A Prospective Study of High-Grade Cervical Neoplasia Risk Among Human Papillomavirus-Infected Women

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Background: **In case–control studies, smoking, parity, and oral contraceptive use have been associated with an increased risk of cervical intraepithelial neoplasia grade 3 (CIN3) and cervical cancer among women who are infected with oncogenic human papillomavirus (HPV). However, these potential risk factors have not been adequately studied in prospective studies.** ***Methods:*** **We studied 1812 women who were enrolled in a 10-year prospective study of cervical neoplasia at Kaiser Permanente in Portland, Oregon, and who at enrollment had tested positive for oncogenic HPV DNA and had responded to a questionnaire that included questions on smoking, oral contraceptive use, and parity. Absolute risks and crude relative risks (RRs) with 95% confidence intervals (CIs) for CIN3 or cervical cancer were computed for three time intervals (0–8, 9–68, and 69–122 months after enrollment) using the Kaplan–Meier method. Conditional logistic regression models were used to control for factors that may have influenced our risk estimates, specifically the cytologic interpretation of baseline Pap smear, number of Pap smears during follow-up, age at enrollment, age at prediagnosis visit, and age at diagnosis. All statistical tests were two-sided.** ***Results:*** **Oral contraceptive use and parity were not associated with risk of CIN3 or cervical cancer. Former smokers, women who smoked less than one pack of cigarettes per day, and women who smoked one or more packs per day had crude RRs for CIN3 or cervical cancer for the entire follow-up period of 2.1 (95% CI = 1.1 to 3.9), 2.2 (95% CI = 1.2 to 4.2), and 2.9 (95% CI = 1.5 to 5.6), respectively, compared with never smokers. In the multivariable model, former smokers, women who smoked less than one pack/day, and women who smoked one or more packs/day had RRs of 3.3 (95% CI = 1.6 to 6.7), 2.9 (95% CI = 1.4 to 6.1), and 4.3 (95% CI = 2.0 to 9.3), respectively, for CIN3 or cervical cancer compared with never smokers.** ***Conclusions:*** **Smoking is associated with an increased risk of invasive cervical cancer in women who are infected with oncogenic HPV. Subsequent studies should examine the role of smoking in the multistage pathogenesis of cervical cancer.** **[J Natl Cancer Inst 2002;94:1406–14]**

Infection of the cervical epithelium by one of 13 cancer-associated (oncogenic) human papillomavirus (HPV) types (i.e., HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) is the primary cause of cervical cancer and its immediate precursor, cervical intraepithelial neoplasia grade 3 (CIN3) *(1–4).* Although HPV is a necessary etiologic agent of cervical cancer, HPV infection alone may not be sufficient to cause cervical cancer. Most HPV infections are transient, sometimes causing only mild cytologic abnormalities and usually becoming undetectable, even by sensitive DNA detection methods, within 1–2 years. However, a few HPV infections persist for more than 2 years and may, if untreated, progress to CIN3 and eventually to cervical cancer.

A number of secondary factors (i.e., HPV cofactors) are thought to influence the likelihood that an HPV infection will persist and progress to cervical cancer. Smoking *(5–11),* multiparity or multiple pregnancies *(5,10–14),* and oral contraceptive use *(6,10,11,13,15)* are the most commonly cited HPV cofactors, and each has been found to increase the risk of CIN3 or cervical cancer in case–control studies. However, case–control studies of HPV cofactors and cervical cancer risk are limited by their lack of an appropriate control group: The HPV DNA-negative control subjects are not at risk of cervical neoplasia, and the age-

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See "Notes" following "References."

matched HPV DNA-positive control subjects may represent an unusual group of older women who either have persistent infections that could soon progress to high-grade neoplasia or have had a recent change in lifestyle that has led to an HPV infection *(8,16)*.

We examined the prospective risk of developing CIN3 or cervical cancer associated with smoking, oral contraceptive use, and parity in a subset of women who were infected with oncogenic HPV and who were enrolled in a long-term natural history study of HPV and cervical cancer risk *(1)*.

