

Seroreactivity to Human Papillomavirus Types 16, 18, 31, and 45 Virus-Like Particles in a Case-Control Study of Cervical Squamous Intraepithelial Lesions

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Serum IgG antibodies to human papillomavirus (HPV) types 16, 18, 31, and 45 virus-like particles were measured in a nested case-control study of cervical squamous intraepithelial lesions. HPV-16 seroreactivity was strongly associated with HPV-16 DNA detection (odds ratio, 9.0; 95% confidence interval, 4.4–19.4), and similar type specificity was observed for HPV-31 and -45. In contrast, seroreactivity to any type was associated with elevated seroreactivity to all others. Among cases and controls, HPV-16 showed the highest seroprevalence, with 23.8% of 80 cases and 10.5% of 258 controls seroreactive to HPV-16 alone, and another 27.5% and 5.4%, respectively, seroreactive to HPV-16 plus other types. Overall, 24 (30.0%) cases and 17 (6.6%) controls were seroreactive to multiple types. These data suggest that seroreactivity to a given type reflects mainly type-specific HPV infection as measured by DNA detection and may also signal past exposure to other types that are now only serologically detected.

Various epidemiologic studies have examined patterns of immunologic response to cervical human papillomavirus (HPV) type 16 infection, the main causal factor in cervical neoplasia, by measuring serum IgG antibodies to HPV-16 virus-like particles (VLPs) [1, 2]. HPV-16 seroreactivity is strongly associated with the repeated detection of HPV-16 DNA in the cervix over time [3–6], suggesting that it is a marker of prolonged infection, which in turn is associated with the development and persistence of squamous intraepithelial lesions (SILs) [7]. A prospective cohort study of 325 female university students, in which cervical HPV-16 DNA and seroreactivity measurements were repeatedly obtained at ~4-month intervals, reported a median seroconversion time of 8.3 months in 25 women who acquired incident HPV-16 infection and a relative risk (RR) of HPV-

16-associated SIL of 5.7 (95% confidence interval [CI], 2.4–13.4) in seroconverters versus nonseroconverters [4].

HPV-16 seroreactivity is also associated with sexual activity and with having multiple sex partners [8–11]. In cytologically normal, HPV-16 DNA-negative women, seroprevalence increases with increasing lifetime number of sex partners, suggesting that HPV-16 seroreactivity, to some degree, can be a marker of past infection [12].

Together with HPV-16, the 4 types most commonly found in cancers worldwide also include HPV-18, -31, and -45 [13], but less is known about the seroepidemiology of the latter types. With few exceptions [8, 14, 15], most VLP serologic studies of cervical neoplasia to date have focused on the most prevalent type, HPV-16. Although animal models have suggested that VLP assays are relatively type specific [16], little is known about the type specificity of VLP assays in seroepidemiologic studies of humans. The main objectives of this case-control study were to examine possible serologic cross-reactivity between types and associations of cervical HPV DNA with seroreactivity to all 4 types.

Materials and Methods

This study was conducted on a subset of women who previously participated in a case-control study of HPV-16 VLP seroreactivity, nested within a large prospective cohort study on cervical neoplasia. Details about the nested case-control serology study are provided elsewhere [3]. That study included 152 incident cases (i.e., 97 with

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Table 1. Prevalence odds ratios (ORs) and 95% confidence intervals (CIs) of human papillomavirus (HPV)–16, -18, -31, and -45 seroreactivity vs. DNA positivity (ever vs. never) of the same type.

