MAJOR ARTICLE

A Population-Based Study of Vaginal Human Papillomavirus Infection in Hysterectomized Women

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We compared point prevalences and determinants of human papillomavirus (HPV) DNA detection by testing enrollment vaginal specimens from hysterectomized women ($n = 569$) and enrollment cervical specimens from nonhysterectomized women ($n = 6098$) ⩾30 years old, using MY09/MY11 L1 consensus-primer polymerase chain reaction. The subjects were participating in a population-based cohort study ($n = 10{,}049$) in Guanacaste, Costa Rica, that was initiated in 1993. Non–cancer-associated HPV types, especially types 61, 71, and 72, were detected more frequently in the vaginal specimens from hysterectomized women (23.7% [95% confidence interval {CI}, 20.3%–27.4%]) than in the cervical specimens from nonhysterectomized women (16.7% [95% CI, 15.7%–17.6%]) ($P = .0001$). There was no difference between the prevalences of cancer-associated HPV types in hysterectomized women and those in nonhysterectomized women; in both groups, the prevalence of HPV DNA was greater in women with multiple lifetime sex partners. We infer from our data that the cervical transformation zone may not be needed for cancer-associated HPV infection but may be uniquely susceptible to HPV-induced carcinogenesis; we also infer that specific phylogenetic groups of HPV (i.e., A3/A4/A15) may have a predilection for vaginal epithelium.

Human papillomavirus (HPV) infection is a common sexually transmitted infection. Although most HPV infections are transient, becoming undetectable within 1–2 years, the persistence of 1 of ~15 cancer-associated HPV types may cause cervical cancer, the second most common malignancy among women worldwide [1–4]. The observation that HPV also is detectable in the vagina but rarely causes cancer has prompted our prevailing model of cervical carcinogenesis, in which the infection of squamous metaplastic cells located in the annulus of tissue between the glandular endocervical and stratified squamous epithelia (the cervical transformation zone) is central to tumorigenesis.

A recent study has suggested that vaginal HPV infections may precede cervical infections [5], but, in that analysis, it was not possible to discount the possibility that HPV detection in the vagina was sometimes a consequence of "contamination" by exfoliated cells of the cervix. Women who have undergone hysterectomy and are without a cervix provide a unique opportunity to examine HPV infection of the vagina in the absence of cervical tissue and, thus, to determine whether the cervix, and specifically the cervical transformation zone, is required for HPV infection and/or carcinogenesis.

During 1993–1994, >10,000 women were enrolled in a population-based natural-history study of HPV infection and cervical neoplasia in Guanacaste, Costa Rica [6]; the study included nearly 600 women who had undergone total hysterectomies before enrollment. We recently completed enrollment HPV DNA testing for the entire cohort of women, using MY09/MY11 L1 con-

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sensus-primer polymerase chain reaction (PCR) [7], and HPV serologic testing for types 16, 18, and 31, using a virus-like particle ELISA [8]. In this analysis of vaginal specimens from self-reported and visually confirmed hysterectomized women, we evaluated (1) HPV DNA prevalence by type, by cancer risk group, and by phylogenetic groupings; (2) HPV seroprevalence; and (3) HPV DNA prevalence stratified by age and sexual behavior and by age and time since hysterectomy. We compared, as a point of reference, the relevant results for the hysterectomized women to the results for the nonhysterectomized women without high-grade cervical neoplasia who spanned a similar age range.

SUBJECTS, MATERIALS, AND METHODS

Study population. A National Cancer Institute–sponsored population-based cohort study of HPV infection and cervical neoplasia was established in Guanacaste, Costa Rica, during 1993–1994 [6]. At enrollment, 10,049 of the 11,742 women who were identified in a door-to-door survey and who were residing in randomly chosen censal segments of Guanacaste agreed to visit one of our study clinics and participate in the enrollment interview. After excluding virgins ($n = 583$) and those women who were unwilling or unable to undergo one ($n = 291$), pelvic examinations were performed on 9175 women. Consent was obtained from all participants in accordance with the guidelines of the US Department of Health and Human Services; the study was approved by the institutional review boards of the National Institutes of Health and of Costa Rica.

Of the eligible participants, 664 women (7.2%) were confirmed to have undergone a hysterectomy before enrollment. We excluded 37 hysterectomized women (8 virgins) who were missing HPV testing results by MY09/MY11 L1 consensus-primer PCR. We further excluded 38 women who had undergone the procedure as the result of cancer diagnosis (organ site unspecified), because of the high probability that many of these hysterectomies might have been the result of cervical cancer; the HPV DNA prevalence in these excluded women was 50%. Either Cervigrams (National Testing Laboratories Worldwide) or, when Cervigrams were missing, DenVu images (DenVu) were available for 564 (95.8%) of the remaining 589 women; expert review (J.J.) revealed that 20 (3.4%) of these hysterectomized women had intact cervices; these women also were excluded (HPV DNA prevalence, 40%). On the basis of the above criteria, 569 hysterectomized women (85.7%) were included in these analyses; they had a median age of 54 years (range, 27–88 years; only 1 woman was <30 years old). We note that this total included 25 women for whom we did not have either a Cervigram or a DenVu image with which to assess whether the cervix had been removed; 10 (40.0%) of these women were positive for HPV DNA.

Data and specimen collection. At enrollment, participants responded to an interviewer-administered risk-factor questionnaire that assessed information on sociodemographic characteristics; sexual, reproductive, and birth-control practices; cigarette smoking; and self-reported history of sexually transmitted diseases [6]. At the pelvic examination, after vaginal pH was measured by use of a pHydrion strip (Micro Essential Laboratories), exfoliated cervical cells were collected, conventional Pap smears were prepared, and the residual cells on the cytology sampler were suspended in PreservCyt (Cytyc), for semiautomated ThinPrep cytology (Cytyc). After the application of acetic acid, Cervigrams were taken (n.b., DenVu digital colposcopy images were collected during colposcopic examinations). An additional cervical-cell specimen was obtained by use of a Dacron swab, which was then placed in 1.0 mL of specimen transport medium (STM; Digene Corporation) and was stored frozen until used for HPV DNA testing [6]. Specimens from hysterectomized women without cervices were collected either from the vaginal cuff (if visible) or from the deepest portion of the vagina, as visualized with a speculum in place.

