A COHORT STUDY OF THE RISK OF CERVICAL INTRAEPITHELIAL NEOPLASIA GRADE 2 OR 3 IN RELATION TO PAPILLOMAVIRUS INFECTION

Laura A. Koutsky, Ph.D., King K. Holmes, M.D., Ph.D., Cathy W. Critchlow, M.S., Claire E. Stevens, M.A., P.A., Jorma Paavonen, M.D., Anna Marie Beckmann, Ph.D., Timothy A. DeRouen, Ph.D., Denise A. Galloway, Ph.D., Debra Vernon, C.T., A.S.C.P., and Nancy B. Kiviat, M.D.

Abstract *Background.* Human papillomavirus (HPV) has been associated with cervical intraepithelial neoplasia, but the temporal relation between the infection and the neoplasia remains unclear, as does the relative importance of the specific type of HPV, other sexually transmitted diseases, and other risk factors.

Methods. We studied prospectively a cohort of 241 women who presented for evaluation of sexually transmitted disease and had negative cervical cytologic tests. The women were followed every four months with cytologic and colposcopic examinations of the uterine cervix and tests for HPV DNA and other sexually transmitted diseases.

Results. Cervical intraepithelial neoplasia grade 2 or 3 was confirmed by biopsy in 28 women. On the basis of survival analysis, the cumulative incidence of cervical intraepithelial neoplasia at two years was 28 percent among women with a positive test for HPV and 3 percent among

those without detectable HPV DNA. The risk was highest among those with HPV type 16 or 18 infection (adjusted relative risk as compared with that in women without HPV infection, 11; 95 percent confidence interval, 4.6 to 26; attributable risk, 52 percent). All 24 cases of cervical intraepithelial neoplasia grade 2 or 3 among HPV-positive women were detected within 24 months after the first positive test for HPV. After adjustment for the presence of HPV infection, the development of cervical intraepithelial neoplasia was also associated with younger age at first intercourse, the presence of serum antibodies to *Chlamydia trachomatis*, the presence of serum antibodies to cytomegalovirus, and cervical infection with *Neisseria gonorrhoeae.*

Conclusions. Cervical intraepithelial neoplasia is a common and apparently early manifestation of cervical infection by HPV, particularly types 16 and 18. (N Engl J Med 1992;327:1272-8.)

THE dramatic decline in the incidence of cervical cancer in this country over the past 40 years has been attributed largely to the widespread use of cytologic screening to detect precancerous cervical lesions that can be treated successfully. These precursor lesions are classified histologically as cervical intraepithelial neoplasia (grades 1 to 3), with lesions of grades 2 and 3 considered to be the most serious.

Although several studies have shown that cervical intraepithelial neoplasia is associated with the detection of human papillomavirus (HPV) DNA,[1-4] these studies have been cross-sectional, precluding analysis of the interval between the detection of HPV DNA and the development of cervical intraepithelial neoplasia. Three prospective cohort studies of women with cervical intraepithelial neoplasia found that the development of higher-grade neoplasia was associated with cervical HPV infection.[5-7] The temporal relation between HPV infection and the appearance of cervical intraepithelial neoplasia grade 2 or 3 in women with negative cytologic tests is still undefined, as is the influence of other risk factors.

METHODS

Study Population and Study Design

From 1984 through 1986, a random-numbers table was used to select adolescents and women presenting to a sexually transmitted disease clinic in Seattle. Eligible adolescents and women were required to be 16 to 50 years old, not pregnant, and not taking antibiotics; in addition, they were required not to have undergone a hysterectomy and to speak English. Of 833 eligible women, 779 (94 percent) were enrolled in a cross-sectional study. Women in this sample who were planning to remain in the area for at least one year and were willing to comply with follow-up were asked to join the longitudinal study and to provide written informed consent (which was approved by the institutional review board of the University of Washington). Of the 779 women, 323 (41 percent) met the entry criteria and consented to be enrolled. Of these, 34 (11 percent) reported previous cervical neoplasia, 81 (25 percent) had smears suggestive of cervical intraepithelial neoplasia or HPV infection, and 21 (6.5 percent) had smears that were not satisfactory for analysis. We consider here the remaining 187 women (58 percent) with no history of cervical intraepithelial neoplasia who had satisfactory smears but had neither HPV-related atypia nor cervical intraepithelial neoplasia. Between May 1987 and December 1989, 60 consecutive women with negative smears and no history of cervical intraepithelial neoplasia were enrolled to augment the earlier group. The rates of cervical intraepithelial neoplasia grade 2 or 3 and HPV infection were similar for the two groups, and the combined results are presented.

