ARTICLES

Epidemiologic Evidence Showing That Human Papillomavirus Infection Causes Most Cervical Intraepithelial Neoplasia

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Background: **Experimental studies have provided strong evidence that human papillomavirus (HPV) is the long-sought venereal cause of cervical neoplasia, but the epidemiologic evidence has been inconsistent.** ***Purpose:*** **Given improvements in HPV testing that have revealed a strong link between sexual activity history and cervical HPV infection, we conducted a large case–control study of HPV and cervical intraepithelial neoplasia (CIN) to evaluate whether sexual behavior and the other established risk factors for CIN influence risk primarily via HPV infection.** ***Methods:*** **We studied 500 women with CIN and 500 control subjects receiving cytologic screening at Kaiser Permanente, a large prepaid health plan, in Portland, Ore. The established epidemiologic risk factors for CIN were assessed by telephone interview. We performed HPV testing of cervicovaginal lavage specimens by gene amplification using polymerase chain reaction with a consensus primer to target the L1 gene region of HPV. Unconditional logistic regression analysis was used to estimate relative risk of CIN and to adjust the epidemiologic associations for HPV test results to demonstrate whether the associations were mediated by HPV.** ***Results:*** **The case subjects demonstrated the typical epidemiologic profile of CIN: They had more sex partners, more cigarette smoking, earlier ages at first sexual intercourse, and lower socioeconomic status. Statistical adjustment for HPV infection substantially reduced the size of each of these case–control differences. Seventy-six percent of cases could be attributed to HPV infection; the results of cytologic review suggested that the true percentage was even higher. Once HPV infection was taken into account, an association of parity with risk of CIN was observed in both HPV-negative and HPV-positive women.** ***Conclusion:*** **The data show that the great majority of all grades of CIN can be attributed to HPV infection, particularly with the cancer-associated types of HPV.** ***Implications:*** **In light of this conclusion, the investigation of the natural history of HPV has preventive as well as etiologic importance. [J Natl Cancer Inst 85:958–964, 1993]**

The well-established association between sexual activity and the development of cervical neoplasia strongly implicates a sexually transmissible etiologic agent (*1,2*). Molecular studies have provided strong evidence that human papillomavirus (HPV) may be this agent (*3*), but the epidemiologic evidence has been weaker (*4,5*). HPV DNA is identified much more frequently in women with cervical neoplasia than in women with normal cervical cytologic diagnoses. Moreover, statistical adjustment for HPV infection has not explained the elevated risk of developing cervical neoplasia in women with multiple sex partners, suggesting that other venereally transmitted agents play an etiologic role (*6-9*). In addition, the estimated proportion of cervical neoplasia attributable to HPV infection in previous studies has been too low for one to conclude that HPV infection causes most cervical neoplasia.

Recently, improved HPV testing methods revealed for the first time a strong link between sexual activity history and cervical HPV infection (*10*). This finding prompted our large case–control study of cervical intraepithelial neoplasia (CIN) and HPV infection, which evaluates whether sexual behavior and the other established risk factors for cervical neoplasia influence risk primarily via HPV infection.

*See "Notes" section following "References."

Subjects and Methods

Study Subjects

We selected 500 case subjects with CIN and 500 control subjects from a cohort of 21 146 women included in an ongoing natural history study of HPV infection at the Kaiser Permanente prepaid health plan in Portland, Ore. This cohort, established between April 1, 1989, and November 2, 1990, was chosen to be representative of all women presenting for routine cervical cytologic (Pap smear) screening at Kaiser Permanente, which serves about one fourth of the adult female population of Portland.

The 500 case subjects were selected from among the 673 women found at enrollment to have cytologic evidence of CIN or condylomalous atypia [a diagnosis now combined with CIN 1 in the Bethesda cytologic classification system (*11*)]. (We excluded from consideration as case subjects the 2819 women [13%] of the cohort who had a medical history of CIN but were currently cytologically negative, often because of ablative treatment.) The 500 control subjects were randomly selected from the 17 654 women with normal cytologic diagnoses and no known history of CIN.