HPV DNA testing. HPV DNA was detected by MY09/M11 L1 consensus-primer PCR, with AmpliTaq Gold polymerase [7, 9]. Testing was performed masked to hysterectomy status. In brief, an aliquot of the STM specimen was lysed, and the specimen DNA was precipitated by use of ammonium acetate/ethanol solution and then pelleted by centrifugation. The DNA pellet was suspended in 10 mmol/L Tris (pH 8.0) containing 0.1 mmol/L EDTA and was stored frozen until used. Thermocycling conditions were as follows: initial denaturation for 9 min at 95°C; 40 cycles of denaturation for 60 s at 95°C, annealing for 60 s at 55°C, and extension for 60 s at 72°C; and final extension for 5 min at 72°C. A 100-cell copy of SiHa HPV DNA positive control, a 2-cell copy of SiHa HPV DNA positive control, and a 100-cell copy of HuH7 (a human hepatoma cell

Table 1. Self-reported reason for hysterectomy.

Reason for hysterectomy	No. (%) of women: Total	No. (%) of women: HPV DNA positive
Unknown[a]	32 (5.6)	6 (18.8)
Bleeding	199 (35.0)	57 (28.6)
Birth control	6 (1.1)	2 (33.3)
Benign tumor	165 (29.0)	49 (29.7)
Other reasons[b]	167 (29.3)	49 (29.3)
Overall	569 (100.0)	163 (28.6)

NOTE. Excluded from these analyses are 37 women who were missing HPV DNA results, 38 women who reported cancer as the reason for their hysterectomy, and 20 women who still had a cervix.

[a] Did not answer or did not know the reason.
[b] The primary other reason was prolapse ($n = 74$; 13.0%).

Table 2. DNA prevalences of human papillomavirus (HPV) types, in hysterectomized women and in nonhysterectomized women without pathology review–confirmed cervical intraepithelial neoplasia grade 2 or higher.

	Prevalence (95% CI), %		
	Hysterectomized women (n = 569)		Nonhysterectomized women (n = 6098)
Category	Crude	Age standardized[a]	
HPV, any type	**28.6** (25.0–32.6)	**32.0** (24.6– 39.3)	22.8 (21.8–23.9)
Cancer-associated types[b]	9.7 (7.4–12.4)	9.5 (5.0–14.1)	9.3 (8.6–10.0)
Non–cancer-associated types	**23.7** (20.3– 27.4)	**26.3** (19.3–33.2)	16.7 (15.7–17.6)
HPV type[c]			
6	0.7 (0.2–1.8)	…	0.5 (0.3–0.7)
11	0.2 (0.0–1.0)	…	0.2 (0.1–0.4)
16	1.8 (0.8–3.2)	1.4 (0.4–2.5)	2.1 (1.8–2.5)
18	1.1 (0.4–2.3)	…	0.9 (0.7–1.1)
26	0.0 (0.0–0.6)	…	0.2 (0.1–0.4)
31	0.4 (0.0–1.3)	…	1.0 (0.8–1.3)
32	0.9 (0.3–2.0)	…	0.3 (0.2–0.5)
33	0.7 (0.2–1.8)	…	0.6 (0.4–0.9)
35	0.4 (0.0–1.3)	…	0.3 (0.2–0.5)
39	0.7 (0.2–1.8)	…	0.6 (0.4–0.8)
40	0.0 (0.0–0.6)	…	0.1 (0.1–0.3)
45	0.9 (0.3–2.0)	…	0.7 (0.5–0.9)
51	1.2 (0.5–2.5)	…	1.5 (1.2–1.9)
52	1.6 (0.7–3.0)	…	1.0 (0.8–1.3)
53	1.9 (1.0–3.4)	3.1 (0.0–7.1)	1.9 (1.5–2.2)
54	0.7 (0.2–1.8)	…	0.3 (0.2–0.5)
55	0.2 (0.0–1.0)	…	0.2 (0.1–0.4)
56	0.4 (0.0–1.3)	…	0.7 (0.5–1.0)
58	1.9 (1.0–3.4)	1.8 (0.6–3.0)	1.2 (0.9–1.5)
59	0.2 (0.0–1.0)	…	0.2 (0.1–0.4)
61	**4.0** (2.6–6.0)	4.1 (2.2–6.0)	2.3 (1.9–2.7)
62	1.8 (0.8–3.2)	1.2 (0.4–2.1)	1.7 (1.4–2.0)
66	0.9 (0.3–2.0)	…	0.5 (0.4–0.8)
67	0.0 (0.0–0.6)	…	0.1 (0.1–0.3)
68	0.2 (0.0–1.0)	…	0.2 (0.1–0.3)
70	2.6 (1.5–4.3)	2.2 (0.9–3.5)	1.6 (1.3–2.0)
71	**3.9** (2.4–5.8)	2.8 (1.5–4.2)	2.5 (2.1–2.9)
72	**1.2** (0.5–2.5)	…	0.2 (0.1–0.4)
73	0.4 (0.0–1.3)	…	0.3 (0.2–0.5)
81	1.6 (0.7–3.0)	…	1.1 (0.9–1.4)
82v	0.2 (0.0–1.0)	…	0.3 (0.2–0.5)
83	1.1 (0.4–2.3)	…	1.2 (1.0–1.5)
84	0.5 (0.1–1.5)	…	0.6 (0.4–0.8)
85	1.2 (0.5–2.5)	…	0.7 (0.5–0.9)
89	0.7 (0.2–1.8)	…	0.2 (0.1–0.4)
AE10[d]	0.4 (0.0–1.3)	…	0.1 (0.1–0.3)
dbmix[e]	2.1 (1.1–3.7)	…	1.0 (0.8–1.3)
Uncharacterized[f]	3.5 (2.2–5.4)	…	2.7 (2.3–3.2)

NOTE. A statistically significant increase ($P<.05$) in HPV DNA prevalence in one group of women, compared with the other group, is indicated in boldface. CI, confidence interval.