The women were examined one month and four months after enrollment and every four months thereafter. The average duration of follow-up was 25 months (range, 1 to 65). Women who completed less than two years of follow-up (131 women) and those who completed more than two years (116 women) did not differ significantly or appreciably with respect to race, age, years of education, number of sexual partners, or age at first intercourse. The clinicians were unaware of the HPV test results, and laboratory personnel were unaware of the results of other laboratory tests and of the clinical findings.

Women for whom a single cytologic smear or colposcopic exami-

nation was consistent with the presence of cervical intraepithelial neoplasia grade 2 or 3, and those for whom two smears were consistent with the presence of cervical intraepithelial neoplasia grade 1 but for whom there was inadequate colposcopic visualization of the squamocolumnar junction, underwent biopsy. Overall, 47 of the 247 women (19 percent) met these criteria for biopsy during follow-up. Of the 47, 41 (87 percent) underwent colposcopically directed biopsy and endocervical curettage, and 28 of these (68 percent) had histologic evidence of cervical intraepithelial neoplasia grade 2 or 3; 11 (27 percent) had cervical intraepithelial neoplasia grade 1 or HPV-related atypia, and 2 (5 percent) had negative histologic findings. Because the outcome of interest was histologic evidence of cervical intraepithelial neoplasia grade 2 or 3, the six women who met the criteria for biopsy but elected to transfer their care were excluded from the analyses. All six women had HPV (of type 16 or 18 in two; of type 31, 33, or 35 in two; and untyped in two). This left a total of 241 women, of whom 28 had cervical intraepithelial neoplasia grade 2 or 3.

Interview and Physical Examination

In standardized interviews the women were asked about their age, education, race, parity, number of sexual partners, age at first coitus, current and past smoking status, contraceptive practices, the current and past occurrence of genital warts, and the results of previous cytologic smears. Standardized unaided and colposcopic examinations were performed of the external genitalia, vagina, and cervix before and after the application of 3 percent acetic acid.

Cytologic, DNA, and Microbiologic Specimens

Epithelial cells collected from the cervical transformation zone and the endocervical canal were placed on separate slides and immediately fixed in 95 percent ethanol. For the detection of HPV DNA, a second sample from the transformation zone was obtained with a Dacron-tipped swab. These samples were placed in sodium dodecyl sulfate (SDS) detergent during the first three years of the study and into Virapap collection tubes (Digene Diagnostics, Silver Spring, Md.) during the last three years.

Plastic-shafted cotton or Dacron swabs and cytobrushes were used at every visit to obtain endocervical and ectocervical specimens, respectively, for the isolation of *Neisseria gonorrhoeae*, cytomegalovirus (CMV), herpes simplex virus (HSV), and *Chlamydia trachomatis*.

Cytologic and Histologic Examinations

Smears were stained with modified Papanicolaou stain and reviewed by one cytotechnologist and one pathologist who were unaware of the clinical or laboratory findings. Standard terminology was used to classify the cytologic and histologic findings.[8-10]

Detection and Typing of HPV DNA

The methods used to detect and type HPV DNA have been described elsewhere.[7,11-13] In brief, specimens obtained for the detection of HPV DNA were screened by dot-filter hybridization.[13] One fifth to one fourth of the aliquots of specimens positive or equivocal for HPV DNA on dot-filter hybridization were retested by Southern transfer hybridization.

During the first three years of the study, cells were removed from swabs by vortexing and were placed in 4 ml of a solution containing 1 percent SDS, 10 mmol of TRIS per liter (pH 7.8), and 1 mmol of EDTA per liter. DNA was purified by two extractions, first with phenol and then with chloroform; it was then precipitated with ethanol, and the concentration was determined fluorometrically. Up to 5 μg of DNA was used; when a smaller amount was available, it was mixed with salmon-sperm DNA to yield 5 μg of DNA in all. DNA was denatured and spotted onto nylon (Nytran) membranes with a miniblot apparatus (Schleicher and Schuell, Keene, N.H.). The controls consisted of 10 and 100 pg each of pBR322 and pHPV 6b, 11, 16, 18, and 31 admixed with salmon-sperm DNA, and cervical DNA from a known negative control. HPV plasmid DNA

