

Human Papillomavirus 16 and 18 L1 Serology Compared across Anogenital Cancer Sites¹

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ABSTRACT

Human papillomavirus (HPV) DNA has been detected in the great majority of cancers of the uterine cervix and anus, whereas the association of HPV DNA with cancer at other anogenital sites has produced less consistent results. This study was designed to compare HPV exposure among anogenital cancer cases and matched controls. Cases (1782) of anogenital cancer diagnosed in the Seattle area from 1978 to 1998 were identified and interviewed. Their responses were compared with those of 2383 age- and sex-matched controls. Blood was drawn at interview from both cases and controls and tested for antibodies to HPV-16 and HPV-18. Tissue blocks were tested for HPV DNA for 649 cases. Serum antibodies to HPV-16 were associated with *in situ* and invasive cancer at all sites among men and women with the exception of *in situ* penile cancer. Anti-HPV-18 antibodies were associated with cancers at all sites among women. The increased risk of cancer associated with HPV-16 seropositivity ranged from odds ratio = 1.8 (95% confidence interval, 1.4–2.5) for adenocarcinoma of the cervix to odds ratio = 5.9 (95% confidence interval, 3.4–10.3) for anal cancer in men. Associations between seroprevalence and cancers were stronger when analyses were restricted to HPV-16- or HPV-18 DNA-positive cases. HPV DNA was detected in >80% of cancers from all sites tested. HPV-16 DNA was the type most frequently detected at all sites (range, 40.9–82.2%). HPV-18 DNA was detected in 44.7% of adenocarcinomas of the cervix but detected much less often (2.6–18.1%) at other sites. These findings support an important role for HPV infection in anogenital cancer at all sites. Differences in the proportion of seropositives among HPV-16 DNA-positive cases by site suggest either that the immune response varies by site or that cancer development may lead to changes in antibody responses in a site-specific fashion.

INTRODUCTION

Cancers of the uterine cervix and anus result, in part, from infection with sexually transmitted HPVs³ (1). HPVs are believed to cause a subset of vulvar, vaginal, and penile cancers as well; however, the detection of HPV DNA in these cancers has varied widely between studies.

To assess the risk of neoplasia due to HPV infection or to examine epidemiological risk factors that may influence the development of HPV-associated cancers, it is important to estimate exposures in both case and control populations in a common geographical setting. Testing for HPV DNA in tumors is an effective method for measuring exposure among cases, but not for determining exposure in controls. Most HPV infections are transient, and natural history studies have

shown that HPV DNA is detectable in cells from the cervix for less than a year in most infected women (2). Therefore, the presence or absence of HPV DNA at a single time point is a poor indicator of lifetime exposure. To mitigate this problem, serological tools to detect HPV antibodies have been developed. The best characterized HPV serology test is an ELISA using HPV capsids [or virus-like particles (3, 4)]. Although serology is not a perfect measure of HPV exposure, the majority of infected women develop type-specific HPV antibodies (3).

Anti-capsid antibodies have been shown to correlate with the detection of HPV DNA in a type-specific fashion and to correlate with the lifetime number of sexual partners (for reviews, see Refs. 3 and 4). Among individuals who develop responses, those antibodies persist for many years (5–7). Anti-capsid antibodies are imperfect indicators of lifetime HPV exposure because serum antibodies to HPV-16 capsids are undetectable in 20–40% of women who test positive for HPV-16 DNA in cells from the cervical mucosa (3, 4).

Case-control and prospective studies have demonstrated an association of HPV serum antibodies with cervical, anal, vulvar, and vaginal cancers (8–14). One study of penile cancer found an association with HPV-16 antibodies (15), whereas another study did not (16). Most studies have focused on HPV-16; however, antibodies to other HPV types have been examined recently (14, 17, 18).

This study was designed to examine, in a single geographically defined population, the relationship between cancer at all anogenital sites and HPV serum antibody responses to HPV-16 and HPV-18 capsids in women and men. Interviews and serological testing were also conducted on age- and gender-matched controls. Tumor specimens were tested for the presence of HPV DNA by PCR. Earlier studies from this project regarding the relationship of antibody reactivity to cervical and vulvar cancer have been reported (8, 9).

MATERIALS AND METHODS

Subject Identification, Eligibility, and Recruitment. This study combines information from six case-control studies of anogenital cancer conducted in the population served by the CSS. The CSS is a population-based cancer registry that is part of the Surveillance, Epidemiology, and End Results program of the United States National Cancer Institute (19). Subjects for the case-control studies of invasive cervical cancer, vulvar cancer, and anal cancer were residents of a three-county area that includes Seattle, Washington. Study subjects for the more rare anogenital cancer sites (adenocarcinoma *in situ* of the cervix, vaginal cancer, and penile cancer) resided in a larger 13-county area that includes the most populous counties in western Washington State.