[a] Standardized to the age distribution of the nonhysterectomized women.

[b] HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

[c] For hysterectomized women, there are 2 missing typing results; for nonhysterectomized women, there are 26 missing typing results.

[d] For hysterectomized women, there are 2 missing typing results; for nonhysterectomized women, there are 4 missing typing results.

[e] HPV types 2, 13, 34, 42–44, 57, 62, 64, 69, 74, 82, and AE9.

[f] HPV DNA positive for an uncharacterized type.

Table 3. DNA prevalences of human papillomavirus (HPV) types grouped by phylogenetic clades, in hysterectomized women and in nonhysterectomized women with pathology review–confirmed cervical intraepithelial neoplasia grade 2 or higher.

	Prevalence (95% CI), %		
	Hysterectomized women (n = 569)		Nonhysterectomized women (n = 6098)
Clade(s)[a]	Crude	Age standardized[b]	
A9/A11	6.7 (4.8–9.1)	7.4 (3.0–11.8)	6.2 (5.5–6.8)
A7	6.2 (4.3–8.5)	8.4 (2.9–13.9)	4.5 (4.0–5.1)
A5/A6	3.9 (2.4–5.8)	4.6 (0.5–8.7)	5.0 (4.4–5.5)
A3/A4/A15	**11.4** (8.9–14.3)	**9.9** (7.2–12.5)	7.3 (6.7–8.0)
A1/A8/A10	3.0 (1.7–4.7)	**6.6** (1.5–12.1)	1.8 (1.5–2.2)

NOTE. A statistically significant increase ($P<.05$) in vaginal HPV DNA prevalence in hysterectomized women, compared with cervical HPV DNA prevalence in nonhysterectomized women, is indicated in boldface. CI, confidence interval.

[a] A9/11: HPV types 16, 31, 33–35, 52, 58, 67, and 73; A7: HPV types 18, 39, 45, 59, 68, 70, and 85; A5/A6: HPV types 26, 51, 53, 56, 66, 69, and 82v; A3/A4/A15: HPV types 61, 71, 72, 81, 83, 84, and 89; A1/A8/A10: HPV types 6, 11, 32, 40, 54, 55, and AE10.

[b] Standardized to the age distribution of the nonhysterectomized women.

line) HPV DNA negative control were used per every 48 specimens tested.

PCR products were analyzed by gel electrophoresis and were then transferred to nylon filters. The filters were hybridized overnight with radiolabeled generic probes for HPV (HPV types 11, 16, 18, 51, 73, and 81 combined). Thereafter, HPV PCR products were typed by dot-blot hybridization, with type-specific oligonucleotide probes for the following HPV types [7, 9]: 2, 6, 11, 13, 16, 18, 26, 31–35, 39, 40, 42–45, 51–59, 61, 62, 64, 66–74, 81–85, 82v (AE2), 89, AE9, and AE10. Probes for HPV types 2, 13, 34, 42–44, 57, 62, 64, 69, 74, 82, and AE9 were combined in dot-blot hybridizations for the detection of rare types (dbmix). A specimen was considered to be HPV positive but uncharacterized if it tested positive for HPV DNA by the radiolabeled generic-probe mix but was not positive for any type-specific probe. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 were considered to be the primary cancer-associated types [1]. HPV types were also grouped according to the following viral phylogenetic clades: (1) A9/A11: HPV types 16, 31, 33–35, 52, 58, 67, and 73; (2) A7: HPV types 18, 39, 45, 59, 68, 70, and 85; (3) A5/A6: HPV types 26, 51, 53, 56, 66, 69, and 82v; (4) A3/A4/A15: HPV types 61, 62, 71, 72, 81, 83, 84, and 89; and (5) A1/A8/A10: HPV types 6, 11, 32, 40, 54, 55, and AE10. Three experienced investigators interpreted each dot-blot result, and discrepancies were resolved by consensus. Two observers evaluated the signal strength (1 [low] to 5 [high]) of the PCR products for the generic-probe set and the type-specific probes, which we used as a crude measure of HPV load [10].

HPV serologic testing. Virus-like particles were prepared in *Trichoplusia ni* cells (High Five; Invitrogen) from recombinant baculoviruses that were expressing either the L1 and L2 gene of HPV types 16 or 31 or the L1 gene alone of HPV type 18; the particles were then purified by density-gradient ultracentrifugation and column-chromatography techniques. ELISAs that were specific for HPV types 16, 18, and 31 were performed as described elsewhere [11], except for the use of an automatic plate washer (Skanwasher 300; Skatron) and a MultiPROBE II robotic liquid-handling system (Packard Instruments), to dilute serum samples 1:10 in 0.5% polyvinyl alcohol (PVA; molecular weight, 30,000–70,000; Sigma) and to add 10 μL of the diluted serum sample to antigen-coated plates containing 100 μL of 0.5% PVA/well.

The cut point for positive results was determined from the reactivity of concurrently tested plasma samples from self-reported virgins, within a batch (n = 200) [8]. The mean and SD of the optical-density values (i.e., signal) for controls were calculated, and values greater than the mean + 2 SDs were excluded. The analysis was repeated until no further optical-density values could be excluded by this criterion, and the cut point for seropositivity was set for each batch at 5 SDs above the mean of this distribution. The κ for interlaboratory agreement between 2 laboratories, for HPV type 16 seropositivity on a subset of 2,998 plasma specimens, was 0.67 (95% confidence interval [CI], 0.64–0.71).