We selected the 1000 subjects on the basis of the original enrollment cytologic diagnoses, regardless of subsequent cylologic review or histopathologic confirmation. The case group included all 181 women in the cohort with enrollment cylologic diagnoses of CIN 1 or greater (23 with CIN 3 or cylologic changes suspicious for cancer, 27 with CIN 2, and 131 with CIN 1) and 319 women chosen randomly from the 492 with condylomalous atypia. Among the control subjects, the enrollment cytologic diagnoses of 413 were negative, and 87 (17.4%) were classified as having "benign reactive atypia"; there was no statistically significant difference between these two control subgroups with regard to HPV test results or demographic characteristics.

Telephone Interview and Cytologic Sampling

All subjects were contacted by telephone. If oral informed consent was obtained, trained interviewers administered a 20-minute telephone questionnaire that measured the established epidemiologic risk factors for CIN except diet.

For HPV testing, a 10-mL cervicovaginal lavage was obtained from the study participants as part of the cervical cytologic screening procedure, with approval from the Kaiser Permanente Institutional Review Board. The lavage consisted of a directed rinse of the cervical os (using sterile physiologic saline administered via a 10-mL syringe with a $3^1/_4$-inch flexible intracatheter extender), recollected after pooling in the posterior vaginal fornix (*12*). The lavage was obtained after the routine one-slide cervical smear, consisting of a cervical scrape performed with an Ayre spatula and a cylobrush sample of the endocervix.

HPV test data and at least some questionnaire data were obtained from 438 (88%) of the case subjects and 424 (85%) of the control subjects. Four in the case group and 10 in the control group were ineligible for study because of a previous hysterectomy or past CIN determined after their selection. Of those eligible, 17 case subjects and 30 control subjects had cervical specimens that were inadequate for HPV testing, mainly because there were no apparent cervical cells, as assessed by the appearance of the samples and the lack of human DNA (β-globin) amplification. An additional 41 case subjects and 36 control subjects refused the interview or were lost to follow-up. In the results presented below, all available data are included; the findings were unchanged when the analysis was restricted to participants with complete data.

Laboratory Analysis

The cervicovaginal lavage specimens were refrigerated within 1 hour of collection and transported to a central laboratory for processing. A 1-mL aliquot was removed and frozen (at −70 °C and kept on dry ice during shipment) until preparation for HPV determination by polymerase chain reaction (PCR)-based analysis, which was performed without knowledge of medical history, cytologic diagnosis, or other data. Of the 1-mL aliquot, 50 μL was prepared for PCR analysis (*13*). Negative controls containing only human DNA (from the K562 cell line; the American Type Culture Collection, Rockville, Md.) were interspersed to monitor potential contamination during sample preparation.

Gene amplification methods including PCR primers, buffer, reaction components, and cycling parameters have been previously described in detail (*14*). Duplicate aliquots of 5 μL (0.05% of the original 10-mL lavage) were tested using a consensus primer PCR method targeting the Ll gene region of HPV (*15,16*). The amplification of a human β-globin gene fragment was used as an internal control for sample integrity. The PCR products were analyzed using dot blot hybridization as previously described (*14*), with minor modifications including the use of a nonradioactive detection method (*17*). Blots were individually hybridized with the generic probe, designed to detect most known HPV types, as well as probes specific for HPV types 6/11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 57, and 59. In addition, we probed for preliminarily identified types known as PAP88, PAP155, PAP238A, PAP251, PAP291, and W13B. Recombinant plasmids were provided by Dr. G. Orth (Institut Pasteur, Paris, France) for HPVs 33, 39, 42, 54, and 55; Dr. K. Shah (the Johns Hopkins University, Baltimore, Md.) for HPV 45; Dr. S. Silverstein (Columbia University, New York, N.Y.) for HPV 51; Dr, W. Lancaster (Wayne State Medical School, Detroit, Mich.) for HPV 52; and Dr. R. Ostrow (University of Minnesota, Minneapolis) for HPV 26.

On the basis of previous work (*18*), HPV types were combined into three groups differing in their association with invasive cervical cancer: high-risk HPV types (16 or 18), intermediate-risk types (31, 33, 35, 39, 45, 51, or 52), and low-risk types (6/11, 42, or unknown and all other types). Multiple infections, which accounted for 35% of infections, were assigned hierarchically to the highest risk category applicable.