was kindly provided by M. Durst, E.-M. de Villiers, L. Gissmann, H. zur Hausen, and A.T. Lorincz. Filters were baked at 80°C for 90 minutes and prehybridized for 3 to 4 hours at 68°C in a solution containing 6× saline sodium citrate buffer (1× is 0.9 mol of sodium chloride and 0.09 mol of sodium citrate per liter [pH 7.0]), 1× Denhardt's solution (0.02 percent Ficoll, 0.02 percent polyvinylpyrrolidone, and 0.02 percent bovine serum albumin [fraction V]),[13] 100 μg of salmon-sperm DNA per milliliter, and 0.1 percent SDS. The filters were hybridized as above (28°C below the melting temperature) for 24 hours with ^{32}P-labeled probes, washed in 2× SDS at 68°C (20°C below the melting temperature), and exposed for autoradiography with intensifying screens. The probes consisted of HPV DNA sequences released from the vector by cleavage with appropriate restriction enzymes and radiolabeled by nick-translation with [^{32}P]nucleoside triphosphates to specific activities of 2×10^8 to 10×10^8 cpm per microgram. For each probe, 1×10^6 cpm per milliliter of hybridization solution was used. Filters were hybridized sequentially, first with HPV 16 and 18, then with HPV 6 and 11, and finally with HPV 31.

During the last three years of the study, the dot-hybridization method used to screen for HPV DNA was Virapap. This includes a mixture of RNA probes to HPV 6, 11, 16, 18, 31, 33, and 35 DNA.[7,11] A total of 1389 specimens were tested by the Virapap method, and a total of 1300 specimens were tested by the dot-blot method originally used. The two methods were found to have comparable sensitivities (Fig. 1). The results obtained separately for the two methods were comparable, and thus only the combined results are presented here.

Southern transfer hybridization[7,11-13] was performed with ^{32}P-labeled DNA probes, with hybridizations first at low stringency (37°C below the melting temperature) with ^{32}P-labeled DNA probes to HVP 6, 11, and 16. The filters were rewashed at high stringency (10°C below the melting temperature) and reexposed to distinguish HPV 6, 11, and 16 from other HPVs. Subsequently, the filters were sequentially probed at high stringency with HPV 16, 18, 31, 33, and 35. In some instances, RNA probes directed against DNA were used. Thirty specimens were typed by Viratype (Digene Diagnostics) with probes to detect HPV 6 or 11, HPV 16 or 18, and HPV 31, 33, or 35.

Microbiologic Analysis

C. trachomatis was isolated in cycloheximide-treated McCoy cells.[14] *N. gonorrhoeae* was isolated and identified by standard methods. HSV and CMV were isolated in human-foreskin fibroblasts.[15] Serum samples were tested for antibodies to HSV,[16] CMV,[17] and *C. trachomatis*.[14]

Statistical Analysis

Kaplan–Meier plots were estimated from the time of the first positive HPV test to the time of the development of cervical intraepithelial neoplasia grade 2 or 3. Follow-up was censored at the time of the last visit for women in whom cervical intraepithelial neoplasia grade 2 or 3 did not develop. These plots were compared with those from the time of enrollment to the time of the development of cervical intraepithelial neoplasia grade 2 or 3, or of censoring in the case of women without a positive HPV DNA test.

Cox regression models in which specific types of HPV infection were treated as time-dependent covariates were used to estimate relative risks and 95 percent confidence intervals. These models permitted an assessment of the risk associated with specific HPV types, with adjustment for the presence of other HPV types, and other risk factors.[18] The proportion of all cases attributable to HPV infection was estimated by the equation

$$(RR - 1/RR) \times P_1 = AP_1,$$

in which RR denotes the relative risk, P_1 the proportion of cases infected, and AP_1 the attributable proportion among the total population of infected and uninfected persons.[19] Because of colinearity between sexually transmitted diseases and variables related to sexual behavior, and because estimates of relative risk associating

HPV infection with cervical intraepithelial neoplasia grade 2 or 3 were not appreciably influenced by any of the potential confounding variables, a stepwise procedure was used to select confounding variables for entry into the final Cox regression models.

RESULTS

Characteristics of the Study Population

The 779 women from whom the cohort was selected have been described elsewhere.[13,20,21] In the cohort of 241 women, the mean (±SD) age at enrollment was 26±7 years; 181 (75 percent) were white, 42 (17 percent) were black, and 18 (7 percent) were members of other racial groups. All the women had had sexual experience, with 148 (61 percent) having had more than 10 partners. The mean age at the time of first intercourse was 17±3 years. Forty-three women (18 percent) were positive for HPV at the time of enrollment, 18 more (7 percent) were positive on the second visit, and 49 more (20 percent) became positive after the second visit. Forty-three women (18 percent) had HPV 16 or 18; 19 (8 percent) had HPV 31, 33, or 35; and 15 (6 percent) had HPV 6 or 11. Forty-four women (18 percent) had HPV infections that could not be identified as one of these types.