Statistical analysis. Prevalence estimates and binomial exact 95% CIs were calculated for each individual HPV type and serotype, for any HPV type or serotype, for categories of cancer-associated HPV types and non–cancer-associated HPV types, and for genetic clades. Prevalences of HPV within categories were stratified by age group (30–34, 35–44, 45–54, 55–64, 65–74, and ⩾75 years). Age-standardized HPV DNA prevalences and seroprevalences in hysterectomized women (n.b., age-standardized prevalences for single types were calculated only for the most prevalent HPV types: 16, 53, 58, 61, 62, 70, and 71) were calculated, and the age distributions of the nonhysterectomized women were used to control for the aforementioned differences in age distributions in groups.

We also stratified cancer-associated and non–cancer-associated HPV DNA prevalences by age group (30–34, 45–64, and ⩾65 years) and by lifetime number of sex partners (1, 2–3, and ⩾4 partners), and stratified HPV DNA prevalences for all types by age group and by time since hysterectomy (<5, 5–14, and ⩾15 years).

Differences in crude and age-standardized HPV DNA and seroprevalences were tested for statistical significance ($P<.05$) by Pearson's χ^2 test and by age-adjusted logistic regression, respectively. Multivariate logistic models also were used to explore determinants of HPV DNA in hysterectomized women. PCR signal strength for the generic-probe set and for the type-specific probes (the strongest signal for any type) were used as crude estimators of total and type-specific virus load, respec-

Table 4. Comparison of polymerase chain reaction (PCR) signal strength, a surrogate for HPV load, in HPV DNA–positive vaginal specimens from hysterectomized (H) women and in HPV DNA–positive cervical specimens from nonhysterectomized women (NH) without pathology review–confirmed cervical intraepithelial neoplasia grade 2 or higher.

PCR signal strength[a]	Any HPV type				Cancer-associated HPV type				Non–cancer-associated HPV type			
	Generic probe[b]		Type-specific probe[c]		Generic probe[d]		Type-specific probe[e]		Generic probe[f]		Type-specific probe[g]	
	NH	H	NH	H	NH	H	NH	H	NH	H	NH	H
1	207 (14.9)	16 (9.8)	252 (18.1)	23 (14.1)	40 (7.1)	2 (3.6)	88 (15.6)	10 (18.2)	174 (17.1)	14 (10.4)	227 (22.4)	21 (15.6)
2	276 (19.9)	21 (12.9)	248 (17.8)	22 (13.5)	91 (16.1)	7 (12.7)	91 (16.1)	6 (10.9)	195 (19.2)	15 (11.1)	193 (19.0)	19 (14.1)
3	273 (19.6)	41 (25.2)	230 (16.6)	24 (14.7)	112 (19.8)	13 (23.6)	85 (15.0)	9 (16.4)	200 (19.7)	32 (23.7)	186 (18.3)	20 (14.8)
4	242 (17.4)	27 (16.6)	280 (20.1)	41 (25.2)	109 (19.3)	11 (20.0)	116 (20.5)	12 (21.8)	165 (16.3)	20 (14.8)	197 (19.4)	37 (27.4)
5	392 (28.2)	58 (35.6)	380 (27.3)	53 (32.5)	214 (37.8)	22 (40.0)	186 (32.9)	18 (32.7)	281 (27.7)	54 (40.0)	212 (20.9)	38 (28.2)
Total	1390	163	1390	163	566	55	566	55	1015	135	1015	135

NOTE. Data are no. (%) of HPV DNA–positive specimens. Signal strength was based on the strongest signal, for a type-specific probe, and on the generic-probe set (which has probes for HPV types 11, 16, 18, 51, 73, and 81), for generic probes.

[a] In the scale of 1–5, 1 is low and 5 is high.
[b] $P_{Trend} = .008$.
[c] $P_{Trend} = .02$.
[d] $P_{Trend} = .4$.
[e] $P_{Trend} = 1.0$.
[f] $P_{Trend} = .0008$.
[g] $P_{Trend} = .002$.

tively, and the differences between the measurements of the 2 groups of women were evaluated by Pearson's χ^2 test and by the Mantel-Haenszel extension test for trend. Differences between the optical densities for type-specific–seropositive hysterectomized women and those for type-specific–seropositive nonhysterectomized women were evaluated for significance by nonparametric analysis of variance (the Kruskal-Wallis test).

RESULTS

The self-reported reasons for hysterectomy are shown in table 1. The primary reported reason for hysterectomy was bleeding ($n = 199$; 35.0%). There were no significant differences in HPV prevalence by the reported reason for hysterectomy (table 1). We compared these women to cohort members 30–94 years old who had not undergone hysterectomy ($n = 6098$; 66.5%), excluding those women with prevalent, pathology review–confirmed cervical intraepithelial neoplasia (CIN) grade 2, CIN3, or cancer (⩾CIN2) but including those women with CIN1 (because we consider CIN1 to be a histopathologic manifestation of a productive HPV infection). The median age of the women in this group was 43 years, which was significantly younger than that for the hysterectomized women ($P = .0001$; Kruskal-Wallis test).

HPV prevalence. HPV DNA was detected more often in vaginal specimens from hysterectomized women (crude, 28.6% [95% CI, 25.0%–32.6%]; age standardized, 32.0% [95% CI, 24.6%–39.3%]) than in cervical specimens from nonhysterectomized women ⩾30 years old (22.8% [95% CI, 21.8%–23.9%]) ($P = .002$, for crude; $P = .006$, for age standardized) (table 2) (n.b., for reference, the HPV DNA prevalence among nonhysterectomized women, including those with pathology review–confirmed ⩾CIN2, was 24.1%). The proportions of hysterectomized women (34.3%) and nonhysterectomized women (30.2%) infected with multiple HPV types did not differ significantly ($P = .3$).