Statistical Analysis

Unconditional logistic regression analysis was used to compute odds ratios as estimates of the relative risk (RR) of CIN, with 95% confidence intervals (CIs). CIs excluding 1.0 indicated statistically significant association. We estimated the association of each epidemiologic factor with and without adjustment for HPV test results. If a strong association (indicated by an elevated RR) disappeared with adjustment for HPV (RR approached 1.0), we inferred that HPV infection mediated the association between that epidemiologic factor and the risk of CIN (*19*). We calculated the attributable proportion, the fraction of disease attributable to HPV, by the following formula: attributable proportion = % HPV positivity in case subjects × (1 − [l/RR]). Because of the age difference between case subjects (median age, 28 years) and control subjects (median age, 34 years), all RR estimates were adjusted for age in sextiles (16-24, 25-28, 29-34, 35-39, 40-46, and 47+ years) or tertiles (16-28, 29-39, and 40+ years) in analyses with few subjects.

Cytology Review and Case Definition

The original cylologic diagnoses were used to define case and control subjects in the main analysis, but the results of possible misclassification of case–control status were addressed by ancillary analyses based on cytology review and histopalhologic confirmation. Two hundred fifty cervical smears were selected for cytology review, including 200 smears from borderline lesions. All available slides classified as benign reactive atypia (borderline controls) were reviewed as well as a sample of condylomatous atypias (borderline cases). In addition, 25 randomly selected negative diagnoses and 25 CIN 1-3 were chosen for review. When the three reviewers (M. E. Sherman, D. R. Scott, R. J. Kurman) did not agree, the results of the primary outside reviewer (M. E. Sherman) were used.

Possible misclassification in case–control status was mainly in the condylomatous atypia category; 26% were reviewed as negative. None of the cases originally diagnosed as CIN were called negative on review. Only two control smears (one benign reactive atypia and one negative) were judged to be definitely misclassified.

Colposcopically directed biopsies, performed according lo standard referral protocol on 363 of the 500 case subjects, confirmed that the case series was a typical unselected U.S. series with regard to severity of disease. Almost all of the unbiopsied case subjects (117/137; 85%) had a cervical cytologic diagnosis of condylomatous atypia. Of the case women

from whom biopsy specimens were taken, 63% had a histopathologic lesion diagnosed as definite CIN (including 28% high-grade, with two invasive cancers).

As a final aspect of case definition, apparently incident (i.e., newly diagnosed) cases did not differ from known prevalent (previously diagnosed) cases with regard to HPV test results or epidemiologic risk profiles (data not shown). Thus, for ease of presentation, data on all case subjects were combined for the main analysis.

Additional HPV Testing

After the HPV data were unmasked and after the data were analyzed, we identified HPV type PAP251 as HPV 58, a type that has been associated with cervical cancer. In addition, we hybridized all amplified specimens with probes specific for HPVs 56 and 68, two other cancer-associated types. These refinements in the HPV-testing protocol would have changed the hierarchical HPV group assignments, from the type 6 group to the type 31 group, for 46 subjects (seven control and 39 case subjects). Because these test results changed after the data were unmasked, we have chosen to present the original, masked results. The conclusions were absolutely unchanged when the data were reanalyzed using the revised HPV results.

Results

HPV Data

HPV DNA was detected much more frequently in case subjects than in control subjects (Table 1). The RRs for CIN were very high for all three HPV type groups but were noticeably higher for the cancer-associated HPV types (type 31 and type 16 groups). Adjustment for the epidemiologic risk factors left the RR estimates in Table 1 unchanged. The analysis of individual HPV types was limited by small numbers; nevertheless, the type-specific RR estimates confirmed the a priori grouping of types into low, medium, and high risk, with the slight exceptions for women with multiple HPV types noted in the "Additional HPV Testing" section. Within the type 6 group, the unknown types of HPV (31 case subjects, 29 control subjects) had the weakest association with risk of CIN (RR = 4). Women with multiple HPV types detected (35% of positives) were more likely to be in the case group (RR = 36) than women with single HPV types (RR = 16).