Subjects and Methods

Cohort

Between April 1, 1989, and November 2, 1990, 23 702 women were enrolled in a natural history study of HPV infection at the Kaiser Permanente Prepaid Health Plan in Portland, OR, as previously described *(17)*. The study was approved by institutional review boards at the National Institutes of Health and Kaiser Permanente. The women were recruited from seven clinics in Portland (two health appraisal clinics and five obstetrics and gynecology clinics) that performed Pap smears. The cohort included approximately 50% of women who had undergone cervical cytologic screening at Kaiser Permanente, which served approximately 25% of the women who lived in Portland when the study was initiated. Subjects were 16 years of age or older. The main analysis cohort, which consisted of 20 810 women, excluded women who refused to participate (n = 1107), women who had undergone hysterectomy (n = 1406), women who had an inadequate specimen for HPV DNA testing (n = 128), women who were 15 years old or younger (n = 67), women who had unsatisfactory or missing baseline cervical Pap smears (n = 85), and women who had undergone colposcopy rather than Pap smear screening at enrollment (n = 99). The main analysis cohort was followed as part of the standard cytologic screening for cervical neoplasia recommended by Kaiser Permanente (Fig. 1).

Enrollment Examination

Each study subject underwent a routine pelvic examination, during which exfoliated cervical cells were collected for Pap smears by using standard methods *(1)*. Cervicovaginal lavages using 10 mL of sterile saline were then performed on consenting individuals in the main cohort to collect specimens for HPV DNA testing *(1)*. Aliquots of the cervicovaginal lavage specimens were stored at –70 °C. Subjects who were willing to answer a questionnaire completed a written, self-administered questionnaire that contained 12 questions about their demographic characteristics, smoking habits, contraceptive practices (including current oral contraceptive use), and parity history. Some subjects, as participants in a prevalent case–control study of 1000 women *(1)*, also completed a more in-depth questionnaire (prevalent case–control questionnaire) on risk factors that was administered in a 20-minute telephone interview by a single trained interviewer after additional oral consent was obtained. For our study, data from the prevalent case–control questionnaire (n = 128) were used when the short questionnaire was missing; the prevalent case–control questionnaire was administered a median of 365 days after the short questionnaire. The