Case Identification. The initial diagnosis dates of the eligible cases, all of whom were identified from hospital and pathology laboratory records by the CSS, varied by site: (a) invasive cervical cancer, 1986; (b) *in situ* adenocarcinoma of the cervix, 1990; (c) vulvar cancer, 1986; (d) anal cancer, 1986; (e) vaginal cancer, 1981; and (f) penile cancer, 1979. The overall participation rate for the in-person interview was 65.1% and ranged from 55.0% (penile cancer cases) to 74.9% (adenocarcinoma *in situ* of the cervix cases). By individual study, the interview response proportions were 65.6% for invasive cervical cancer, 74.9% for adenocarcinoma *in situ*, 68.2% for vulvar cancer, 63.1% for anal cancer, 57.3% for vaginal cancer, and 55.0% for penile cancer patients.

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³ The abbreviations used are: HPV, human papillomavirus; OR, odds ratio; RDD, random digit dialing; ROC, receiver operator characteristic; CI, confidence interval; CSS, Cancer Surveillance System; ICDO, International Classification of Diseases for Oncology.

Reasons for nonresponse included patient death (7.3%), physician refusal (7.5%), and patient refusal (20.1%). Squamous cell invasive (ICDO 8010, 8012, and 8070–8077) or *in situ* and invasive adenocarcinoma histologies (ICDO 8140) were included in the analysis of cervical cancer; for the other sites, only cases with squamous cell cancers were included in this study (ICDO 8010, 8051–8081, and 8094). Cases who had their blood drawn at the time of interview were available for this analysis. Blood samples were collected from 91.7% of case subjects who were interviewed.

Control Identification. The population-based controls were identified by use of RDD (20). Simple unrestricted RDD was used to recruit all male controls (20). Female controls were selected using simple unrestricted RDD before 1996. The Waksberg-Mitofsky modification of RDD with a clustering factor (denoted “k” by Waksberg) of two residences/sampling unit was used to recruit controls beginning in 1996 (21). Each telephone number selected was called at least nine times at different times of the day and week during a 3-week period before the number was abandoned. Telephone numbers that resulted in refusals (refusal to answer the screening questions or to receive a letter describing the study) or contact with an answering machine in all nine attempts were called again by a different interviewer 3–5 months later. Forty-eight percent of these numbers were successfully screened on the second attempt. One-step recruitment was used (22), with a stratified sampling design that recruited controls evenly throughout the ascertainment period into 5-year age and county strata to approximate the age and county distribution of the cases.

Eligibility requirements included residence in 1 of the 13 counties of western Washington State, having a working telephone, residing in a noninstitutionalized setting, and the ability to communicate in English. Only women with intact uteri were eligible as control subjects for the cervical cancer studies.

A household census was conducted for the randomly selected residential phone numbers called. The screening response rate for male controls was 94%, and the interview response rate was 66%, for an overall response rate of 62%. For female controls, the screening response rate was 92%, and the interview response rate was 68%, for an overall response rate of 62.6%. Blood samples were collected from 88.3% of control subjects who were interviewed.

Data Collection. A team of interviewers administered a detailed interview in a standardized way to case and control subjects. Information covered in the interview included demographic characteristics, reproductive, sexual, birth control, and smoking histories. Case subjects were asked to refer to a time before diagnosis when answering questions. Control subjects were matched to the case subject on the year of diagnosis and then assigned a randomly chosen month. After the interview, all subjects were asked to provide a serum sample. Case subjects were asked to sign a consent form that would allow us to retrieve tumor blocks for testing.

Laboratory Tests. Sera were sent in batches to the serology laboratory, and all available samples were tested. Serum antibodies reactive with HPV-16 and HPV-18 capsids were detected using an antigen capture antibody ELISA as described previously (6). Briefly, capsids were produced using HPV-16 L1 and HPV-18 L1 recombinant vaccinia viruses and purified on cesium chloride gradients. Capture antibodies were kindly provided by Dr. Neil Christensen (University of Pennsylvania Hershey Medical Center, Hershey, PA). Human sera were tested in triplicate with and without capsids by researchers blinded to case-control status. Bound human IgG was detected using a goat antihuman alkaline-phosphatase-conjugated antibody (Roche Diagnostics, Indianapolis, IN) and developed with Sigma alkaline-phosphatase substrate 104. Positive controls (a pool of HPV-16 or HPV-18 reactive sera) were included on each plate. For each serum, a value was calculated as follows: average of the natural log of the absorbance values from the three wells containing antigen minus the average of the natural log of the absorbance values from the three wells with no antigen.

Tissue blocks were available for 80% of the case subjects, and two different PCR methods were used to detect HPV DNA. Samples tested before 1996 used a method that was subsequently found to be less sensitive (23) and were not included in this report to minimize misclassification. The newer method for HPV detection was a PCR-based assay carried out as described previously (24). Briefly, PCR was performed using L1 consensus primers (My09/My11; Ref. 25) and primers specific for the E6 open reading frames of HPV-16 and HPV-18. The identity of the PCR products was confirmed by Southern hybridization, and the L1 consensus products were typed by restriction frag-

ment analysis (26). There were HPV DNA PCR results available for 649 of the 1782 (36.4%) case subjects included in this analysis.