Of the 66 women with typed HPV infections, 11 (17 percent) had more than one type of HPV DNA detected during follow-up: 5 had HPV 16 or 18 plus HPV 31, 33, or 35; 5 had HPV 16 or 18 plus HPV 6 or 11; and 1 had HPV 31, 33, or 35 plus HPV 6 or 11. In the Kaplan–Meier analyses, all but the last of these women (i.e., 10 of 11) were included in one group until they became positive for the second type of HPV, at which point they were included in the cohort of women with HPV 16 or 18 plus other types of HPV. The one woman who was positive for HPV 6 or 11 plus HPV 31, 33, or 35 (who did not have cervical intraepithelial neoplasia grade 2 or 3) was included in the cohort with

Figure 1. Hybridization of Various Amounts of HPV 16 DNA with a Mixture of HPV 16 and 18 ³²P-Labeled DNA Probes (Panel A) and with the Virapap Probes (Panel B).

The Virapap probes are a mixture of ³²P-labeled RNA probes directed against HPV 6, 11, 16, 18, 31, 33, and 35 DNA.
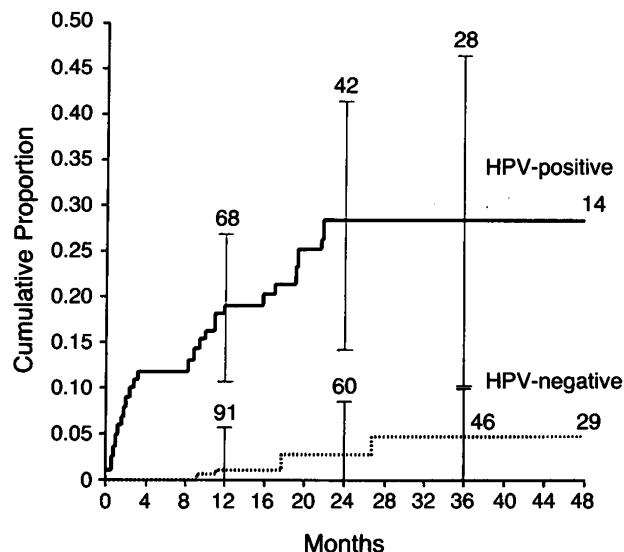

Figure 2. Cumulative Proportion of 241 Women with Negative Cervical Cytologic Tests on Enrollment in Whom Biopsy-Confirmed Cervical Intraepithelial Neoplasia Grade 2 or 3 Developed.

The analysis began at the time of the first positive HPV DNA test for the HPV-positive group, and at the time of enrollment for the HPV-negative group. Ninety-five percent confidence intervals are shown at one, two, and three years for the cumulative proportion with cervical intraepithelial neoplasia grade 2 or 3. Numbers indicate the number of women without biopsy-confirmed cervical intraepithelial neoplasia grade 2 or 3 who were included in the follow-up at the intervals shown. One woman with two negative tests for HPV DNA and a second woman with eight such tests tested positive for HPV DNA (HPV 16 or 18) on the same day that biopsy-confirmed cervical intraepithelial neoplasia grade 2 or 3 was discovered.

HPV 6 or 11 and was censored when she became positive for HPV 31, 33, or 35. Neither the continued inclusion of this woman in the cohort with HPV 6 or 11 nor her transfer to the cohort with HPV 31, 33, or 35 changed the results substantially.

Of the 28 women in whom cervical intraepithelial neoplasia grade 2 or 3 developed, 27 (96 percent) had cytologic evidence (colposcopic in the case of 1 woman) of cervical intraepithelial neoplasia grade 2 or 3, and 1 woman had two smears that showed cervical intraepithelial neoplasia grade 1 and an inadequate colposcopic examination before biopsy. Only 10 of these 28 women (36 percent) had a smear that showed cervical intraepithelial neoplasia grade 1 before they had a smear that showed cervical intraepithelial neoplasia grade 2 or 3.