Main Analysis

Women in the case group demonstrated the typical epidemiologic profile of CIN (RR #1 in Table 2). On average, relative to control subjects, those in the case group had more sex partners, earlier ages at first intercourse, less education and lower family incomes, and smoked cigarettes more often. They were marginally more likely than control subjects to have used oral contraceptives. All of these characteristics were associated with HPV infection.

Several of the epidemiologic risk factors could be explained, even prior to consideration of the HPV test results, as correlates of sexual behavior, particularly as correlates of increasing lifetime numbers of sex partners (RR #2 in Table 2). For example, the associations of smoking and early age at first intercourse with risk of CIN were explained by taking lifetime numbers of sex partners into account. However, sexual behavior did not explain the associations of risk with education and income.

Adjustment for the HPV test results (RR #3 in Table 2) explained most of the risk of CIN associated with increasing lifetime numbers of sex partners and correlates of sexual behavior, as well as the associations of risk with education and income. Adjustment for HPV status generally resulted in very modest residual associations that were not statistically significant. As a notable exception, adjustment for HPV infection uncovered a moderately strong association between increasing parity and risk of CIN.

Among HPV-positive women, there was no evidence that sexual activity, education, income, or smoking remained associated with risk of CIN (Table 3). Some persistently elevated RRs were seen in the HPV-negative stratum, most notably for sexual behavior and low income. Increasing parity appeared to be a risk factor for CIN in both the HPV-negative and HPV-positive groups.

Ancillary Analyses

We repeated the analyses shown in Tables 2 and 3, including only the 309 case subjects without a documented history of CIN. The results were similar; in fact, the associations with sexual behavior and low income were even better explained by HPV infection when using the more restrictive case definition. For example, restricted to case subjects with no history of CIN, an age-adjusted RR of 2.6 for an income of less than $20 000 (compared with $50 000 or more) became 1.2 when HPV test results were taken into account.

We also repeated the main analyses restricting the case group to the most stringent case definition possible, namely,

Table 1. Relative risks of CIN according to HPV test results*

HPV test result†	Case subjects		Control subjects		RR	95% CI
	No.	%	No.	%		
Negative	89	18.9	373	82.3	1.0	—
Types 6, 11, 42, or other or unknown type	108	22.9	52	11.5	8.7	5.8-13.0
Types 31, 33, 35, 39, 45, 51 or 52	117	24.8	15	3.3	33.0	18.0-59.0
Types 16 or 18	158	33.5	13	2.9	51.0	28.0-94.0
Total	472		453			

*CIN included a cytologic diagnosis of condylomatous atypia or CIN 1-3.
†HPV typing results grouped hierarchically according to risk when multiple types were present: 16 group > 31 group > 6 group.

Table 2. Estimated relative risks of CIN for the established epidemiologic risk factors, showing the effects of adjustment for HPV test results

Risk factor	No. of case subjects	No. of control subjects	RR #1*	RR #2†	RR #3 (95% CI)‡
Lifetime No. of sex partners					
1	40	113	1-	1-	1-
2	34	57	1.6	1.6	1.0 (0.5-2.1)
3-5	127	116	2.6§	2.4§	1.2 (0.6-2.1)
6-9	117	70	4.2§	4.1§	1.8 (1.0-3.4)
10+	117	73	4.4§	4.4§	1.8 (1.0-3.3)
Age at first intercourse					
21+	49	84	1-	1-	1-
19-20	70	94	1.2	0.9	1.1 (0.6-2.0)
17-18	118	130	1.3	0.8	1.0 (0.6-1.8)
5-16	199	123	1.9§	0.8	1.4 (0.8-2.5)
Education					
Postgraduate	37	53	1-	1-	1-
College graduate	56	82	0.8	0.9	0.8 (0.4-1.6)
Some college	182	176	1.2	1.2	1.0 (0.5-1.9)
High school graduate	119	112	1.1	1.0	0.8 (0.4-1.6)
<High school graduate	56	30	1.8	1.5	1.2 (0.5-2.8)
Family income per annum					
⩾$50 000	63	104	1-	1-	1-
$40-49 000	43	72	1.0	0.9	0.7 (0.4-1.4)
$30-39 000	73	104	1.0	1.1	1.0 (0.6-1.8)
$20-29 000	107	92	1.6§	1.5	1.4 (0.8-2.4)
<$20 000	154	69	2.9§	2.2§	1.6 (0.9-2.8)
Smoking					
Never	227	260	1-	1-	1-
Past	68	100	0.9	0.7	1.0 (0.6-1.6)
Current	157	92	1.7§	1.1	1.2 (0.8-1.8)
Oral contraceptive use					
Never used	37	62	1-	1-	1-
Past use	163	196	1.5	1.2	1.5 (0.8-2.7)
Timing of use unknown	154	125	1.5	1.1	1.4 (0.7-2.7)
Current use	89	56	1.6	1.3	1.3 (0.6-2.8)
Parity					
0 live births	188	148	1-	1-	1-
1	78	80	1.0	1.0	1.4 (0.8-2.4)
2	99	124	1.1	1.1	1.9 (1.1-3.3)
3	54	58	1.4	1.7	2.5 (1.3-4.8)
4+	33	42	1.3	1.3	2.8 (1.3-5.9)