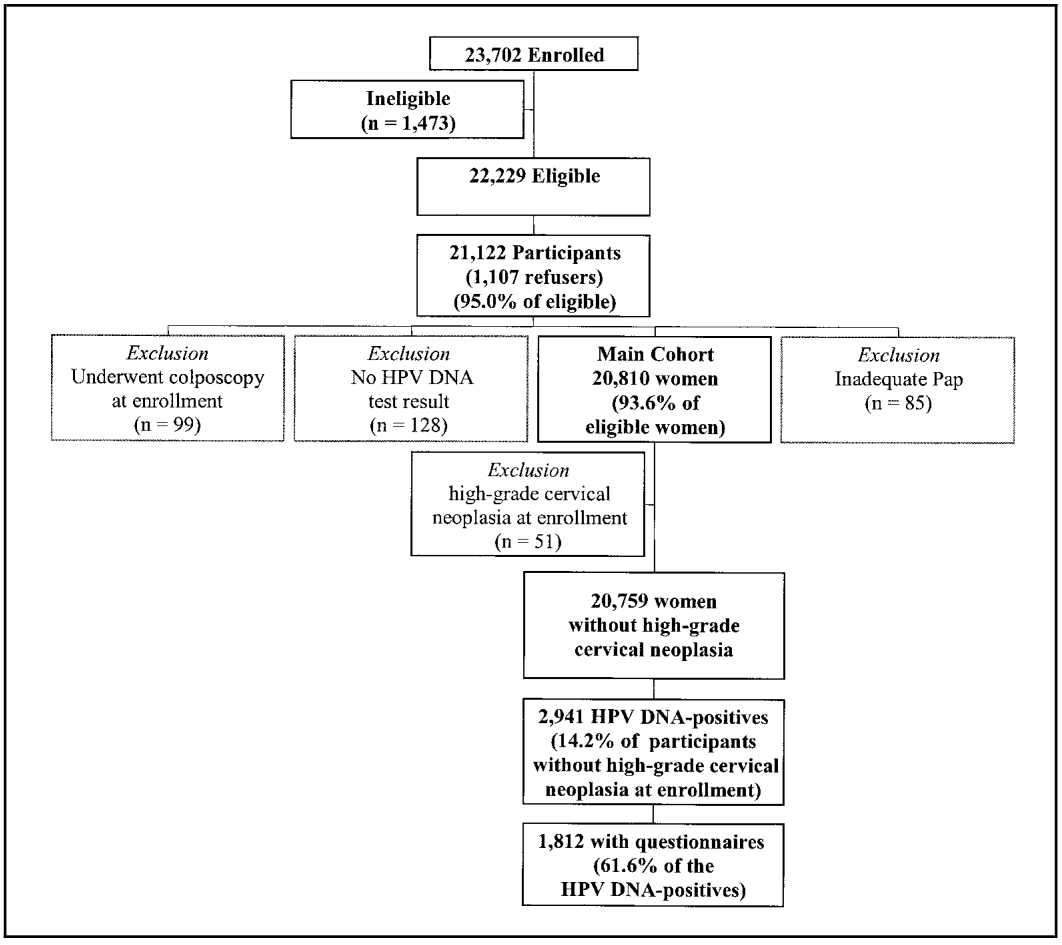

Fig. 1. CONSORT diagram for the prospective study of cervical neoplasia at Kaiser Permanente in Portland, OR, 1989–1999.

analytic subcohort for this study consisted of the 1812 women who were HPV DNA-positive, had no evidence of high-grade squamous intraepithelial lesions (HSIL) at enrollment, and had completed either of the questionnaires. The prevalent case–control questionnaire did not inquire about current oral contraceptive use. Thus, there were 1790 responses to the question about smoking history, 1789 responses to the question about the history of parity, and only 1675 responses to the question about current oral contraceptive use. In addition to reviewing the questionnaire data, we also reviewed computerized medical records to identify women who had a pre-enrollment history of cervical abnormalities or were treated for cervical disease at Kaiser Permanente to examine the possible influences of these variables on the risk of CIN3 or cervical cancer.

Follow-up

During the study period, Pap smears were routinely performed at Kaiser Permanente gynecologic clinics for women who returned for their annual Pap test and had not been screened for cervical cancer in the previous 9 months or when there was clinical suspicion of a cervical neoplasia that would have prompted a colposcopic examination. Women in the analytic subcohort underwent a median of four Pap smears during the course of the study, and 82% of the women in this subcohort of 1812 women had at least one Pap smear during the follow-up period. (By comparison, subjects in the full cohort also underwent a median of four Pap smears during the course of the study, with 83% of the subjects having at least one Pap smear during the follow-up period.) Women were followed for up to 122 months; those with negative Pap smears at enrollment had a median follow-up of over 6 years. Patients with Pap smears interpreted as abnormal were managed according to standard practice guidelines.

Pathology

Results of Pap smears were originally reported by using a classification that preceded the development of the Bethesda System classification guidelines for cervical cytology. Those results were therefore reclassified according to the 1991 Bethesda System guidelines *(18)* as follows: Smears reported as "normal" or "benign reactive atypia" were reclassified as "negative for intraepithelial lesion or malignancy (negative)," except for smears reported as benign reactive atypia with mention of "koilocytotic atypia" or "suggestive of condyloma," which were reclassified as "atypical squamous cells (ASC)." Also classified as ASC were smears originally reported as "severe reactive atypia." Smears originally reported as mild cervical dysplasia were reclassified as low-grade squamous intraepithelial lesion (LSIL), and those reported as moderate or severe cervical dysplasia were reclassified as HSIL. Histologic diagnoses were converted into CIN nomenclature. Specifically, severe dysplasia and carcinoma *in situ* were categorized as CIN3. Of note, a diagnosis of CIN2 was the standard threshold for treatment, but health plan physicians also treated some cases of CIN1 at their discretion.

Women with rigorously defined histopathologic CIN3 or cervical cancer (including endocervical adenocarcinoma *in situ*) were designated as case patients. To avoid misclassifying women who had less severe lesions as case patients, we restricted our case patient group to women who received 1) histopathologic diagnoses of CIN3 or cervical cancer on the basis of two different clinical specimens obtained on different dates; 2) original diagnoses of histopathologic CIN3 or worse that were confirmed, upon review, as being at least CIN2; or 3) original diagnoses of histopathologic CIN2 that were called CIN3 or worse upon review. The reviews were performed by a single pathologist (D.R. Scott) who applied conservative criteria. In total, 171 (0.8%) women from the main analysis cohort fulfilled this definition of case patient, including 26 (0.1%) women who had invasive cervical carcinoma. For women who received a histopathologic diagnosis of CIN3 or worse at multiple times, the time of diagnosis was defined as the date of the first histopathologic diagnosis of CIN3 or worse.