Data Analysis. Cut points were determined by ROC analysis as described previously (27). Values of sera from female or male controls who had reported having one or no lifetime sexual partners were treated as true negatives, and sera from cases who tested HPV-16 or HPV-18 DNA positive were true positives for this test. The specificity of the assay was set to 90% because some proportion of subjects with one partner may have been infected with HPV. The cut points used for serum samples from women in these analyses were 0.35 for HPV-16 and 0.26 for HPV-18; for men, the cut point for HPV-16 was set at 0.38. This yielded sensitivities of 41.8%, 50.6%, and 43.1%, respectively, for detection of HPV-16 (in women and men) and HPV-18 antibodies. We were unable to determine a HPV-18 L1 cut point for men by this method because only 2 of 50 men tested for HPV DNA had HPV-18 in their tumor tissue. This method of cut point determination produced a different prevalence of HPV antibodies but similar relative risks as compared with those obtained using methods previously used by this group (9, 28). The previous cut point method relied on virgin women as negative controls, and a comparable male population was not available. The ROC method we use in this report is similar to the cutoff values determined for virus-like particle ELISA in other studies (27).

The relative risk of cancer was estimated by calculating ORs by using multiple logistic regression. Subjects with missing values for any variables in a model were excluded from that model. Neither inclusion of the matching variables (age and reference year) nor inclusion of socioeconomic variables (income and education) affected the ORs, but age was included in the logistic regression models as a continuous variable.

RESULTS

Demographics of the Study Population. The variation in the matching variables (gender, age, county of residence, and reference year) for cases and controls is detailed in Table 1. Education level and income are also presented to describe the population. The patients with squamous cell cancer had lower levels of education than controls or women with adenocarcinomas. More male controls (42.8%) than female controls (34.5%) were in the highest income bracket. Among the cancer patients, the highest income levels were reported by the women with adenocarcinoma of the cervix (39.6%) followed by men with penile cancer (28.2%) and women with vulvar cancer (28.1%). The income distribution was shifted toward the lowest levels among patients with any type of squamous cell cancer compared to controls.

Prevalence of HPV-16 Antibodies among Anogenital Cases and Controls. The relative risk of anogenital cancers associated with HPV-16 and HPV-18 seropositivity is presented in Tables 2 and 3. Seropositivity to HPV-16 capsids showed a significant association with anogenital cancer at all sites when *in situ* and invasive cancers were considered together (Table 2). The highest association between seropositivity and cancer was observed among men with anal cancer (OR, 5.9; 95% CI, 3.4–10.3). A lower association was observed among penile cancer cases (OR, 2.2; 95% CI, 1.4–3.6). This lower association was not the result of a lower proportion of penile cases containing HPV-16 DNA because 69.7% of these cases tested HPV-16 DNA positive (Table 4) compared with 76.3% of cancers from men with anal cancer. The detection of HPV-16 antibodies among female cases was similar among women with vulvar, vaginal, and anal cancers, with ORs ranging from 4.4 (95% CI, 2.9–6.7) for anal cancer to 4.8 (95% CI, 3.3–6.9) for vaginal cancer. Detection of HPV-16 antibodies was somewhat lower among women with squamous cell cervical cancer (OR, 2.7; 95% CI, 2.1–3.5) and lower still among women with adenocarcinoma of the cervix (OR, 1.8; 95% CI, 1.4–2.5). The lower HPV-16 seroprevalence among adenocarcinoma relative to squamous cell carcinoma of the cervix mirrored HPV-16 DNA detection among those cancers (see Table 4). The lower prevalence of HPV-16 seropositivity among squamous cell cervical cancer

Table 1 Age, residence, income, and education status of anogenital cases and controls