Non–cancer-associated HPV types were detected more frequently in vaginal specimens from hysterectomized women (crude, 23.7% [95% CI, 20.3%–27.4%]; age standardized, 26.3% [95% CI, 19.3%–33.2%]) than in cervical specimens from nonhysterectomized women (16.7% [95% CI, 15.7%–17.6%]) ($P < .0005$, for crude; $P = .001$, for age standardized). However, there was no difference between the detection of cancer-associated HPV types in vaginal specimens from hysterectomized women (crude, 9.7% [95% CI, 7.4%–12.4%]; age standardized, 9.5% [95% CI, 5.0%–14.1%]) and that in cervical specimens from the nonhysterectomized women (9.3% [95% CI, 8.6%–10.0%]).

In regards to the 48 individual HPV types assayed, hysterectomized women were more likely to be positive for types 58, 61, 70, 71, 72, and 85 and the composite of 2, 13, 34, 42–44, 57, 62, 64, 69, 74, 82, and AE9 than were nonhysterectomized women (table 2). The differences in crude prevalence were significant only for types 61, 71, and 72; after age standardization, there was a significant difference in type 72 prevalence and a marginally significant difference in type 61 ($P = .05$). When grouped by genetically related types (table 3), HPV types of the A3/A4/A15 phylogenetic clades (types 61, 71, 72, 81, 83, 84, and 89) were more prevalent in vaginal specimens from hysterectomized women (crude, 11.4% [95% CI, 9.1%–14.4%]; age standardized, 9.9% [95% CI, 7.2%–12.5%]) than in cervical specimens from nonhysterectomized women (7.3% [95% CI, 6.7%–8.0%]) ($P < .0005$, for crude; $P = .008$, for age standardized). HPV types in the A1/A8/A10 clades (types 6, 11, 32,

Table 5. Seroprevalences of human papillomavirus (HPV) types 16, 18, and 31, in hysterectomized women and in nonhysterectomized women without pathology review–confirmed cervical intraepithelial neoplasia grade 2 or higher.

	Hysterectomized women (n = 569)			Nonhysterectomized women (n = 6098)		
		Positive (95% CI), %				
HPV type	No. tested	Crude	Age standardized[a]	No. tested	Positive (95% CI), %	P[b]
16	567	13.4 (10.7–16.5)	10.6 (8.0–13.3)	6061	16.5 (15.5–17.4)	.06
18	566	17.7 (14.6–21.1)	20.3 (13.5–27.0)	6056	16.9 (16.0–17.9)	.7
31	567	15.7 (12.8–19.0)	19.5 (12.8–26.2)	6056	17.0 (16.0–17.9)	.4
16, 18, or 31	566	30.6 (26.8–34.5)	33.6 (26.1–41.0)	6056	32.1 (30.9–33.3)	.5

NOTE. CI, confidence interval.

[a] Standardized to the age distribution of the nonhysterectomized women.

[b] To compare differences between the crude seropositivity in hysterectomized women and that in nonhysterectomized women, Pearson's χ^2 test was used.

40, 54, 55, and AE10) were more prevalent in vaginal specimens from the hysterectomized women than in cervical specimens from the nonhysterectomized women ($P = .05$, for crude; $P = .04$, for age standardized). In contrast, there were no apparent differences in the prevalences of HPV types in the A5/A6 and A9/A11 clades.

There was no significant difference between the distribution of PCR signal strength of cancer-associated HPV infections in the nonhysterectomized women and that in the hysterectomized women (table 4) (n.b., although PCR signal strength is a qualitative evaluation—and therefore not a true measure of virus load—and is subject to variability due to DNA purity, specimens were tested masked to hysterectomy status, and thus there is no bias in the interpretation of these data). Among specimens positive for a non–cancer-associated HPV type, there was a strong trend for the PCR signal strength of specimens from the hysterectomized women to be greater than that of specimens from the nonhysterectomized women ($P_{\text{Trend}} = .0008$, for the generic-probe set; $P_{\text{Trend}} = .002$, for the maximum signal strength for a type-specific probe). When restricted to single non–cancer-associated infections, this association of signal strength and hysterectomy status persisted ($P_{\text{Trend}} = .002$, for the generic-probe set; $P_{\text{Trend}} = .04$, for the maximal signal strength.

Hysterectomized women had a marginally lower HPV type 16 seroprevalence (13.4% [95% CI, 10.7%–16.5%]), compared with the nonhysterectomized women (16.5% [95% CI, 15.5%–17.4%] ($P = .06$), but this difference was not significant after age adjustment. There were no significant differences between the crude or age-standardized seroprevalences of HPV types 18 and 31 or of seroprevalence for any of the 3 types (table 5). There also were no differences, for any single HPV type, between the optical densities of the ELISAs with seropositive results for the hysterectomized women and those for the nonhysterectomized women (data not shown).

HPV DNA prevalence by age, sexual behavior, and time since hysterectomy. The prevalence of non–cancer-associated HPV types was higher in hysterectomized women than in nonhysterectomized women for the age groups 30–34 ($P = .3$), 35–44 ($P = .001$), 45–54 ($P = .07$), and 55–64 years ($P = .2$), which explains the differences between the age-specific prevalences for any HPV type in hysterectomized women and those for any HPV type in nonhysterectomized women (figure 1). In contrast, the prevalences of cancer-associated HPV types in various age groups did not differ by hysterectomy status.

We next examined whether vaginal infection in hysterectomized women was associated with sexual behavior. In a stratified analysis considering age group (30–44, 45–64, or ⩾65 years), lifetime number of sex partners (1, 2–3, or ⩾4 partners), and prevalence of HPV by risk category (cancer-associated or non–cancer-associated), the prevalence of vaginal HPV infection in hysterectomized women, like the prevalence of cervical infection in nonhysterectomized women, was generally higher in women with >1 lifetime sex partner, for each age group and for each HPV category (figure 2). The small numbers in these groups may explain the notable exceptions—specifically, cancer-associated HPV in the age groups for older hysterectomized women.