HPV type by DNA	HPV type by serology							
	16 (<i>n</i> _{pos} = 116)		18 (<i>n</i> _{pos} = 31)		31 (<i>n</i> _{pos} = 69)		45 (<i>n</i> _{pos} = 48)	
	<i>n</i> _{pos} ^a	OR (95% CI)	<i>n</i> _{pos} ^a	OR (95% CI)	<i>n</i> _{pos} ^a	OR (95% CI)	<i>n</i> _{pos} ^a	OR (95% CI)
16 (<i>n</i> _{pos} = 47)	34	9.0 (4.4–19.4)	1	0.2 (0.02–1.5)	7	0.8 (0.3–2.1)	5	0.9 (0.3–2.4)
18 (<i>n</i> _{pos} = 17)	8	2.4 (0.8–7.1)	3	2.8 (0.5–10.9)	5	2.2 (0.6–6.8)	5	3.4 (0.9–11.0)
31 (<i>n</i> _{pos} = 21)	12	3.7 (1.4–10.2)	1	0.6 (0.02–4.0)	14	12.2 (4.3–37.1)	6	3.3 (1.0–9.7)
45 (<i>n</i> _{pos} = 10)	4	1.7 (0.4–7.4)	1	1.4 (0.03–10.5)	3	2.2 (0.4–9.8)	7	20.5 (4.4–125.9)

NOTE. Data include 80 cases, 258 random controls, and 74 HPV DNA-positive controls (*n* = 412).

^a No. of HPV DNA-positive (underlined) women in each row who were seroreactive to each HPV VLP type.

cervical SILs confirmed by an expert pathologist panel on the basis of review of cytologic and histologic material and 55 considered by the expert panel to represent equivocal SIL) and 688 cytologically normal controls (total, 840) matched by age, follow-up time, and other factors. However, because of the large number of additional assays required to test for multiple-type seroreactivity, only a subset of 412 women was selected for the present study.

The 412 included 80 of the previous 97 SIL cases (17 with insufficient serum were excluded) and 332 of the previous 688 controls. The 55 previous cases with equivocal SIL were excluded because their HPV-16 seroprevalence was shown earlier to be similar to that of the controls. Of the 332 controls, 258 were randomly selected, and an additional 74 were selected because they had a positive HPV DNA test for any type at cohort enrollment or follow-up. The 74 nonrandom DNA-positive controls were included to ensure a sufficient number of multiple-type seroreactive subjects for the cross-reactivity analysis.

Serum samples were collected only at the diagnostic visit when SIL versus control status was determined. Sera were newly tested for seroreactivity to HPV-18, -31, and -45 VLPs and, simultaneously, were retested for seroreactivity to HPV-16 VLPs to obtain results under the same laboratory conditions as the multiple-type testing. The κ statistic comparing original (i.e., from 1995) and retested HPV-16 seroreactivity results was 0.70, indicating good agreement, as in a previous interlaboratory comparison [17]. Testing was done by ELISA [1]. Each sample was tested 4 times for each VLP type, including one set of duplicates tested on the same microtiter plate and a second set tested on a different plate. As an indication of laboratory variability, the median coefficient of variation for HPV-16 seroreactivity was 19%. All 4 ELISA optical density (OD) values were then averaged for each sample.

DNA extracts from cervicovaginal lavage specimens, obtained at cohort enrollment and the subsequent diagnostic visit, were tested for known and novel genital HPV types by MY09/11 consensus primer polymerase chain reaction (PCR), followed by oligonucleotide hybridization of PCR products to type-specific probes. All DNA samples were tested for HPV types 6/11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51–56, 58, 59, 68, PAPI55, PAP238A, PAP291, and W13B, whereas additional tests for HPV-2, -13, -32, -34, -61, -62, -64, -66, -67, -69, -70, -72, -AE2, -AE7, and -AE8 were conducted on a subgroup [18–20]. Subjects completed an epidemiologic questionnaire at the diagnostic visit covering sexual behavior and other factors.

Seroreactivity cut points were calculated for the averaged OD values. A subgroup of women was identified who were presumed

unlikely to have had a previous genital HPV infection (all HPV DNA-negative, cytologically normal controls who reported 0–1 lifetime sex partners on the follow-up study questionnaire). The cut point of seroreactivity for a given HPV type was defined as the low-risk subgroup’s overall mean OD value + (2.5 × SD). On the basis of these cut points, ~3% of the low-risk controls were seroreactive to any given HPV VLP type.