*RR estimates adjusted for age in sextiles.

†RR estimates adjusted for age in sextiles, lifetime number of sex partners, age at first intercourse, education, income, smoking, oral contraceptive use, and number of live births, categorized as shown in the table.

‡RR estimates adjusted for age in sextiles and HPV test results categorized as shown in Table 1.

§95% confidence intervals exclude 1.0, analogous to statistical significance at the $P<.05$ level.

newly diagnosed, biopsy-confirmed CIN excluding condylomatous atypia. Despite the increased imprecision of the RR estimates because of smaller numbers, the results were fundamentally the same as those in Tables 2 and 3.

In addition, we analyzed the data pertaining to condylomatous atypia, CIN 1, and CIN 2–3 as three distinct case groups (Table 4). All three disease categories were strongly associated with both increasing lifetime numbers of sex partners and HPV positivity, particularly positivity to types 16 and 18. Multivariate analyses including both lifetime numbers of sex partners and HPV test results pointed to HPV infection as the primary risk factor for newly diagnosed condylomatous atypia, CIN 1, and CIN 2-3, although the analysis of CIN 2-3 was imprecise because of small numbers.

In a similar grade-specific analysis (separating condylomatous atypia, CIN 1, and CIN 2-3), HPV-positive current smokers were almost three times as likely to have CIN 2-3 than HPV-positive women who had never smoked (RR = 2.7; 95% CI = 1.1-6.5).

Proportion of Cases Attributable to HPV Infection

We estimated the proportion of cases attributable to HPV infection, for all grades of disease combined and for the three grade categories separately. For the entire case group, the attributable proportion was 0.76, indicating that 76% of all cases of CIN in the study population could be attributed to HPV infection. The comparable figures for condylomatous atypia, CIN 1, and CIN 2-3 were 0.70, 0.90, and 0.88, respectively.

The results of cytologic review tended to increase the proportion of cases of condylomatous atypia attributable to HPV infection, because HPV prevalence increased with

Table 3. Estimated relative risks of CIN for selected epidemiologic risk factors in HPV-negative and HPV-positive women

