect of number of past sexual partners of the women was still present (OR = 2.6; 95% CI = 1.0–2.7) and remained in the multivariate model. The characteristics of the partner, including his age in relation to the woman and whether he used condoms or visited prostitutes were not associated with HPV appearance, but there was a 1.6-fold increased risk if the woman reported that her partner had other partners during his relationship with her (95% CI = 1.0–2.6; Table 2). There was also a nonsignificant 1.7-fold (95% CI = 0.92–3.0) increased risk if the woman reported that she did not know whether her partner had other partners, an association that became weaker after adjustment. We did not observe any association with the duration of the relationship or with frequency of sexual intercourse.

Among women who reported no sexual partners during the window of interest (Table 3), weak or moderate lymphocyte proliferation response upon stimulation with PHA, compared with strong response, was the only factor significantly associated with HPV appearance. We evaluated the association between immune response and HPV appearance in 3 different age groups (44–54, 55–64, and ≥65) and did not observe differences by age (data not shown).

Table 4 presents attributable risk estimates for the different factors identified in this study. A risk factor related to recent sexual behavior was reported by 64% of cases and was associated with a 1.5-fold increased risk of HPV appearance; 21% of new HPV infections among older women could be explained by this factor. A risk factor related to past sexual behavior was also reported

Table 2. Distribution and risk of appearance of an HPV infection associated with sexual behavior factors of partners of women with one partner during the study window. Study of women aged 45 to 75 years in Guanacaste, Costa Rica

	Controls	Cases	OR[a]	P_{trend}	Multivariate OR[b]	P_{trend}
No. of past sexual partners						
0	116	87	1		1	
1	46	47	1.3 [0.80–2.2]		1.3 [0.76–2.1]	
2+	40	48	**2.6 [1.0–2.7]**	**0.05**	**1.7 [1.0–2.9]**	**0.04**
Age of the partner						
Same age or older than the woman	166	141	1			
Younger than the woman	33	39	1.4 [0.84–2.4]			
Condom use by partner						
Never	142	134	1			
Sometimes	43	41	1.0 [0.62–1.7]			
Often/always	17	7	0.44 [0.17–1.1]	0.21		
Other partners of the partner during the relationship with the woman						
No	60	38	1			
Yes	98	96	**1.6 [1.0–2.6]**		1.5 [0.92–2.6]	
Don't know	44	48	1.7 [0.92–3.0]		1.5 [0.84–2.8]	
Sex with prostitutes						
No	50	54	1			
Yes	45	40	0.89 [0.50–1.6]			
Don't know	107	88	0.80 [0.49–1.3]			
Duration of relationship						
>1 y	201	178	1			
≤1 y	1	4	4.4 [0.49–40.5]			
Frequency of sexual intercourse						
0–4 per mo	56	51	1			
5–8 per mo	87	70	0.88 [0.54–1.5]			
9+ per mo	59	61	1.1 [0.66–1.9]	0.64		

[a]Adjusted for matching variables (age and time in study).
[b]Adjusted for age, time in study, number of past sexual partners, OC use, lymphocyte proliferation in response to HPV-16 VLPs, and other partners of the partner.

Table 3. Distribution and risk of appearance of an HPV infection associated with sociodemographic, sexual, reproductive, lifestyle, and immune factors among women who report no sex in the period of HPV appearance. Study of women aged 45 to 75 years in Guanacaste, Costa Rica

	Controls	Cases	OR[a]	P_{trend}
Marital status[b]				
Married	4	8	1	
Separated	15	15	0.37 [0.08–1.6]	
Widowed	17	12	0.25 [0.05–1.1]	
Single	18	18	0.46 [0.11–1.9]	
Time since last menstrual period				
<1 y	7	10	1	
1.1–5 y	8	2	0.19 [0.03–1.2]	
>5 y	39	4	0.78 [0.20–3.1]	0.95
No. of past sexual partners				
1	20	19	1	
2+	34	34	1.1 [0.51–2.5]	
No. of pregnancies				
0–2	9	7	1	
3–5	20	11	0.67 [0.19–2.3]	
6–8	9	13	2.3 [0.59–8.9]	
9+	16	22	2.2 [0.64–7.7]	0.05
Smoking				
Never	42	40	1	
Former	10	8	0.84 [0.30–2.4]	
Current	2	5	2.6 [0.47–14.9]	0.48
OC use				
Never	34	27	1	
Ever	20	26	1.6 [0.71–3.7]	
Injectable contraceptive use				
Never	45	39	1	
Ever	9	14	1.7 [0.64–4.4]	
Cervical atrophy identified on cytology[b]				
None	18	25	1	
Mild	6	5	0.51 [0.13–2.1]	
Moderate	14	10	0.53 [0.18–1.5]	
Severe	16	13	0.44 [0.16–1.3]	0.12
Lymphocyte proliferation in response to HPV-16 VLPs[b]				
Strong	29	21	1	
Middle	12	14	1.6 [0.59–4.1]	
Weak	12	17	1.9 [0.72–4.8]	0.18
Lymphocyte proliferation in response to PHA[b]				
Strong	29	15	1	
Middle	16	25	**3.0 [1.2–7.5]**	
Weak	8	12	2.9 [0.94–8.8]	**0.03**

[a]Adjusted for matching variables (age and time in study).
[b]At the time of OW study visit.

by 64% of cases and was associated with a 1.5-fold increased risk of HPV appearance; 21% of new HPV infections among older women were attributable to this factor. Finally, weak lymphocyte proliferation response was observed for 31% of cases and was associated with a 1.6-fold increased risk of HPV appearance; 12% of new HPV infections among older women could be explained by this factor. These 3 factors together explain 45% of HPV appearances.