HPV DNA Testing

Frozen (–70 °C) aliquots of the cervicovaginal lavage specimens were tested for oncogenic HPV DNA by Digene Corporation (Gaithersburg, MD) using the Hybrid Capture 2 HPV DNA Test with probe set B, a microplate assay that uses RNA probes and a chemiluminescent signal to detect hybridization between RNA probes and HPV DNA *(19)*. This assay was performed masked to the clinical results at Digene Corporation, according to the manufacturer's specifications. The Hybrid Capture 2 Test is a Food and Drug Administration-approved diagnostic test for HPV that has sensitivity similar to that of polymerase chain reaction-based methods that use consensus sequence oligonucleotide primers to detect HPV DNA *(20–22)*. The ratio of the continuous outcome (relative light units [RLU]) was compared to the mean RLU of the positive control (1 pg of HPV16 DNA) tested in triplicate, resulting in RLU per positive control (RLU/PC). A sample that gave a value of 1 RLU/PC or greater was considered positive for HPV DNA. Log units of the RLU/PC values that were greater than 1 (e.g., 1.0–9.9 RLU/PC, 10.0–99.9 RLU/PC, and >100.0 RLU/PC) were used as semiquantitative measures of viral load among HPV DNA-positive women *(23)*.

HPV DNA-Positive Subcohort

A total of 2941 women tested positive for HPV DNA and did not have HSIL or worse at enrollment. Of these 2941 women, 88 (3.0%) developed CIN3 or cervical cancer during the follow-up period. Our analyses focused on the analytic subcohort of 1812 HPV DNA-positive women (61.6% of the eligible HPV DNA-positive women). Sixty-eight (3.8%) of those women developed CIN3 (58 women) or cervical cancer (10 women) during the course of the 10-year study. The women in this analytic subcohort were younger (mean age of 32 years, range = 16–84 years) than the women in the full cohort of 23 702 (mean age of 36 years, range = 16–94), which is consistent with increased prevalence of HPV infection in younger women *(24)*.

Statistical Analysis

For the univariate longitudinal analysis, we divided follow-up time into an initial period of the first 9 months after enrollment followed by 10 consecutive 1-year intervals for a total time of 122 months (the final time interval, at the completion of the study, was 5 months). These time intervals roughly paralleled the intervals at which women returned to the clinic for their annual Pap smears. We suspected that the Pap smears that were performed within 9 months (i.e., 0–8 months) of enrollment were probably prompted by a previously-known cytologic abnormality or by the occurrence of suspicious symptoms (e.g.,

vaginal discharge or inflammation) that a woman might have had during that time.

To examine the association between potential HPV cofactors and prospective risk of developing CIN3 or cervical cancer, we calculated absolute risks and crude relative risks (RRs) with 95% confidence intervals (CIs) for each time interval using the Kaplan–Meier method. To increase the sample size within time intervals, we consolidated the first five yearly visits into one time interval that included months 9 through 68 after enrollment and the next five yearly visits into another time interval that included months 69 through 122 after enrollment. Thus, absolute risks and RRs are presented for each of these two time intervals as well as for months 9 through 122 after enrollment and the entire 122-month follow-up period.

We also used a multivariable model to examine the associations between smoking, oral contraceptive use, and past number of live births and the development of CIN3 or cervical cancer while controlling for potential confounding by age, underlying prevalent disease, and screening patterns. Conditional logistic regression was used to calculate odds ratios (ORs) with 95% CIs as an estimate of the RR of CIN3 or cervical cancer. Case patients who developed CIN3 or cervical cancer were matched to control subjects by the cytologic interpretation of baseline Pap smears (negative or ASC/LSIL diagnoses), age (±1 year) at enrollment, the number of Pap smears they underwent during follow-up, age (±6 months) at the visit preceding the diagnosis of CIN3 or cervical cancer, and age (±6 months) at diagnosis of CIN3 or cervical cancer. Using these matching criteria, 12 of the 68 case patients had fewer than two matching control subjects (seven case patients had one matching control subject and five case patients had no matching control subjects). For these 12 case patients only, we relaxed the matching criterion of baseline age for control subjects by half-year increments, up to a maximum of ±5 years of the case patient's age. The matching algorithm described above and the relaxation thereof resulted in a median number of control subjects per case patient of 11.5 (range = 1–139), with five case patients having only a single matched control subject. Of the 926 women initially designated as control subjects, 898 never developed CIN3 or worse during follow-up. The remaining 28 original control subjects developed CIN3 or cervical cancer during follow-up and were subsequently matched to other control subjects. The 926 control subjects were used to create 1528 matches (mean = 2 matches per control subject, median = 1 match per control subject, range = 1–7 matches per control subject) for the 68 case patients.