To assess possible antigenic cross-reactivity between types, prevalence odds ratios (ORs) of seroreactivity were obtained by DNA status of the same or other types (ever vs. never DNA positive during study) and by seroreactivity status of other types. The 74 nonrandom controls were pooled with cases and random controls in this analysis.

After evaluating cross-reactivity, the prevalence of seroreactivity to each HPV VLP type and to multiple types was determined for cases and controls. Prevalence ORs of seroreactivity and exact 95% CIs [21] were modeled with number of times DNA positive for the same type during the study (twice vs. once vs. never) and number of lifetime sex partners as independent variables. The 74 nonrandom controls were excluded so as to not bias prevalence estimates and ORs.

Because the number of matched sets from the original cohort who were eligible for, and subsequently sampled into, this serology study was small, an unmatched analysis was conducted by use of unconditional logistic regression. However, adjustment in the models for the matching factors, including age, follow-up time, and other factors both individually and together, did not notably alter the results, and, therefore, the unadjusted ORs are reported. For these analyses, ORs are presented for ease of interpretation of seroreactivity associations. The more common the HPV type, however, the more the OR would overestimate the true RR.

Results

Assessment of serologic cross-reactivity. In table 1, the associations of type-specific seroreactivity and DNA positivity are shown. For example, in the first row of table 1, the OR of HPV-16 seroreactivity in HPV-16 DNA-positive versus -negative women was compared in magnitude with the ORs of HPV-18, -31, and -45 seroreactivity in HPV-16 DNA-positive versus -negative women. Viral DNA positivity and seroreactivity were compared among all cases and controls combined, including those with multiple HPV types. The association of HPV-16

seroreactivity with HPV-16 DNA was relatively strong (OR, 9.0; 95% CI, 4.4–19.4), in contrast to null associations of HPV-18, -31, and -45 seroreactivity with HPV-16 DNA (table 1). Stronger associations of seroreactivity with DNA of the same type were also observed for HPV-31 and -45 but not for HPV-18 (table 1). Similar patterns were observed when the nonrandom HPV DNA-positive controls were excluded or when the random controls, the nonrandom HPV DNA-positive controls, and the cases were analyzed separately (data not shown).

Overall, 34 (72.3%) of 47 HPV-16 DNA-positive women were HPV-16 seropositive, 3 (17.6%) of 17 HPV-18 DNA-positive women were HPV-18 seropositive, 14 (66.7%) of 21 HPV-31 DNA-positive women were HPV-31 seropositive, and 7 (70.0%) of 10 HPV-45 DNA-positive women were HPV-45 seropositive (table 1). In a previous analysis [3], repeated HPV-16 DNA positivity was associated with increased HPV-16 seroreactivity. This analysis was repeated here with HPV-31. Although numbers were small and 95% CIs overlapped, the ORs of seroreactivity to HPV-31 VLPs increased with increasing numbers of positive HPV-31 DNA tests from 7.7 (1.9–30.2) in women who were HPV-31 DNA-positive once (at cohort enrollment or follow-up) to 23.1 (1.8–121.7) in the repeatedly HPV-31 DNA-positive women. Adjustment for median days of follow-up (<604 vs. ≥604) did not alter the result. Small numbers of repeatedly HPV-18 ($n = 1$) and -45 ($n = 1$) DNA-positive women precluded the evaluation of similar trends for these types.

ORs of seroreactivity to 1 type given seroreactivity to another type are shown in table 2. Seroreactivity to HPV-16, -18, -31, or -45 was strongly associated with seroreactivity to any other of the 4 main HPV types, with prevalence ORs highest for HPV-18 versus -45 (OR, 13.7; 95% CI, 5.7–32.8) and HPV-31 versus -45 (OR, 15.6; 95% CI, 7.5–32.8). Similar patterns were observed when the nonrandom HPV DNA-positive controls were excluded.