Characteristic	Cases							Controls	
	Cervix		Vulvar	Vaginal	Anal (female)	Anal (male)	Penile	Female	Male
	SCC ^a n (%)	Adenocarcinoma n (%)	SCC n (%)	SCC n (%)	SCC n (%)	SCC n (%)	SCC n (%)	n (%)	n (%)
Age (yrs)									
<40	223 (46.1)	161 (52.8)	162 (30.3)	30 (21.4)	10 (9.2)	29 (33.0)	13 (10.7)	639 (35.3)	78 (13.5)
40–59	195 (40.3)	122 (40.0)	247 (46.2)	53 (37.9)	52 (47.7)	31 (35.2)	47 (38.8)	704 (38.9)	245 (42.3)
≥60	66 (13.6)	22 (7.2)	126 (23.6)	57 (40.7)	47 (43.1)	28 (31.8)	61 (50.4)	466 (25.8)	256 (44.2)
Reference year									
≤1989	163 (33.7) ^b	39 (12.8) ^b	117 (21.9) ^b	37 (26.4) ^c	15 (13.8) ^b	12 (13.6) ^b	52 (43.0) ^d	673 (37.2) ^c	250 (43.2) ^d
1990–1994	184 (38.0)	135 (44.3) ^f	243 (45.4)	61 (43.6)	50 (45.9)	30 (34.1)	43 (35.5)	556 (30.7)	132 (22.8)
1995–1998	137 (28.3)	131 (43.0)	175 (32.7)	42 (30.0)	44 (40.4)	46 (52.3)	26 (21.5)	580 (32.1)	197 (34.0)
County									
King	248 (51.2)	159 (52.1)	319 (59.6)	75 (53.6)	62 (56.9)	63 (71.6)	48 (39.7)	951 (52.6)	227 (39.2)
Pierce	146 (30.2)	54 (17.7)	109 (20.4)	23 (16.4)	21 (19.3)	13 (14.8)	17 (14.0)	323 (17.9)	74 (12.8)
Snohomish	90 (18.6)	50 (16.4)	107 (20.0)	9 (6.4)	26 (23.8)	12 (13.6)	14 (11.6)	320 (17.7)	78 (13.5)
Other	0 (0.0)	42 (13.8)	0 (0.0)	33 (23.6)	0 (0.0)	0 (0.0)	42 (34.7)	215 (11.9)	200 (34.5)
Education									
High school or less	234 (48.4)	96 (31.5)	254 (47.5)	73 (52.1)	46 (42.2)	39 (44.3)	58 (47.9)	630 (34.8)	188 (32.5)
More than high school	250 (51.6)	209 (68.5)	281 (52.5)	67 (47.9)	63 (57.8)	49 (55.7)	63 (52.1)	1179 (65.2)	391 (67.5)
Income									
<\$15,000	131 (27.2)	41 (13.5)	117 (22.2)	41 (29.5)	38 (35.8)	23 (26.4)	18 (15.4)	248 (14.0)	40 (7.0)
\$15,000–\$30,000	137 (28.5)	69 (22.8)	147 (28.0)	49 (35.2)	25 (23.6)	24 (27.6)	38 (32.5)	522 (29.4)	130 (20.6)
\$30,000–\$45,000	96 (20.0)	73 (24.1)	114 (21.7)	19 (13.7)	17 (16.0)	20 (23.0)	28 (23.9)	392 (22.1)	159 (27.7)
≥\$45,000	117 (24.3)	120 (39.6)	148 (28.1)	30 (21.6)	26 (24.5)	20 (23.0)	33 (28.2)	612 (34.5)	246 (42.8)

^a SCC, squamous cell carcinoma.

^b The first reference year category includes subjects from 1986–1989.

^c The first reference year category includes subjects from 1981–1989.

^d The first reference year category includes subjects from 1978–1989.

^f The first reference year for *in situ* adenocarcinoma is 1990.

relative to vaginal, vulvar, and anal cancer did not correlate with a lower detection of HPV-16 DNA.

With the exception of penile cancer, HPV-16 seroprevalences tended to be higher among *in situ* cases than among invasive cases (Table 2). There was also an increase in the proportion of seropositive cases when analyses were restricted to HPV-16 DNA-positive tumors; however, the increases varied considerably from site to site. Most surprising was that the HPV-16 seroprevalence among vulvar cancer cases was unchanged when only HPV-16 DNA-positive tumors were considered (43.0%). The highest association between HPV-16 seropositivity and HPV DNA 16-positive cancers was observed for men with *in situ* anal cancer (OR, 16.0; 95% CI, 4.6–55.7). Very high associations were also observed for HPV-16 DNA-positive cases of *in situ* vaginal cancer (OR, 13.1; 95% CI, 5.6–30.4) and invasive penile cancer (OR, 11.6; 95% CI, 2.7–49.4). There was the suggestion that

among women with anal cancer and adenocarcinoma of the cervix, the lower HPV-16 seroprevalence among invasive cases relative to *in situ* cases was reversed when the analyses were restricted to HPV-16 DNA-positive cases. A similar trend was not observed for other cancer sites (Table 2).

Prevalence of HPV-18 Antibodies among Anogenital Cancer Cases and Controls. The associations between HPV-18 seropositivity and anogenital cancers are shown in Table 3. Female anogenital cancer cases were significantly more likely to be HPV-18 seropositive than controls at all sites. The highest association was observed for adenocarcinoma of the cervix (OR, 3.6; 95% CI, 2.7–4.7). The association of HPV-18 seropositivity with cancer at other sites in women ranged from 2.7 (95% CI, 1.7–4.1) for anal cancer to 2.0 (95% CI, 1.6–2.6) for vulvar cancer cases. The high proportion of HPV-18 seropositives among adenocarcinoma cases