We also considered the effect that time since hysterectomy has on overall HPV prevalence (figure 3). In each of the 3 age groups, there was increased HPV prevalence associated with longer times since hysterectomy (5–14 years and ⩾15 years, compared with <5 years), although only the difference for the age group 45–64 years was statistically significant ($P = .03$). There were no obvious differences in these patterns when stratified by HPV risk category.

In a multivariate logistic model, the presence of vaginal HPV DNA in hysterectomized women was positively associated with a higher lifetime and recent number of sex partners, time since hysterectomy, and living with one's husband only part of the

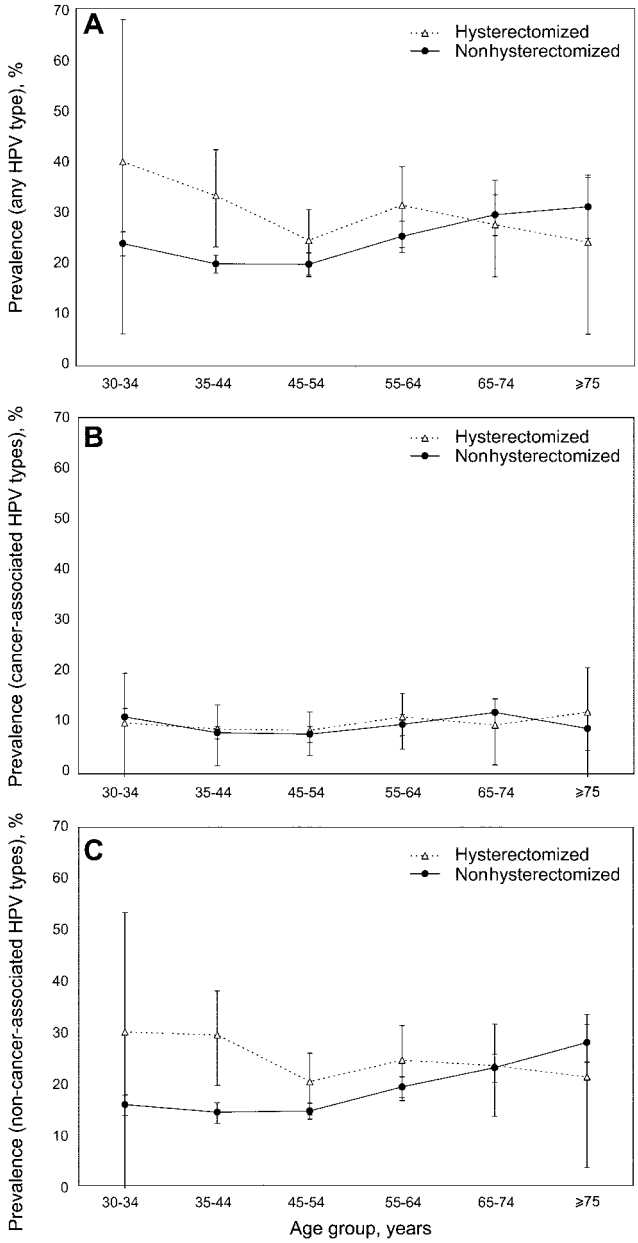

Figure 1. Age group–specific human papillomavirus (HPV) prevalences for any HPV type *(A),* cancer-associated HPV types *(B),* and non–cancer-associated HPV types *(C),* for hysterectomized women and for nonhysterectomized women without pathology review–confirmed cervical intraepithelial neoplasia grade 2 or higher.

year. It was negatively associated with number of pregnancies (data not shown).

DISCUSSION

To our knowledge, this is the first population-based study of HPV prevalence in hysterectomized women, and it permitted an examination of vaginal infection without the influence or confounding effects of cervical tissue. In our analysis, we demonstrated that (1) non–cancer-associated HPV types were detected more frequently in the vaginal specimens from the women who had undergone a total hysterectomy than in the cervical specimens from nonhysterectomized women of similar age; (2) detection of cancer-associated HPV types was similar in these 2 groups; and (3) the presence of vaginal HPV DNA in hysterectomized women (and of cervical HPV DNA in nonhysterectomized women) was related to a higher lifetime number of sex partners, a result suggesting that these HPV infections were sexually transmitted. These vaginal infections with non–cancer-associated HPV types were less likely to cause any indication of cytologic abnormalities (i.e., equivocal or more-severe cytologic interpretations) than were cervical infections, despite the tendency for higher PCR signal strengths (suggestive of high virus loads) in the vaginal specimens, compared with the cervical specimens. Cancer-associated HPV infections, including infections with type 16, were similarly prevalent but were less likely to cause any indication of cytologic abnormalities in the vaginal specimens from hysterectomized women than they were in the cervical specimens from nonhysterectomized women. It is uncertain how many of these vaginal infections were persistent, how many were acquired before hysterectomy, and what impact, if any, hysterectomy itself has on HPV prevalence or on the risk of vaginal and vulvar cancer.

In each age group (30–44, 45–64, and ⩾65 years), the HPV DNA prevalence for any type was higher in the substrata with longer times since hysterectomy. We infer from these data that the presence of HPV in the vaginas of hysterectomized women cannot be explained by exfoliated infected cervical epithelial residing in the vagina after surgical removal of the cervix. Although we cannot rule out the husbands' having new sex partners as being the causative factor, it is particularly noteworthy that HPV DNA prevalence was very high in the oldest age group, whose members we expect to be less likely to have new sex partners. Given the relationship that prevalence has with duration and incidence, we argue that some vaginal infections may benignly persist for extended periods of time and might be considered to be commensal infections.