Cumulative Incidence of Cervical Intraepithelial Neoplasia Grade 2 or 3

The two-year cumulative incidence of cervical intraepithelial neoplasia grade 2 or 3 from the time of the first positive test for HPV DNA (or from the time of enrollment in the case of women who remained negative for HPV DNA) was 28 percent for women with a positive test for HPV DNA, as compared with only 3 percent for women without HPV infection (Fig.
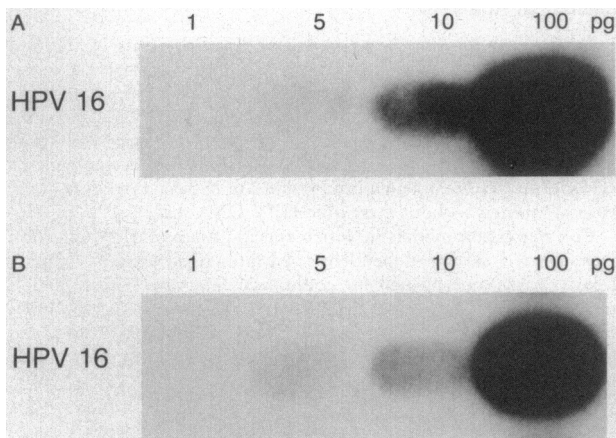
2). After 24 months, no additional cases of cervical intraepithelial neoplasia grade 2 or 3 were detected among the 41 women with HPV infection, and only one additional case was detected (at 27 months) among the 59 women who remained negative for HPV, despite an average of 16 months of additional follow-up. Cervical intraepithelial neoplasia grade 2 or 3 developed in 24 women with HPV infection and in 4 women without it (Table 1).

Of the 28 women with cervical intraepithelial neoplasia grade 2 or 3, 10 (36 percent) had cervical intraepithelial neoplasia grade 3. Of these 10, 5 had HPV 16 or 18 (including 1 with carcinoma in situ); 1 had HPV 31, 33, or 35; 3 had untyped HPV infections; and 1 had no detectable HPV DNA. None were infected with HPV 6 or 11.

When the analyses were restricted to the 144 women with negative HPV DNA tests on both of their first two visits, the cumulative incidence of cervical intraepithelial neoplasia grade 2 or 3 was 32 percent among the 49 women with newly detected HPV infection, as compared with 7 percent among the 95 women who remained HPV-negative. Similarly, when the analyses were restricted to the 105 women with negative cytologic smears and negative tests for HPV DNA on their first two visits, the subsequent two-year cumulative incidence of cervical intraepithelial neoplasia grade 2 or 3 was 23 percent among the 35 women in whom HPV infection developed, as compared with 2 percent among the 70 who remained negative for HPV DNA.

Among the 110 women with HPV infection, the cumulative two-year incidence of cervical intraepithelial neoplasia grade 2 or 3 from the time of the first type-specific positive test was 49 percent for women with HPV 16 or 18 plus other types of HPV; 39 percent for women with HPV 16 or 18 only; 22 percent for women with HPV 31, 33, or 35 only; 26 percent for women with HPV 6 or 11 only; and 8 percent for women with untyped HPV infections (Fig. 3).

Association between the Risk of Cervical Neoplasia and HPV Infection

Women with cervical HPV infection were 11 times more likely than those without such infection to have cervical intraepithelial neoplasia grade 2 or 3 (95 percent confidence interval, 3.7 to 31; attributable risk, 78 percent). Much of this excess risk was associated with HPV 16 or 18 infection (relative risk, 11; 95 percent confidence interval, 4.6 to 26; attributable risk, 52 percent). The risk of cervical intraepithelial neoplasia

Table 1. Cox Regression Analysis of the Relation between HPV Infection and Biopsy-Confirmed Cervical Intraepithelial Neoplasia Grade 2 or 3.

VARIABLE	INITIAL COHORT*†	BIOPSY-CONFIRMED NEOPLASIA†	RELATIVE RISK (95% CI)‡		ATTRIBUTABLE PROPORTION
			UNADJUSTED	ADJUSTED	
	no. of women				
HPV DNA					
Any	110	24	11 (3.9–33)	11 (3.7–31)	0.78
None	198	4	1.0	1.0	
HPV DNA type					
16 or 18	33 (10)	12 (4)	11 (4.7–25)	11 (4.6–26)	0.52
31, 33, or 35	13 (6)	3 (1)	2.5 (0.8–7.6)	2.9 (0.9–9.3)	0.09
6 or 11	9 (6)	2 (3)	3.5 (1.3–9.6)	3.5 (1.2–10)	0.13
Untyped	50	3	2.0 (0.5–7.6)	2.0 (0.5–7.6)	0.05
None	198	4	1.0	1.0	
No. of tests positive for HPV DNA					
≥3	9	4	27 (6.3–112)	26 (6.5–112)	0.14
2	24	6	11 (3.2–41)	12 (3.3–44)	0.20
1	53	14	9.1 (3.0–28)	9.1 (3.0–28)	0.44
0	127	4	1.0	1.0	

*The size of each cohort varied as women became positive for HPV DNA, as cervical intraepithelial neoplasia grade 2 or 3 developed, and as the women were no longer included in the study (see Fig. 1 and 2).