We used data obtained from the enrollment questionnaire to calculate ORs for CIN3 or cervical cancer that were associated with smoking behavior (never smoker, former smoker, smoked <1 pack of cigarettes/day, smoked ⩾1 pack of cigarettes/day), the number of live births (0, 1–2, ⩾3 births), and oral contraceptive use at enrollment (yes or no). To test for statistically significant dose-response relationships (i.e., P_{trend}), covariates were treated as continuous variables and tested as to whether the resulting β coefficient was a value other than zero. Standard contingency table methods with Pearson χ^2 tests and multivariable adjustment were used to examine whether other possible covariates (e.g., viral load and past medical history) were associated with outcome and to examine the relationships between matching criteria, covariates, and outcome. Nonparametric analysis of variance tests (Kruskal–Wallis) were used to test for differences in follow-up time by covariate status. Pearson χ^2 tests were also used to evaluate differences in occurrence of CIN3 versus cancer by time interval. All statistical tests were two-sided. Statistical analyses were performed using SAS, version 8.2 (SAS Institute, Cary, NC) and STATA, version 7.0 (STATA Corporation, College Station, TX).

RESULTS

Of the 2941 women who tested positive for oncogenic HPV DNA by the Hybrid Capture 2 Test and had no cytologic evidence of HSIL or worse at baseline, 1812 (61.6%) completed a questionnaire and thus made up our study subcohort. Women who had a medical history of mild cervical neoplasia were more likely to participate in our study, partly as the direct result of their participation in a prevalent case–control study *(1)*. Accordingly, over the entire duration of follow-up, responders to the questionnaires were twice as likely to develop CIN3 or cervical cancer (RR = 2.1, 95% CI = 1.3 to 3.5) as those who did not respond to the questionnaire.

Among the 1790 women in the HPV DNA-positive subcohort who answered the question on race/ethnicity, 1668 (93.2%) were white, 64 (3.6%) were African-American, 47 (2.6%) were of Asian or Pacific Island descent, and 11 (0.6%) were Native American (Table 1). Approximately 50% of the women in the HPV DNA-positive subcohort had an annual income between $20 000 and $50 000, and nearly 70% had either some college education (41.7%) or were college graduates or had some postgraduate education (28.0%). The primary reason most (56.3%) women gave for the clinic visit that led to their enrollment in this study was for a routine checkup.

Total follow-up time (i.e., the time from enrollment to final visit) differed for subjects according to their cofactor status. For example, the median follow-up times for women who smoked one or more packs per day (n = 1002 days) or less than one pack per day (n = 1317 days) were shorter than those for women who were former smokers (n = 1845 days) or nonsmokers (n = 1896 days) (P<.001, Kruskal–Wallis test). The median follow-up time for women who used oral contraceptives at baseline (n = 1160 days) was shorter than that for nonusers (n = 2094 days) (P<.001, Kruskal–Wallis test). The median follow-up time for women with a past history of at least three live births (n = 2429 days) was longer than that for women with a past history of one or two live births (n = 2019 days) or that for nulliparous women (n = 1195 days) (P<.001, Kruskal–Wallis test).

There was no statistically significant difference between the total follow-up times for women who were diagnosed with cervical cancer and women who were diagnosed with CIN3. By time interval, 29 cases (five cancers and 24 CIN3) were diagnosed in the 0–8 month time interval, 33 cases (4 cancers and 29 CIN3) were diagnosed in the 9–68 month time interval, and 6 cases (1 cancer and 5 CIN3) were diagnosed in the 69–122 month time interval (P = .8, Pearson's χ^2 test). Case patients were more likely than control subjects to have their baseline Pap smears interpreted as ASC (19.1% versus 11.1%, respectively) or LSIL (16.2% versus 5.1%, respectively) rather than as normal (64.7% versus 83.8%, respectively) (P<.001, Pearson's χ^2 test).