We offer 2 hypotheses that could explain the higher prevalence of non–cancer-associated HPV types in hysterectomized women versus that in nonhysterectomized women. First, we note that vaginal sampling in hysterectomized women exclusively collects squamous epithelial cells, whereas cervical sampling collects squamous, columnar, and metaplastic epithelial cells. Despite the close proximity and continuity of these 2 anatomic locations, we cannot rule out that this difference in cell sampling, rather than hysterectomy status per se, may explain differences in prevalence estimates. Studies of vaginal self-sampling for HPV testing also have shown an increased prevalence for non–cancer-associated types, compared with the prevalence observed with physician-directed sampling of the

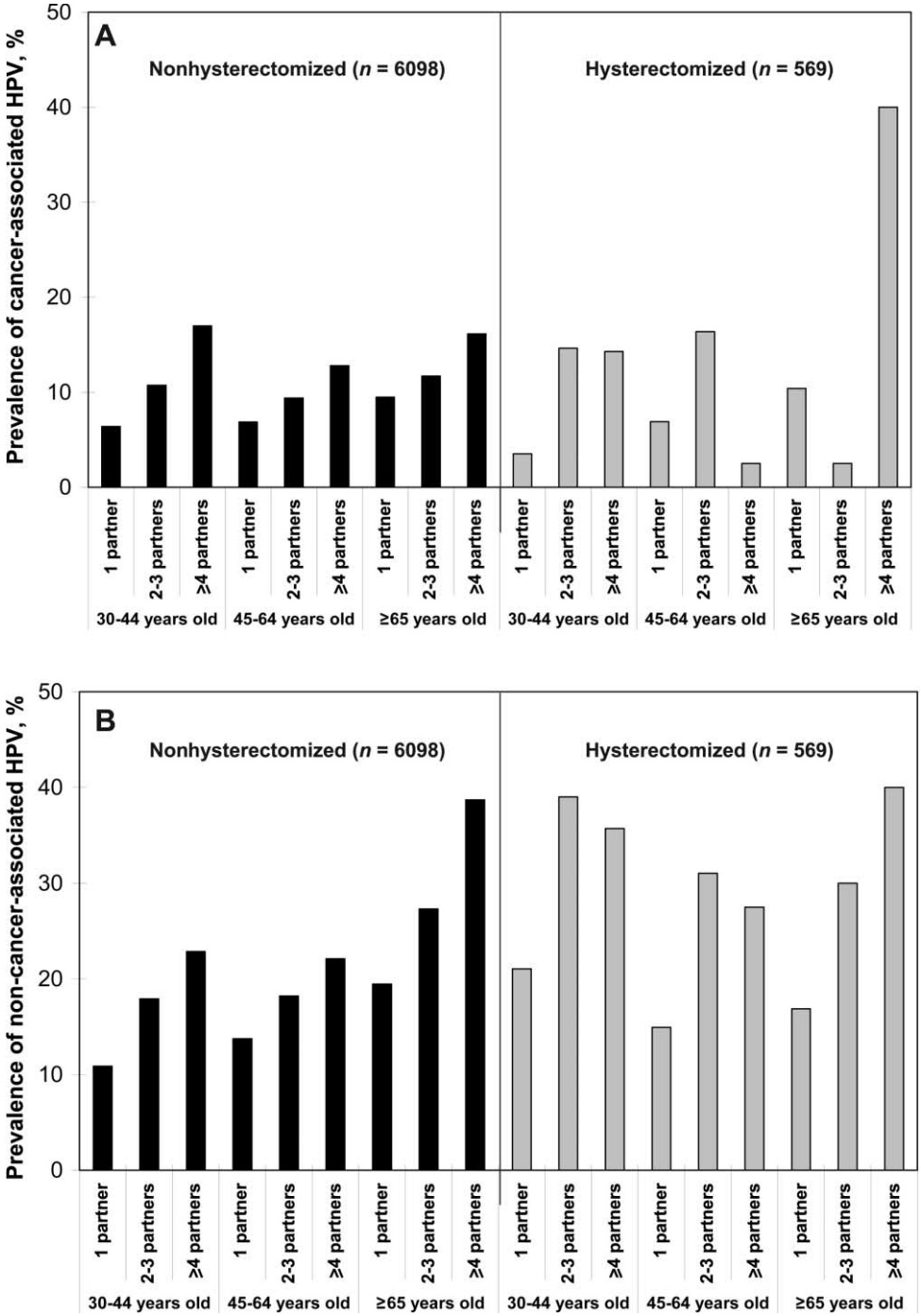

Figure 2. Prevalences of cancer-associated human papillomavirus (HPV) types *(A)* and of non–cancer-associated HPV types *(B),* in strata defined by both age (30–44, 45–64, and ⩾65 years) and lifetime no. of sex partners (1, 2–3, and ⩾4 partners), for hysterectomized women and for nonhysterectomized women without pathology review–confirmed cervical intraepithelial neoplasia grade 2 or higher.

ectocervix [12]. Thus, there may be tropism of non–cancer-associated HPV types for vaginal squamous epithelial cells, especially those of the A3/A4/A15 clades, compared with squamous metaplastic cells of the cervical transformation zone, where nearly all HPV-induced cancer (by cancer-associated types) in the lower genital tract occur. In support of this explanation, we note the following evidence: (1) the prevalences of non–cancer-associated HPV types in older (⩾55 years) hysterectomized women and in postmenopausal, nonhysterectomized women for whom the squamocolumnar junction had migrated into the endocervix were similar; (2) the prevalence of non–cancer-associated HPV was higher in the self-reported