†Numbers in parentheses refer to 11 women with more than one type of HPV, 4 of whom had cervical intraepithelial neoplasia grade 2 or 3. One of five women with HPV 16 or 18 plus HPV 31, 33, or 35 had cervical intraepithelial neoplasia grade 2 or 3, as did three of five women with HPV 16 or 18 plus HPV 6 or 11. The one woman who had HPV 31, 33, or 35 plus HPV 6 or 11 did not have cervical intraepithelial neoplasia grade 2 or 3.

‡CI denotes confidence interval. Risks were adjusted for early age at the time of first intercourse, gonococcal infection, and antibodies to chlamydia in addition to specific types of HPV DNA.

grade 2 or 3 was also elevated, but to a significantly lesser extent, among women with other types of HPV infections (Table 1). Each additional positive HPV DNA test was associated with an increased risk of cervical intraepithelial neoplasia grade 2 or 3.

From the time of enrollment until the study end point, vulvar condylomas were detected in 11 women, none of whom had cervical intraepithelial neoplasia grade 2 or 3.

Risk Associated with Other Cervical Infections

After adjustment for HPV infection, the development of cervical intraepithelial neoplasia grade 2 or 3 was associated with antibodies to C. trachomatis, antibodies to CMV, and cervical infection with N. gonorrhoeae (Table 2). Neither the detection of antibodies to HSV-2 nor the isolation of HSV from the cervix was associated with the development of cervical intraepithelial neoplasia grade 2 or 3.

Other Risk Factors

Younger age at first intercourse, but not the number of sexual partners, was associated with cervical intraepithelial neoplasia grade 2 or 3. In fact, an inverse relation was seen between the number of partners and the development of cervical intraepithelial neoplasia (Table 2). This was related to the finding that older women reported more partners and older age at the time of first intercourse than did younger women but that older women were less likely to have cervical intraepithelial neoplasia grade 2 or 3. Ten or more partners were reported by 58 of 72 women 30 to 47 years of age (81 percent), as compared with 25 of 47 adoles-

cents and women 16 to 19 years of age (53 percent). Among women with 10 or more partners, cervical intraepithelial neoplasia grade 2 or 3 was found in only 4 of 58 women 30 to 47 years of age (7 percent), as compared with 5 of 25 women 16 to 19 years of age (20 percent). First intercourse at the age of 16 or under was reported by only 28 of 72 women 30 to 47 years of age (39 percent), as compared with 37 of 47 women 16 to 19 years of age (79 percent) (odds ratio, 5.8; 95 percent confidence interval, 2.6 to 13).

DISCUSSION

In this cohort of women with initially negative cytologic smears, cervical HPV infection was the most important determinant of the risk of cervical intraepithelial neoplasia grade 2 or 3, with an estimated 78 percent of cases attributed to HPV detected by the methods that we used and 52 percent of them attributable specifically to infection by HPV 16 or 18. Cervical intraepithelial neoplasia grade 2 or 3 was a surprisingly early and common manifestation of HPV infection in this study, with 100 percent of HPV-associated cases detected within 24 months after the initial detection of HPV DNA, and with cervical intraepithelial
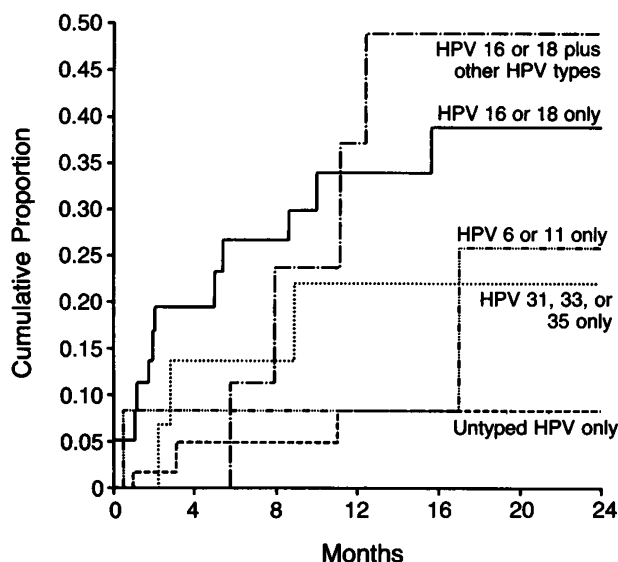

Figure 3. Cumulative Proportion of 110 Women with HPV DNA in Whom Biopsy-Confirmed Cervical Intraepithelial Neoplasia Grade 2 or 3 Developed, from the Time of the First Detection of HPV DNA in a Cervical Specimen.