We calculated the cumulative incidence of CIN3 or cervical cancer among women in the subcohort according to smoking status, the number of past live births, and oral contraceptive use as reported at enrollment. Former smokers, women who smoked less than one pack per day, and women who smoked one or more

Table 1. Sociodemographic characteristics of the human papillomavirus DNA-positive subcohort

Characteristics	n (%)
Age, y (N = 1812)	
<30	964 (53.2)
30–39	431 (23.8)
⩾40	417 (23.0)
No. of Pap smears (N = 1812)	
<4	793 (43.8)
4–6	479 (26.4)
⩾7	540 (29.8)
Past history of cervical neoplasia (N = 1812)	
No	1467 (81.0)
Yes	345 (19.0)
Reason for clinic visit (N = 1662)	
Routine checkup	935 (56.3)
Health problem	400 (24.1)
Birth control	327 (19.7)
Education (N = 1781)	
High school diploma or less	540 (30.3)
Some college	743 (41.7)
College graduate or more	498 (28.0)
Income, $ (N = 1680)	
<20 000	640 (38.1)
20–50 000	816 (48.6)
⩾50 000	224 (13.3)
Smoking (cigarettes) (N = 1790)	
Never	973 (54.4)
Past smoker	342 (19.1)
<1 pack per day	285 (15.9)
⩾1 pack per day	190 (10.6)
Currently pregnant (N = 1749)	
No	1620 (92.6)
Yes	129 (7.4)
Number of live births (N = 1789)	
0	810 (45.3)
1–2	718 (40.1)
⩾3	261 (14.6)
Currently using oral contraceptives (N = 1675)	
No	1149 (68.6)
Yes	526 (31.4)
Marital status (N = 1682)	
Married	742 (44.1)
Never married	586 (34.8)
Divorced	354 (21.0)
Race (N = 1790)	
White	1668 (93.2)
Black	64 (3.6)
Asian/Pacific Island	47 (2.6)
American Indian/Eskimo	11 (0.6)

packs per day had consistently higher risks of CIN3 or cervical cancer throughout the follow-up period than nonsmokers (Table 2). During the entire follow-up period, 5.0% of former smokers (RR = 2.1, 95% CI = 1.1 to 3.9), 5.3% of women who smoked less than one pack of cigarettes per day (RR = 2.2, 95% CI = 1.2 to 4.2), and 6.8% of women who smoked one or more packs of cigarettes per day (RR = 2.9, 95% CI = 1.5 to 5.6) developed CIN3 or cervical cancer compared with 2.4% of the women who never smoked. These risk estimates did not change when we excluded women who developed CIN3 or cervical cancer within the first 9 months of the follow-up period. Stratification of the subcohort by the median age of the current smokers (younger than 34 years versus 34 years old or older), by the estimated age at menopause (younger than 50 years versus 50 years old or older), and by the cytologic interpretation of the baseline Pap smears (normal versus ASC or LSIL) did not change these risk estimates (data not shown).

The risk of CIN3 or cervical cancer was not associated with the number of live births a woman had had before enrollment (RR = 0.70, 95% CI = 0.31 to 1.6 for the entire follow-up period for women who had had three or more live births) (Table 3) or with the use of oral contraceptives at enrollment (RR = 0.84, 95% CI = 0.49 to 1.5 for the entire follow-up period for women who used oral contraceptives) (Table 4). Stratification of the subcohort by the cytologic interpretation of the baseline Pap smear (normal versus ASC or LSIL) did not change the estimates of risk for women who had one or more live births or for those who used oral contraceptives at enrollment (data not shown). These estimates of risk did not change when we excluded from the analysis the women who developed CIN3 or cervical cancer within the first 9 months after enrollment or when we stratified the subcohort according to the median age (i.e., 34 years) of the women who reported using oral contraceptives at enrollment (data not shown).

The risk of CIN3 or cervical cancer among this group of HPV-infected women was not statistically significantly associated with viral load (as measured by the RLU/PC value of the Hybrid Capture 2 Test), past history of cervical neoplasia, age, income, pregnancy at enrollment, reason for attending the clinic (birth control, health problems, or routine checkup), ethnicity, marital status, nonhormonal contraceptive use (spermicide, barrier, or intrauterine device), or level of education (data not shown).