Seroreactivity to single versus multiple VLP types in cases and controls. Of the 80 SIL cases and 258 random controls (total, 338), 111 (32.8%) were seroreactive to ≥1 HPV VLP type, including 24.3% seroprevalence for HPV-16, 7.4% for HPV-18, 13.6% for HPV-31, and 8.6% for HPV-45 VLPs (table 3). Among the cases, seroprevalence ranged from 51.3% for HPV-16 to 11.2% for HPV-18, 25.0% for HPV-31, and 17.5% for HPV-45. For the random controls, seroprevalence for the respective types was 15.9%, 6.2%, 10.1%, and 5.8%. Overall, 82 (24.3%) of 338 cases and random controls were identified by serologic testing for HPV-16; an additional 29 (8.6%) were identified by further testing for HPV-18, -31, and -45 VLPs.

Reactivity to multiple types of HPV VLPs was relatively common among seroreactive cases and random controls as shown in part in table 3. Of the 111 seroreactive women, 70 (63.0%) reacted to only 1 VLP type, 41 (37.0%) to ≥1 type, and 9 (8.1%) to all 4 types. The percentage seroreactive to multiple types was higher among cases than random controls

Table 2. Prevalence odds ratios (ORs) and 95% confidence intervals (CIs) of human papillomavirus (HPV)-16, -18, -31, and -45 seroreactivity vs. seroreactivity to other type.

HPV type by serology	HPV type by serology		
	16	18	31
	OR (95% CI)	OR (95% CI)	OR (95% CI)
18	5.4 (2.4–12.9)		
31	6.1 (3.4–11.1)	9.0 (3.9–21.0)	
45	6.0 (3.0–12.1)	13.7 (5.7–32.8)	15.6 (7.5–32.8)

NOTE. Data include 80 cases, 258 random controls, and 74 HPV DNA-positive controls ($n = 412$).

(table 3). In total, 24 (30.0%) of the 80 SIL cases and 17 (6.6%) of the 258 random controls showed serologic evidence of exposure to ≥1 HPV type. Those reacting to multiple VLP types were more likely to have reported multiple sex partners than those reacting to only 1 VLP type (Mantel-Haenszel χ^2 test for trend, $P = .03$). The OR of multiple- versus single-type seroreactivity was 1.7 (95% CI, 0.4–11.0) in women with 2–5 sex partners relative to 0–1 sex partners. The respective OR for women with ≥6 versus 0–1 sex partners was 3.5 (0.8–21.7).

Discussion

In this study of seroreactivity to VLPs of the 4 main cancer-associated HPV types, HPV-16 showed the highest seroprevalence (24.3%) among cases and controls combined. An additional 8.6% of the total study sample was seroreactive to HPV-18, -31, and/or -45 VLPs in the absence of HPV-16 seroreactivity. Although these estimates may vary by population, the results suggest that in epidemiologic studies, important additional information on seroprevalence of cancer-associated HPV infection is gained by serologic testing for other major types besides HPV-16.

In total, 37.0% of 111 seroreactive women, including 30.0% of SIL cases and 6.6% of random controls, showed serologic evidence of exposure to multiple HPV types, confirming similar DNA data that coinfection or serial infection with ≥1 cancer-associated HPV type is relatively common. Higher multiple-type seroprevalence in cases indicates that women who develop incident SIL are cumulatively exposed to more HPV types than those who do not. The significant trend of multiple-type seroreactivity in women with increasing numbers of lifetime sex partners implies that multiple-type seroprevalence might be related to nonmonogamous sexual behavior. HPV-16, -18, and -33 seroreactivity were previously associated with increasing lifetime sex partners in Swedish women [8].