Ten of the women (9 percent) had HPV 16 or 18 plus other types of HPV; 37 (34 percent) had HPV 16 or 18 only; 15 (14 percent) had HPV 31, 33, or 35; 14 (13 percent) had HPV 6 or 11; and 50 (45 percent) had positive HPV DNA tests that could not be typed by Southern transfer hybridization. Of the 110 women, 16 were included in more than one cohort at different times. For example, four women were positive for HPV 16 or 18 DNA alone for a few months and then had a positive test for another type of HPV, at which time they were included in the cohort with HPV 16 or 18 plus other types of HPV. No cases of biopsy-confirmed cervical intraepithelial neoplasia grade 2 or 3 occurred after 24 months among the 40 women who completed additional follow-up.

Table 2. Cox Regression Analysis of the Relative Risk for Biopsy-Confirmed Cervical Intraepithelial Neoplasia Grade 2 or 3.

CHARACTERISTIC*	BIOPSY-CONFIRMED NEOPLASIA		ADJUSTED RELATIVE RISK (95% CI)†
	ABSENT (N = 213)	PRESENT (N = 28)	
Mean (±SD) age (yr)	26.1±6.5	23.6±5.5	0.9 (0.9–1.1)
Mean (±SD) age (yr) at first intercourse	16.7±3.0	15.4±2.5	0.9 (0.8–1.0)
	no. (% of group)‡		
Race			
White	162 (76)	19 (68)	1.0
Other	51 (24)	9 (32)	1.7 (0.8–3.7)
High school education			
No	90 (42)	17 (61)	1.0
Yes	123 (58)	11 (39)	0.5 (0.2–1.1)
Cigarette smoking			
Never smoked	84 (39)	11 (39)	1.0
Exsmoker	45 (21)	4 (14)	1.3 (0.6–2.9)
Current smoker	84 (39)	13 (46)	0.5 (0.2–1.5)
Parity			
0	158 (74)	17 (61)	1.0
≥1	55 (26)	11 (39)	2.0 (0.9–4.2)
Birth control used			
None	63 (30)	8 (29)	1.0
Oral contraceptives	65 (31)	8 (29)	0.9 (0.4–2.1)
Other	85 (40)	12 (43)	0.6 (0.2–1.7)
No. of sexual partners			
1–5	12 (6)	3 (11)	1.0
6–10	43 (20)	5 (18)	0.4 (0.1–1.5)
11–20	63 (30)	10 (36)	0.2 (0.1–0.7)
>20	95 (45)	10 (36)	0.2 (0.0–0.6)
HSV-2 antibodies§			
Negative	87 (57)	11 (55)	1.0
Positive	65 (43)	10 (45)	1.0 (0.5–2.3)
HSV culture			
Negative	206 (97)	27 (96)	1.0
Positive	7 (3)	1 (4)	0.5 (0.1–4.0)
C. trachomatis antibodies			
Negative	95 (45)	7 (25)	1.0
Positive	118 (55)	21 (75)	2.4 (1.0–5.7)
C. trachomatis culture			
Negative	186 (87)	22 (79)	1.0
Positive	27 (13)	6 (21)	1.1 (0.5–2.8)
N. gonorrhoeae culture			
Negative	198 (93)	21 (75)	1.0
Positive	15 (7)	7 (25)	3.1 (1.3–7.3)
CMV antibodies§			
Negative	56 (35)	3 (14)	1.0
Positive	104 (65)	19 (86)	3.6 (1.1–12)
CMV cultures			
Negative	191 (90)	22 (79)	1.0
Positive	22 (10)	6 (21)	1.7 (0.7–4.2)

*Includes exposures or infections detected at enrollment and during follow-up.

†Adjusted for the detection of cervical HPV infection. CI denotes confidence interval.

‡Because of rounding, not all percentages sum to 100.

§Serum was available for HSV-antibody testing from 173 women and for CMV-antibody testing from 182 women.

neoplasia grade 2 or 3 developing in 28 percent of women with cervical HPV infection (including 39 percent of those with HPV 16 or 18 infection only) during this interval.