Risk factor	RR in HPV-negative women (95% CI)*	RR in HPV-positive women (95% CI)*
Lifetime number of sex partners		
1	1-	1-
2	1.4 (0.6-3.3)	0.3 (0.1-1.4)
3-5	1.2 (0.6-2.6)	0.6 (0.2-2.4)
6-9	2.5 (1.2-5.4)	0.8 (0.2-3.2)
10+	2.0 (0.9-4.3)	1.0 (0.3-3.9)
Age at first intercourse		
21+	l-	1-
19-20	1.1 (0.6-2.4)	1.0 (0.3-3.0)
17-18	0.8 (0.4-1.7)	1.3 (0.5-3.7)
5-16	2.4 (1.1-5.1)	0.9 (0.3-2.2)
Education		
Postgraduate	1-	1-
College graduate	0.8 (0.3-2.0)	0.8 (0.2-3.0)
Some college	1.4 (0.7-3.1)	0.6 (0.2-1.8)
High school graduate	0.9 (0.4-2.1)	0.6 (0.2-2.0)
<High school graduate	1.7 (0.6-5.2)	0.8 (0.2-2.9)
Family income per annum		
⩾$50 000	1-	1-
$40-49 000	1.0 (0.4-2.5)	0.4 (0.1-1.1)
$30-39 000	1.2 (0.6-2.6)	0.8 (0.3-2.4)
$20-29 000	2.4 (1.2-5.1)	0.6 (0.2-1.5)
<$20 000	2.4 (1.1-5.5)	0.8 (0.3-2.0)
Smoking		
Never	1-	1-
Past	1.0 (0.5-1.7)	1.2 (0.5-2.7)
Current	1.4 (0.7-2.5)	1.2 (0.7-2.2)
Oral contraceptive use		
Never used	1-	1-
Past use	2.0 (0.9-4.6)	0.7 (0.2-2.7)
Timing of use unknown	2.4 (1.0-5.8)	0.5 (0.1-1.8)
Current use	2.1 (0.6-7.5)	0.5 (0.1-2.0)
Parity		
0 live births	1-	1-
1	1.8 (0.7-4.4)	1.1 (0.5-2.2)
2	2.1 (0.9-4.9)	1.7 (0.8-3.8)
3	3.5 (1.4-8.6)	1.5 (0.6-4.1)
4+	2.9 (1.1-8.0)	2.7 (0.5-14.5)

*RR estimates adjusted for age in sextiles.

increasing certainty of cytopathologic confirmation. Specifically, cases of condylomatous atypia judged on review to be negative, equivocal, or definite were 52%, 70%, and 92% HPV positive, respectively. Thus, the total proportion of cases attributable to HPV infection (as well as the RR estimates relating HPV to risk of CIN) would be even greater if cytopathologic misclassification of condylomatous atypia (false-positive borderline cases) were eliminated.

Discussion

The association between HPV infection and CIN now satisfies all of the accepted criteria for assessing causality from epidemiologic studies (*20,21*). These criteria include strength and consistency of the epidemiologic association, time sequence, specificity of the association, and coherence with other known facts (biologic and epidemiologic) about the risk factor and disease. Accordingly, the relationship between HPV and CIN is strong, and the association has been verified in numerous case–control studies conducted throughout the world (*22*). The cohort studies with longest follow-up show an elevated risk of development of apparently new CIN within the first few years (*23* and Schiffman MH, Manos MM, Hoover RN, et al.: unpublished data). HPV infection appears to cause a specific set of mucocutaneous tumors, including anogenital tumors and, to a lesser extent, some subtypes of head and neck neoplasia (*24*). Biologic plausibility has been proven by abundant experimental data demonstrating the oncogenic potential of HPV (*25,26*).

With the present investigation, a causal role for HPV infection was shown to be thoroughly consistent with long-standing epidemiologic knowledge of cervical neoplasia. In particular, HPV infection was shown to explain the sexual risk factors for CIN, as well as most other behavioral and demographic determinants. As the one exception, increasing parity appeared to be an HPV-independent risk factor for CIN, a finding that is consistent with some previous epidemiologic work (*27*) but requires confirmation in additional studies.

A major difficulty in all studies of CIN is precise definition of the disease. We examined risk in relation to the severity (grade) of CIN, the reliability of the cytologic diagnosis, the correlation of cytologic with histopathologic diagnosis, and our best estimate of the duration of the condition (newly diagnosed versus previously diagnosed disease). HPV infection was strongly associated with the entire spectrum of CIN and largely accounted for the established behavioral risk factors for CIN.

Some risk factors appeared to persist partially, especially among HPV-negative women, suggesting that a minority of CIN cases arise without HPV, perhaps with a distinct epidemiology involving other venereal agents. However, the residual associations could also result in part from error in the HPV measurements (residual confounding). Despite the improved HPV test used in this investigation, HPV measurement error remains because of problems with the collection of optimal cervical specimens, difficulties in testing for such a large family of incompletely characterized viruses, and the inherent limitations of a single HPV test in defining infection (*28*).