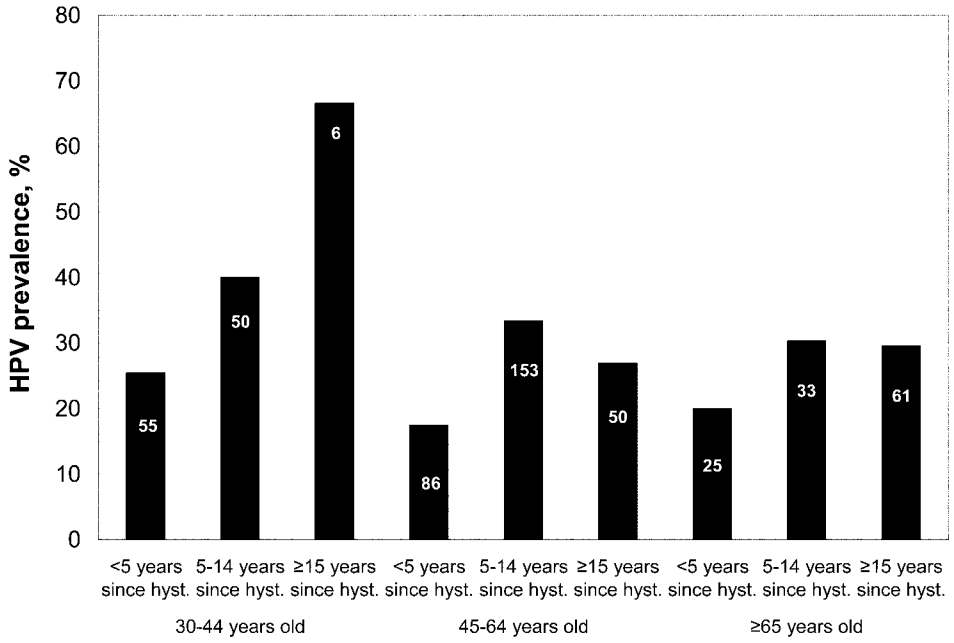

Figure 3. Prevalence of HPV infection of any type, in strata defined by both age (30–44, 45–64, and ⩾65 years) and time since hysterectomy (hyst.) (<5, 5–14, and ⩾15 years). The nos. in the bars indicate the no. of women in that strata.

menopausal women (19.8%) than in the women who still had menstrual cycles (14.8%) ($P < .0005$) (data not shown); (3) the occurrence of cytologic abnormalities in the nonhysterectomized women ⩾55 years old was similar (6.0%) to that in hysterectomized women (6.4%), which was ~50% less common than was the occurrence of cytologic abnormalities in nonhysterectomized women <55 years old (12.6%) ($P < .0001$) (data not shown); and (4) the prevalences of the A3/A4/A15 clades in hysterectomized women <55 years old (11.4%), in hysterectomized women ⩾55 years old (11.5%), and in nonhysterectomized women ⩾55 years old (10.8%) were similar.

Second, it is possible that hysterectomized women had engaged in different sexual behaviors or had selected male sex partners from different risk groups. Gross differences in the sexual practices of hysterectomized and nonhysterectomized women do not appear to explain the differences in HPV DNA prevalence, because hysterectomized women were more likely, rather than less likely, to have been monogamous recently than were nonhysterectomized women (35.7% vs. 21.9%) ($P < .0005$). On the basis of the observed high seroprevalence of herpes simplex virus 2 in monogamous, Guanacastecan women [13], we infer that, in our study population, the transmission of HPV is highly dependent on male behavior. However, we note that the difference in DNA prevalence was for non–cancer-associated types only, which argues against this explanation, because both cancer-associated and non–cancer-associated HPV types are sexually transmitted. The comparability of past HPV exposure, as measured by HPV serologic testing (seroprevalence and optical density among seropositive specimens), for the 2 groups of women further suggests the similarity between the groups, their hysterectomy status notwithstanding.

In addition to general screening, HPV testing is being considered for follow-up of patients who are treated for high-grade cervical neoplasia and cancer, to detect recurrence [14–18]. Such an approach may warrant caution and further consideration, given the prevalence of cancer-associated HPV in the vaginas of hysterectomized women. In the context of treatment for ⩾CIN2, we can infer from our data that a significant number of women (~10%) would test positive for cancer-associated HPV years after treatment, resulting in a large number of additional referrals and causing undue worry in these patients. Type-specific detection in the follow-up cervical specimen of the HPV type that had caused the precancerous or cancerous lesion may result in a more specific test of recurrence, but this strategy is dependent on whether vaginal infection of the same type is present at the time of treatment and on whether it persists for extended periods of time beyond treatment. However, the current FDA-approved clinical HPV DNA test is not type specific, and type-specific PCR assays are not yet sufficiently standardized, reliable, and reproducible for the clinical management of primary and secondary cervical precancer and cancer. Thus, physicians using posttreatment HPV DNA testing to monitor for recurrent cervical neoplasia should be aware of the high prevalence of cancer-associated HPV types in the vagina.

In summary, we found that HPV infections were highly prevalent in hysterectomized women, with no significant differences between the prevalences of cancer-associated types. To date, the prevalence of HPV, by age group and by risk group, in hyster-

ectomized women in other populations has not been reported. The increased prevalence of non–cancer-associated types in hysterectomized women versus that in nonhysterectomized women is unexplained but could be the result of differences in viral-tissue tropism, of the sampling of different locations of the genital tract, or of differences in genital-tract physiology. However, the observed increase in the prevalence of non–cancer-associated HPV with age such that the prevalence in older nonhysterectomized women is approximately the same as that in hysterectomized women (as previously observed in this population [6, 19] and in other populations [20–22]) is not apparent in all populations [23, 24]. We also cannot rule out differences in our 2 subpopulations in Guanacaste. Studies of other populations, with follow-up measurements, are needed to resolve some of these questions about vaginal HPV infections. On the basis of the present study's data, we suggest that the cervix, and specifically the cervical transformation zone, may not be required for cancer-associated HPV infection but may be predisposed to carcinogenic transformation by cancer-associated HPV.

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