Table 2 Association of HPV-16 seropositivity with anogenital cancers

Cancer type	Cases	Controls	OR (95% CI) ^a	HPV-16 DNA-positive cases	OR (95% CI) ^a
	n (% seropositive)	n (% seropositive)		n (% seropositive)	
SCC ^b invasive/cervix	484 (31.6)	1189 (14.6)	2.7 (2.1–3.5)	96 (44.8)	4.7 (3.1–7.3)
Adenocarcinoma/cervix	305 (24.9)	1275 (15.0)	1.8 (1.4–2.5)	54 (33.3)	2.8 (1.5–5.0)
<i>In situ</i>	174 (25.3)	797 (16.6)	1.7 (1.1–2.5)	39 (30.8)	2.2 (1.1–4.6)
Invasive	131 (24.4)	1189 (14.6)	1.9 (1.3–3.0)	15 (40.0)	3.8 (1.3–10.8)
SCC ^b vulvar	535 (43.0)	1539 (14.6)	4.5 (3.8–6.1)	156 (43.0)	4.4 (3.1–6.2)
<i>In situ</i>	436 (45.2)	1539 (14.6)	4.8 (3.6–6.0)	135 (43.7)	4.5 (3.1–6.4)
Invasive	99 (33.3)	1539 (14.6)	3.1 (2.0–4.9)	21 (38.1)	3.7 (1.5–9.1)
Vaginal	140 (43.6)	1804 (14.6)	4.8 (3.3–6.9)	34 (64.7)	10.9 (5.3–22.4)
<i>In situ</i>	105 (43.8)	1804 (14.6)	4.7 (3.1–7.1)	26 (69.2)	13.1 (5.6–30.4)
Invasive	35 (42.9)	1804 (14.6)	4.8 (2.4–9.5)	8 (50.0)	6.3 (1.6–25.6)
Anal (female)	109 (42.2)	1326 (15.2)	4.4 (2.9–6.7)	37 (48.7)	5.6 (2.8–10.9)
<i>In situ</i>	32 (50.0)	1326 (15.2)	5.7 (2.8–11.6)	7 (42.9)	4.2 (0.9–18.9)
Invasive	77 (39.0)	1326 (15.2)	3.8 (2.3–6.3)	30 (50.0)	5.9 (2.8–12.4)
Anal (male)	88 (52.3)	218 (15.6)	5.9 (3.4–10.3)	29 (62.1)	8.9 (3.8–20.6)
<i>In situ</i>	41 (65.9)	218 (15.6)	10.7 (5.0–23.0)	15 (73.3)	16.0 (4.6–55.7)
Invasive	47 (40.4)	218 (15.6)	3.6 (1.8–7.3)	14 (50.0)	5.3 (1.8–16.2)
Penile	121 (24.0)	579 (12.6)	2.2 (1.4–3.6)	23 (43.5)	5.3 (2.2–12.4)
<i>In situ</i>	69 (20.3)	579 (12.6)	1.8 (0.9–3.4)	15 (33.3)	3.4 (1.1–10.3)
Invasive	52 (28.5)	579 (12.6)	2.9 (1.5–5.6)	8 (62.5)	11.6 (2.7–49.4)

^a Age adjusted.

^b SCC, squamous cell carcinoma.

Table 3 Association of HPV-18 seropositivity with anogenital cancers

Cancer type	Cases		OR (95% CI) ^a	HPV-18 DNA-positive cases	
	n (% seropositive)	Controls n (% seropositive)		n (% seropositive)	OR (95% CI) ^a
SCC ^b invasive cervical	480 (26.3)	1184 (14.8)	2.1 (1.6–2.7)	27 (33.3)	2.9 (1.3–6.6)
Adenocarcinoma and ACIS	305 (38.7)	1271 (15.1)	3.6 (2.7–4.7)	59 (64.4)	10.8 (6.2–19.0)
<i>In situ</i>	174 (46.0)	799 (18.7)	4.1 (2.8–5.8)	44 (63.6)	8.6 (4.5–16.6)
Invasive	131 (29.0)	1184 (14.8)	2.4 (1.6–3.6)	15 (66.7)	11.6 (3.9–34.4)
SCC vulvar	534 (24.9)	1532 (14.1)	2.0 (1.6–2.6)	13 (38.5)	3.8 (1.2–11.7)
<i>In situ</i>	435 (25.8)	1532 (14.1)	2.1 (1.6–2.7)	12 (33.3)	3.0 (0.9–10.2)
Invasive	99 (21.2)	1532 (14.1)	1.7 (1.0–2.8)	1 (100.0)	
Vaginal	140 (26.4)	1802 (14.3)	2.2 (1.5–3.2)	3 (66.7)	
<i>In situ</i>	105 (27.6)	1802 (14.3)	2.3 (1.5–3.6)	1 (100.0)	
Invasive	35 (22.9)	1802 (14.3)	1.8 (0.8–4.0)	2 (50.0)	
Anal (female)	109 (33.0)	1322 (15.4)	2.7 (1.7–4.1)	5 (40.0)	3.5 (0.6–21.4)
<i>In situ</i>	32 (43.8)	1322 (15.4)	4.2 (2.1–8.6)	3 (66.7)	
Invasive	77 (28.6)	1322 (15.4)	2.1 (1.2–3.6)	2 (0.0)	