An alternative explanation for the high multiple-type seroprevalence observed in this study is that antibodies to 1 HPV type cross-reacted with VLPs of another type, resulting in inflated seroprevalence estimates. If cross-reactivity was an overwhelming factor, the strength of the association of HPV-16 seroreactivity with HPV-18, -31, or -45 DNA positivity should

Table 3. Single and multiple-type seroprevalence of human papillomavirus (HPV)-16, -18, -31, and -45 in squamous intrathelial lesion (SIL) cases and random controls (*n* = 338).

HPV type	SIL cases (<i>n</i> = 80)		Controls (<i>n</i> = 258)		Total seroreactive to ≥1 of 4 types <i>n</i> (%)
	Seroreactive to this type only <i>n</i> (%)	Seroreactive to this plus other type <i>n</i> (%)	Seroreactive to this type only <i>n</i> (%)	Seroreactive to this plus other type <i>n</i> (%)	
16	19 (23.8)	22 (27.5)	27 (10.5)	14 (5.4)	82 (24.3)
18	2 (2.5)	7 (8.8)	5 (1.9)	11 (4.3)	25 (7.4)
31	2 (2.5)	18 (22.5)	11 (4.3)	15 (5.8)	46 (13.6)
45	0	14 (17.5)	4 (1.6)	11 (4.3)	29 (8.6)
≥1 of 4 types	—	—	—	—	111 ^a (32.8)

^a Data include 47 cases and 64 controls.

have approached that of HPV-16 seroreactivity with HPV-16 DNA positivity. However, stronger associations of seroreactivity with viral DNA of the same type were generally observed for the respective types, with the exception of HPV-18 (table 1). Other studies have similarly reported that the association of HPV-16 seroreactivity is stronger with HPV-16 DNA than with DNA of other types and that HPV-16 seroreactivity associations with other DNA types may reflect dual or past infection [4–6]. In this study, the association of HPV-18 seroreactivity versus HPV-18 DNA was lower than the other ORs of seroreactivity versus same-type DNA. Although this may suggest that the response to HPV-18 is not type specific or that the HPV-18 VLPs do not retain type-specific epitopes, it is also possible that a stronger underlying association between HPV-18 seroreactivity and HPV-18 DNA was obscured by high background reactivity in the HPV-18 ELISA, resulting from relatively low HPV-18 L1 protein expression. Thus, low VLP yields might make these particles more difficult to isolate and more impure.

Hypothetically, greater cross-reactivity between genetically similar HPV types, such as between HPV-16 and -31 or between HPV-18 and -45, would have produced relatively stronger seroreactivity versus DNA associations for these particular combinations of types. However, stronger associations were not exclusive to genetically similar pairs (table 1). Seroreactivity to any 1 HPV type was strongly associated with seroreactivity to any other type (table 2), although again, the relatively stronger associations were not limited to genetically similar pairs. Several prospective studies have shown that levels of serum IgG antibodies to HPV-16 VLPs remain relatively stable over time [4, 6, 22]. Cumulative exposure to multiple HPV types with persisting seroreactivity over time may therefore explain some of the elevated ORs of seroreactivity versus different type DNA or versus different type seroreactivity. However, these associations may still reflect some cross-reactivity.

In conclusion, serologic testing for antibodies to VLPs of the main cancer-associated HPV types provides a relatively type-specific biomarker of exposure to HPV infection and of repeated cervical HPV DNA positivity in epidemiologic studies. Seroprevalence estimates in SIL cases suggest that cumulative

exposure to multiple cancer-associated genital HPV types is common: nearly one-third tested positive for ≥1 HPV type. Additional research is recommended to estimate seroprevalence and further evaluate VLP assay type specificity of other cancer-associated types, such as HPV-33, -35, -39, -52, and -56. Epidemiologically, cross-reactivity may be more easily distinguished from true multiple-type seroreactivity in prospective studies with repeat measurements of HPV DNA and antibodies where a temporal sequence of type-specific seroconversion following new DNA detection could be observed.

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