Discussion

It is now known that in many populations, a second peak in HPV prevalence is observed in older women. This

Table 4. Attributable risk for factors associated with HPV appearance. Study of women aged 45 to 75 years in Guanacaste, Costa Rica

Risk factor category	% of cases with the risk factor ($N = 252$)	Adjusted OR[a]	Adjusted attributable risks
Recent sexual behavior[b]	63.9	1.5 [1.0–2.1]	21.3%
Past sexual behavior[c]	63.9	1.5 [1.1–2.2]	21.3%
Immune response[d]	31.4	1.6 [1.1–2.4]	11.8%
All 3 risk factors			45.4%

[a]Adjusted for age, time in study, recent sexual behavior, past sexual behavior and immune response.
[b]More than 1 partner within the window and/or the woman reported that the partner in that period of time had other partners or that she did not know.
[c]Two or more past sexual partners.
[d]Low cell/lymphocyte proliferation in response to HPV-16 VLPs.

peak has several possible explanations (described in the Introduction section) that include sexual behavior factors related to exposure, physiologic changes that occur with aging and impact HPV detectability, a cohort effect, and immunologic factors that affect the ability of a woman to control infections. Most studies that have evaluated determinants of HPV at older ages to date have focused on individual factors (15, 17, 18, 27). The present analysis has the advantage of being conducted within a well-characterized population and of having the ability to evaluate various possible determinants of HPV among older women simultaneously.

Limitations of our study should be discussed. They include the fact that immunologic measurements were conducted on biological specimens collected on average 3 years after HPV detection and the fact that HPV infections that appeared after enrollment into our cohort but cleared prior to the 5–6 year visit could not be evaluated. The first limitation listed likely resulted in misclassification of the immunologic status a woman at the time of HPV detection, which would have led to an attenuation of the observed effects (i.e., the magnitude of immunologic associations might be stronger than those reported herein). The second limitation listed might also have resulted in the attenuation of observed effects if a proportion of women selected as controls had in fact acquired HPV infections that cleared before the 5–6 year visit used to define cases and controls. This later limitation is likely to also have resulted in a reduction in statistical power and in the precision of our estimates, as a study that included as cases women with infections that were first detected after enrollment but not evident at years 5–6 would have increased study size. It is reassuring to note, however, that women included as cases in our study ($N = 252$) and those who acquired HPV during follow-up but cleared the infection before years 5–6 ($N = 159$) were comparable with respect to measured sociodemographic and behavioral factors including number of lifetime sexual partners, marital status, and educational level (data not shown).

The most salient findings from our analyses of older women were the observations that sexual behavior of women and their partners were predictors of HPV appearance and that among women who reported no sexual activity during the period when HPV appearance was documented, a weakened immunologic response was the only significant predictor of HPV. Overall, 21% of HPV infections detected in our study were attributable to sexual behavior around the time of acquisition; 12% of infections were attributable to a weakened immune response. These findings 1) confirm the sexual route of HPV infections among older women, 2) suggest that reduced immunologic fitness is an important determinant of HPV infection among older women, and 3) indicate the possible existence of "latent" HPV infections that become detectable among older women even in the absence of reexposure through sexual activity. The main factor associated with this detection of "latent" infections was weak immune response. The concept that HPV infections might become "latent" (i.e., undetectable by current, highly sensitive PCR-based methods of detection) and that they reappear when host immunologic responses are unable to control the infection is supported by previous reports, including one from our study population (15) and a second study among HIV-positive women that reported new HPV detection in 22% of sexually inactive HIV-positive women with low $CD4^+$ counts (28).

Interestingly, in our study, lifetime number of sexual partners was a significant risk factor for HPV acquisition even after control for sexual behavior around the time of HPV appearance; 21% of HPV infections detected in our study were attributable to this particular risk factor. It is not clear to us why this is the case, although both biological and nonbiological explanations for this finding exist. If real, our observation would suggest that women with a history of multiple sexual partners are at greater risk of having acquired HPV in the past, and therefore at greater risk of reactivation of a latent HPV infection, even in the absence of recent sexual behaviors that would put

them at risk of exposure to new HPV infections. Alternatively, the finding could reflect residual confounding of recent sexual behavior factors by past/more distant sexual behavior factors as a result of imperfect assessment of sexual behavior via epidemiologic questionnaires.

It should be noted that the present study did not evaluate the clinical relevance of HPV infections detected among older women. None of the women in our study had evidence of cervical precancer or cancer, and the majority of HPV infections detected were with nononcogenic HPV types. This suggests that although HPV infections are common among older populations (whether due to new infections or reactivation of previously acquired infections), they might not necessarily be associated with risk of cancer development, and this should be considered when evaluating the benefit of HPV vaccination among older women. In fact, in a recent report, older women with newly detected HPV infections were as likely to clear their infections as young women who acquire HPV infections and at very low risk of progression of precancer/cancer (19).

In summary, our study confirms the sexual route of transmission of HPV infection at older ages, identifies immunologic responses as important determinants of HPV infections, and suggests that some "newly detected" HPV infections could represent reactivations of previously established but undetectable infections among women with weakened immunologic fitness.

Disclosure of Potential Conflicts of Interest

The authors do not have any conflict of interest to report.

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