^a Age adjusted.^b SCC, squamous cell carcinoma; ACIS, adenocarcinoma *in situ*.

could be attributed to the higher frequency of HPV-18 DNA detected in those tumors (see Table 4). Differences in the HPV-18 seroprevalence at other cancer sites could not be attributed to differences in the proportion of HPV-18 DNA-positive tumors at those sites. Women with *in situ* disease tended to have a higher HPV-18 seroprevalence than women with invasive disease at all sites. This pattern was particularly apparent among women with adenocarcinoma of the cervix (46.0% of *in situ* cases were HPV-18 seropositive compared with 29.0% of invasive cases) and among women with anal cancer (43.8% of *in situ* cases were HPV-18 seropositive compared with 28.6% of invasive cases).

As with HPV-16 serology, the association of HPV-18 antibodies with cancer increased at all sites when the cases were restricted to subjects with HPV-18 DNA in their tumor tissue. The relative risk of HPV-18 DNA-positive adenocarcinoma of the cervix associated with HPV-18 seropositivity was 10.8 (95% CI, 6.2–19.0). An increase in HPV-18 seroprevalence was also observed at other sites [*e.g.*, vaginal and anal (female) cancers], although HPV-18 DNA was rarely detected in the tumor tissue. Among women with adenocarcinoma of the cervix, the difference in HPV-18 seroprevalence previously noted between *in situ* and invasive cases was greatly reduced when only HPV-18 DNA cases were considered.

To determine whether a more sensitive or specific test could be achieved, the results for the two serology tests were combined (Table 5). In these analyses, subjects were categorized as seronegative for both HPV-16 and HPV-18, seropositive for HPV-16 only, seropositive for HPV-18 only, or seropositive for both HPV-16 and HPV-18. The associations of HPV-16/HPV-18 seropositivity with cancers in women ranged from 6.8 (95% CI, 4.1–11.5) for vaginal and anal

cancer to 4.1 (95% CI, 2.7–6.2) for squamous cell cervical cancer. However, the sensitivity of the combined results was quite poor, and none of the interaction terms were significant. The highest proportion of female cases seropositive for HPV-16 and HPV-18 was among vaginal cases: 25 of 140 cases (17.9%) were positive for both HPV types.

Detection of HPV DNA in Anogenital Cancers. HPV DNA was detected in paraffin-embedded tissue blocks of anogenital cancers using the L1 and E6 primer sets (Table 4). More than 85% of tumors from all sites were found to be HPV DNA positive. HPV DNA was detected most frequently in tumor tissue from women with anal cancers [43 of 45 (95.6%) positive] and least frequently among invasive squamous cell vulvar cancers [30 of 38 (79%) positive]. The highest proportion of HPV-16 DNA-positive tumors was again from women with anal cancer (82.2%), followed by tumor tissues from men with anal cancer and women with *in situ* squamous cell vulvar cancer (76.3% and 74.6%, respectively). Among cervical specimens, squamous cell cancers were more frequently HPV-16 DNA positive than were adenocarcinomas (64.4% and 40.9%, respectively). A high proportion of vaginal and penile cancers was HPV DNA positive (90.7% and 81.8%, respectively), with a high proportion of HPV-16 DNA-positive cases (63% and 69.7%, respectively).

HPV-18 DNA was detected most frequently in adenocarcinomas of the cervix, with 59 of 132 (44.7%) positive samples. The next highest proportion of HPV-18 DNA-positive tumors was seen in cervical squamous cell cancers, with 27 of 149 (18.1%) positive samples. HPV-18 was detected in less than 6% of penile, invasive vulvar, and anal (male) cancers. Of interest was the high frequency of detection of

Table 4 HPV DNA prevalence in incident anogenital cancers^a

Cancer type	Tested (N)	HPV positive (%)	HPV-16 (%)	HPV-18 (%)	HPV-30s ^b (%)	Other HPV ^c (%)	No. of multiple positive cases ^d (%)
Cervical SCC ^e	149	89.3	64.4	18.1	6.7	8.0	8.0
Cervical Adeno ^f	132	81.8	40.9	44.7	1.5	8.3	12.1
Vulvar SCC	219	89.0	71.2	5.9	9.6	7.3	5.0
<i>In situ</i>	181	91.2	74.6	6.6	8.8	7.2	6.1
Invasive	38	79.0	55.3	2.6	13.2	7.9	0.0
Vaginal	54	90.7	63.0	5.6	3.7	27.8	9.3
Anal (total)	64	93.8	79.7	9.4	6.2	10.8	10.9
Women	45	95.6	82.2	11.1	6.7	8.9	11.1
Men	38	94.7	76.3	5.3	7.9	13.2	7.9
Penile	33	81.8	69.7	3.0	6.0	12.1	9.1

^a Restricted to subjects with either a HPV-16 and/or HPV-18 serology result.^b Includes HPV-31, -33, -35, and -39.^c Includes HPV-6, -45, -52, -54, -58, -66, -72, -73, and unknown HPV types.^d Multiple positive cases counted more than once for HPV-16, HPV-18, HPV-30s, and other HPV types.^e SCC, squamous cell carcinoma.^f Includes adenocarcinoma, invasive, and *in situ* but excludes adeno-squamous cell invasive cancers (*n* = 36).