If HPV causes most CIN, infection (even as assessed by the one-time detection of HPV DNA) is still much more common than the prevalence of CIN (*5*). The remaining etiologic questions relate, therefore, to cofactors for HPV persistence and progression to CIN among women exposed to HPV infection. In this context, low- and high-grade CIN must be considered separately.

With the potential exception of parity, we uncovered no cofactors for low-grade CIN among HPV-positive women. It is possible that all genital HPV infections might be sufficient to cause small and transient foci of low-grade CIN that disappear under immune surveillance influenced by both environmental and host factors. Transient infections, i.e., transient foci of CIN, could easily be missed in the

Table 4. Estimated relative risks of condylomatous atypia, CIN 1, and CIN 2-3 associated with lifetime numbers of sex partners and HPV lest results

Risk factor	No. of control subjects	Condylomatous atypia			CIN 1			CIN 2-3		
		No. of case subjects	RR*	RR (95% CI)†	No. of case subjects	RR*	RR (95% CI)†	No. of case subjects	RR*	RR (95% CI)†
Lifetime number of sex partners										
1-2	170	56	1-	1-	14	1-	1-	4	1-	1-
3-5	116	78	1.8‡	1.0 (0.6-1.7)	38	3.4‡	1.5 (0.6-3.9)	11	3.8‡	1.3 (0.3-5.4)
6+	143	144	2.8‡	1.6 (1.0-2.6)	63	4.7‡	1.7 (0.7-4.3)	27	7.5‡	2.1 (0.6-7.6)
HPV test result										
Negative	373	74	1-	1-	10	1-	1-	5	1-	1-
Types 6, 11, 42, or other or unknown types	52	72	7.7‡	6.4 (3.9-10.0)	27	21.0‡	24.0 (9.3-60.0)	9	17.0‡	10.0 (3.0-36.0)
Types 31, 33, 35, 39, 45, 51, or 52	15	69	26.0‡	21.0 (11.0-41.0)	43	120.0‡	130.0 (47.0-370.0)	5	33.0‡	22.0 (4.8-97.0)
Types 16 or 18	13	82	37.0‡	33.0 (16.0-70.0)	45	140.0‡	200.0 (68.0-570.0)	31	290.0‡	180.0 (49.0-630.0)

*RR estimates adjusted for age in tertiles.
†RR estimates mutually adjusted for age, HPV test results, and lifetime numbers of sex partners.
‡95% confidence intervals exclude 1.0, analogous to statistical significance at the P<.05 level.

examination of annual or less frequent cervical smears; thus, the determinants of HPV persistence might be the risk factors for the clinical diagnosis of low-grade CIN. Future epidemiologic studies of low-grade CIN might, therefore, focus on evolving measurements of HPV immunity, in the context of frequently repeated cytologic sampling and HPV testing.

Our ability to study the development of high-grade CIN was limited by small numbers of cases. Nevertheless, the results of the present investigation confirm that the development of high-grade CIN is linked to certain types of HPV. In addition, the data suggest that smoking might be a cofactor for the development of high-grade CIN, concordant with previous studies of cervical cancer and high-grade CIN (*2*). Finally, the data do not rule out the existence of another sexually transmitted agent as a cofactor in the development of high-grade CIN (*2*). It is worth considering that the cofactors for CIN progression could be different from the established epidemiologic risk factors for cervical neoplasia, which are mainly explained by HPV.

In addition to searching for HPV cofactors, it will be important to examine the geographic diversity of HPV and to learn whether the same types of HPV infection are responsible for most cervical neoplasia worldwide. Such international efforts are already under way. It also will be important to study the cases of cervical neoplasia not related to HPV infection. Defining such cases convincingly in epidemiologic projects will require near-perfect HPV tests that may not yet exist. However, the existence of HPV-negative cervical neoplasia has been supported by recent molecular biologic (*26*) and epidemiologic (*29*) studies. A crucial question is whether HPV-negative CIN gives rise to HPV-negative invasive cancer.

Such questions of HPV natural history now have preventive as well as etiologic importance, given that HPV testing will eventually become one aspect of improved cervical cancer-screening programs.

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