Biases related to selection, detection, observers, recall, and loss to follow-up were minimized by enrolling all the study subjects from the same clinic population; having clinicians and cytopathologists be unaware of the results of HPV tests; testing all women uniformly at regular intervals for evidence of neoplasia, HPV, and other sexually transmitted diseases; and collecting

behavioral and clinical information with a standardized protocol.

Although the participants were tested for HPV an average of 6.3 times (range, 2 to 16), additional cervical HPV infections may have been missed. In a separate cross-sectional survey of women attending our clinic, the use of probes for HPV types 42 through 45, 51, 52, and 56 added about 15 percent to the number of HPV infections detected by the methods used in the present study.[22] Furthermore, tests based on the polymerase chain reaction appear to be more sensitive than unamplified Southern or dot-hybridization tests for detecting HPV infection.[23] However, the clinical relevance (in terms of either the short-term risk of cervical intraepithelial neoplasia or the ultimate risk of cancer) of detecting HPV by polymerase chain reaction among women with negative Southern or dot-hybridization tests is uncertain. Reeves and colleagues[3] found that the risk of cervical cancer was associated with the intensity of the HPV DNA hybridization signal. The detection of HPV by methods less sensitive than the polymerase chain reaction could prove to be a better predictor of the risk of cervical cancer; comparative studies are needed.

Many HPV infections in this study could not be typed. These probably include infections by the types we tested for, as well as by other types. That these unclassified HPV infections were only weakly predictive of cervical intraepithelial neoplasia grade 2 or 3 may reflect low levels of viral expression or perhaps the presence of small amounts of contaminating HPV DNA from vaginal or vulvar infections. Finally, the small number of infections with type 6, 11, 31, 33, or 35 precludes a precise definition of the risk of cervical intraepithelial neoplasia grade 2 or 3 presented by these types.

The results of this prospective cohort study support those of several case–control and cross-sectional studies that showed an association between HPV 16 or 18 infection and cervical neoplasia grade 2 or 3.[24-27] Furthermore, three previous cohort studies of women with cervical intraepithelial neoplasia found that the development of more abnormal cytologic or histologic findings was associated with HPV, particularly HPV 16.[5-7] Our study further demonstrates that these observations apply to women with negative cytologic smears and no history of cervical intraepithelial neoplasia, that the time from the detection of HPV to the development of cervical intraepithelial neoplasia grade 2 or 3 is remarkably short, and that past or present cervical infection by certain other sexually transmitted pathogens remains associated with cervical intraepithelial neoplasia grade 2 or 3 after adjustment for HPV infection. The latter finding could indicate that HPV infection is more difficult to detect among women with these other infections, or that other cervical infections interact with HPV to increase the risk of cervical intraepithelial neoplasia grade 2 or 3. The small size of our cohort precluded any analysis of interactions. Larger cohort studies would be required for this purpose.

The association of cervical neoplasia with early first intercourse but not with the number of sexual partners bears greater similarity to findings from Latin America[3,28] than to those from North America,[29] Asia,[30,31] or Europe.[32,33] The effects of age, age at first intercourse, number of sexual partners, experience with former HPV infections, and the development of cervical intraepithelial neoplasia grade 2 or 3 in relation to a current HPV infection may be complex. For example, the younger women seen in our clinic had had intercourse earlier than the older women, but the younger women had fewer partners. Perhaps fewer partners mean fewer previous infections by genital HPV and a lower level of immunity to such infection. If acquired immunity reduces the probability of cervical neoplasia with a subsequent cervical HPV infection, then younger women with fewer partners and fewer previous HPV infections could have the highest risk of cervical intraepithelial neoplasia during any given HPV infection.

Several questions arise from these findings as topics for future research on the natural history and clinical management of cervical HPV infections. To what extent does the rapid development of cervical intraepithelial neoplasia grade 2 or 3 found in our study depend on intercourse at a young age and on frequent exposure to other sexually transmitted cervical STD pathogens? Can these observations be replicated in other populations? After the development of an intraepithelial lesion with a cervical HPV infection, what is the subsequent natural history of that HPV infection? What is the subsequent risk of invasive cervical cancer?

Assessment of the potential usefulness of screening for cervical HPV infection as an adjunct to cytologic screening depends not only on an analysis of the cost effectiveness of such a strategy (as compared with that of more frequent cytologic screening in various populations), but also on the answers to these underlying questions.

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