Table 5 Combined association of HPV-16 and/or HPV-18 antibodies with anogenital cancers

Cancer type	HPV serology status	Cases	Controls	OR (95% CI) ^a	P ^b
		n (%)	n (%)		
SCC ^c invasive cervix	Seronegative	259 (54.0)	883 (74.6)	1.0	0.56
	Seropositive for HPV-16 only	95 (19.8)	125 (10.6)	2.6 (1.9–3.5)	
	Seropositive for HPV-18 only	70 (14.6)	128 (10.8)	1.9 (1.3–2.6)	
	Seropositive for both types	56 (11.7)	47 (4.0)	4.1 (2.7–6.2)	
Adenocarcinoma invasive and <i>in situ</i> cervix ^d	Seronegative	149 (48.8)	940 (74.1)	1.0	0.35
	Seropositive for HPV-16 only	38 (12.5)	137 (10.8)	1.7 (1.1–2.5)	
	Seropositive for HPV-18 only	80 (26.2)	139 (11.0)	3.6 (2.6–5.1)	
	Seropositive for both types	38 (12.5)	53 (4.2)	4.5 (2.9–7.1)	
SCC vulvar	Seronegative	252 (47.2)	1158 (75.5)	1.0	0.42
	Seropositive for HPV-16 only	149 (27.9)	159 (10.4)	4.4 (3.4–5.7)	
	Seropositive for HPV-18 only	53 (9.9)	151 (9.8)	1.6 (1.1–2.3)	
	Seropositive for both types	80 (15.0)	65 (4.2)	5.7 (4.0–8.1)	
Vaginal	Seronegative	67 (47.9)	1356 (75.5)	1.0	0.68
	Seropositive for HPV-16 only	36 (25.7)	184 (10.2)	4.2 (2.7–6.6)	
	Seropositive for HPV-18 only	12 (8.6)	180 (10.0)	1.4 (0.7–2.6)	
	Seropositive for both types	25 (17.9)	77 (4.3)	6.8 (4.1–11.5)	
Anal (female)	Seronegative	46 (42.2)	980 (74.2)	1.0	0.30
	Seropositive for HPV-16 only	27 (24.8)	137 (10.4)	4.4 (2.6–7.5)	
	Seropositive for HPV-18 only	17 (15.6)	140 (10.6)	2.5 (1.4–4.5)	
	Seropositive for both types	19 (17.4)	63 (4.8)	6.8 (3.7–12.5)	

^a Age adjusted.^b P for adding interaction term between sera HPV-16 status and sera HPV-18 status.^c SCC, squamous cell carcinoma.^d Excludes adenocarcinoma SCC invasive cancers (n = 36).

HPV of other types (27.8%) in vaginal cancers and HPV-30 (13.2%) in invasive vulvar cancers.

DISCUSSION

Studies that compare risk factors for anogenital cancer development in one geographical area are rarely undertaken but can reveal important clues to the etiologies of these cancers (14, 29). The data presented here underscore the importance of HPV infection in the development of most anogenital cancers, regardless of anatomical site. Variation in the seroprevalence of HPV antibodies across sites may indicate important differences in the relationship between HPV infection and cancer development.

All anogenital cancers were significantly associated with HPV serum antibodies when *in situ* and invasive disease were considered together. HPV-18 seropositivity was most strongly associated with adenocarcinoma of the cervix, as reported previously (Refs.17 and 28; OR, 10.8; 95% CI, 6.2–19.0 among invasive cases positive for HPV-18 DNA). This finding agrees with the higher rate of HPV-18 DNA detection in these cancers when compared with squamous cell cancers as observed here and in other studies (30). For squamous cell cancers at the cervix and cancer at all other anogenital sites, HPV-16 seropositivity was more strongly associated with cancer than was HPV-18 seropositivity. This corresponded to the higher proportion of HPV-16 DNA-positive squamous cell cancers.

Among cancers that tested positive for HPV-16 DNA, the association of HPV-16 serology was very strong for several cancer types. The strong associations (with relative risks greater than 10) for invasive penile, *in situ* anal (male), and *in situ* vaginal cancers were notable. Combining the HPV-16 and HPV-18 serology results produced a more specific test, in that there were relatively few sera positive for both HPV-16 and HPV-18 among the controls (Table 5). For this reason, the association of cancer in women at all sites increased over the use of either assay alone. However, the test became less sensitive, with less than 20% of the cases being seropositive for both HPV-16 and HPV-18.

Although there was an association between HPV serology and cancer at all sites, there were obvious differences in the HPV seroprevalence among cases across sites. Overall, differences in seroprevalence at the various sites were not explained by differences in the

detection of HPV DNA at those sites. The one exception to this was the concordance between the detection of HPV-18 DNA and HPV-18 seroprevalence among women with adenocarcinoma of the cervix. The observed variation in seroprevalence by site might have resulted because seropositivity served as a marker for the etiological agent (HPV-16 or HPV-18) among some cases and as a surrogate marker among other cases. It has been proposed that HPVs are the etiological agents for most cervical cancers and for a smaller subset of vulvar, vaginal, and penile cancers (1). An alternative but not mutually exclusive hypothesis is that variation in seroprevalence by site reflects differences in the immune response to HPV infections at those sites or differences in immune suppression during cancer development at those sites. This last hypothesis is supported by a recent report noting that there were site-specific differences in the risk of anogenital cancer among AIDS patients (31). The association between HPV-16 serology and penile and vaginal cancers (especially those containing HPV-16 DNA) strongly supports the contention that HPV infection plays an important role in cancer development at those sites.

We and others have previously reported a higher seroprevalence among women with *in situ* vulvar (9, 13) and cervical cancer (27) relative to women with invasive cancer at these sites. A similar pattern was seen here among anal cancers (in men and women) but not among penile cases, again pointing to possible differences in the etiology of these cancers. It has been proposed that the lower HPV-16 seroprevalence among invasive cancers resulted from a loss of capsid gene expression in those cases, but not among *in situ* cases (9, 27). However, in this study, where serum was drawn after removal of tumors, the time since surgery did not appear to affect seroprevalence (data not shown). Another possibility is that immune suppression may be involved in the progression from *in situ* to invasive disease and that this occurs more frequently at certain anogenital sites.

It is well-known that HPV serology underestimates HPV exposure. Even when analyses were restricted to cases in which HPV-16 or HPV-18 DNA was detected in tumor tissues, only 33.3–73.3% of cases were seropositive. This is consistent with other studies in which the detection of serum antibodies was examined among HPV-16 DNA-positive women (32–34). The failure to detect type-specific antibodies in patients whose tumors contained HPV DNA of that type may have occurred because the antibody test was insensitive or

reflected an underlying deficiency in the immune response to HPV infection. The lack of detectable antibodies might also indicate that antibodies to L1 eventually wane in the absence of exposure to L1 in the neoplastic tissue for an extended length of time. In any case, misclassification by the serological test would have resulted in an underestimation of the true association between HPV infection and cancer.

In this study, HPV DNA was detected in a high proportion of tumors from all anogenital sites. The highest proportion of HPV DNA-positive tumors was among anal cancer samples from women (95.6%), and the lowest proportion of HPV DNA-positive tumors was seen in invasive vulvar cancers (79.0%). As expected, HPV-16 DNA was the most frequently detected type at all sites, with the notable exception of adenocarcinomas of the cervix, in which HPV-18 DNA was the most frequently detected type [as seen by others (1)].

The HPV DNA prevalence in cervical and anal cancers found here was similar to that seen in previous studies (1). Bosch *et al.* (30) reported a somewhat higher HPV prevalence in a worldwide survey of cervical cancer (93%), perhaps due to the use of fresh tumor biopsies. However, in this study of archival specimens, the proportion of HPV-positive squamous cell cervical cancers attributable to HPV-16 (64.4%) is similar to that seen in many other studies (1). In anal cancer, the proportion of HPV-16 DNA-positive tumors (76.3% and 82.2% among male and female cases) confirms a recent report by Frisch *et al.* (35).

The reported frequency of HPV-16 DNA detection in vulvar neoplasia has been variable. Recent estimates have varied between 18% (36) and 57% (37). In this study, 71.2% of vulvar cancers were found to contain HPV-16 DNA; however, among invasive cases, 55.3% were HPV-16 DNA positive. A study that classified the tumors by squamous cell subtype found that the seroprevalence was 18.2% in controls, 22.2% in women with invasive keratinizing vulvar cancer, 50.0% in women with invasive basaloid or warty vulvar cancer, and 59.1% in women with *in situ* vulvar cancer (38).

Reports on HPV DNA detection in vaginal and penile cancers have also been inconsistent. In penile cancer studies that used PCR to detect HPV DNA, the proportion of HPV-16-positive cancers varied from 12% to 82% (39, 40). In vaginal cancers, the percentage of HPV-16-positive tumors detected by PCR was reported to be 57% (41). In this study, 63.0% of vaginal and 69.7% of penile cancer tumors were HPV-16 DNA positive.

In summary, our study reconfirms the association between HPV and risk of cervical, anal, and vulvar cancers. Cancers of the penis and vagina were shown here to have a similarly strong relationship with the detection of HPV. Among anogenital cancer cases, HPV seroprevalence varied by cancer site. The variation could not, in general, be attributed to the proportion of HPV-positive tumors detected at each site. At most sites, HPV serum antibodies were less frequently detected in invasive cancers than in *in situ* cancers.

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