We also performed multivariable analyses using conditional logistic regression to examine the association of these covariates with CIN3 or cervical cancer. Case patients and control subjects were matched on cytologic interpretation of baseline Pap smear, age, and screening patterns to control for these possibly confounding variables (Table 5). The risk of CIN3 or cervical cancer was not associated with a woman's use of oral contraceptives at enrollment or the number of births she had before enrollment. The risk of CIN3 or cervical cancer among women infected with oncogenic HPV was higher for former smokers (OR = 3.3, 95% CI = 1.6 to 6.7), for women who smoked less than one pack per day at enrollment (OR = 2.9, 95% CI = 1.4 to 6.1), and for women who smoked one pack or more per day at enrollment (OR = 4.3, 95% CI = 2.0 to 9.3) than it was for those who did not smoke. When we excluded the four women who were diagnosed with adenocarcinoma *in situ,* which has been reported to be negatively associated with smoking *(9),* the risk estimates for former smokers (OR = 3.9, 95% CI = 1.9 to 8.1), women who smoked less than one pack per day at enrollment (OR = 3.0, 95% CI = 1.4 to 6.7), and women who smoked one or more packs per day at enrollment (OR = 5.0, 95% CI = 2.3 to 11.0) increased compared with that for women who did not smoke, but those increases were not statistically significant (data not shown). In a model that adjusted for oral contraceptive use and number of past births, the risk of CIN3 and cancer for women who smoked one or more packs/day was further strengthened, albeit statistically nonsignificantly (OR = 5.7, 95% CI = 2.4 to 13) compared with the risk for women who did not smoke.

To address the concern that the risk estimates associated with smoking might reflect the presence of prevalent disease that was missed by the Pap smears, we performed a subset analysis on the 19 case patients who developed CIN3 or cervical cancer after the

Table 2. Absolute risks and relative risks (RRs) of CIN3 or cervical cancer (with corresponding 95% confidence intervals [CIs] associated with smoking habits in each time interval of follow-up*

Time interval, mo	Smoking status†	No. of women seen‡	No. of women diagnosed with CIN3 or cancer	Absolute risk, %	RR (95% CI)
0–8	Never	957	9	0.94	1.0 (referent)
	Former	338	6	1.8	1.9 (0.68 to 5.3)
	<1 pack/day	285	6	2.1	2.2 (0.80 to 6.2)
	⩾1 pack/day	186	8	4.3	4.6 (1.8 to 12)
9–68					
	Never	763	12	1.6	1.0 (referent)
	Former	262	10	3.8	2.4 (1.1 to 5.6)
	<1 pack/day	202	6	3.0	1.9 (0.72 to 5.0)
	⩾1 pack/day	142	5	3.5	2.2 (0.80 to 6.3)
69–122					
	Never	464	2	0.43	1.0 (referent)
	Former	158	1	0.63	1.5 (0.13 to 16)
	<1 pack/day	111	3	2.7	6.3 (1.1 to 37)
	⩾1 pack/day	69	0	0.0	0.0
9–122					
	Never	783	14	1.8	1.0 (referent)
	Former	269	11	4.1	2.3 (1.1 to 5.0)
	<1 pack/day	212	9	4.2	2.4 (1.0 to 5.4)
	⩾1 pack/day	144	5	3.5	1.9 (0.71 to 5.3)
0–122					
	Never	973	23	2.4	1.0 (referent)
	Former	342	17	5.0	2.1 (1.1 to 3.9)
	<1 pack/day	285	15	5.3	2.2 (1.2 to 4.2)
	⩾1 pack/day	190	13	6.8	2.9 (1.5 to 5.6)

*CIN3 = cervical intraepithelial neoplasia grade 3.

†As reported on the enrollment questionnaire.

‡The number of women seen represents counts of women from the analytic subcohort (n = 1812) who were screened by Kaiser Permanente in the defined follow-up interval. Thus, a summation of the numbers in the 0–8 month, 9–68 month, and 69–122 month intervals will exceed 1812 because many women visited during more than one interval.

Table 3. Absolute risks and relative risks (RRs) of CIN3 or cervical cancer (with corresponding 95% confidence intervals [CIs]) associated with the history of parity in each time interval of follow-up*

Time interval, mo	No. of live births†	No. of women seen‡	No. of women diagnosed with CIN3 or cancer	Absolute risk, %	RR (95% CI)
0–8	0	801	12	1.5	1.0 (referent)
	1–2	708	13	1.8	1.2 (0.56 to 2.7)
	⩾3	256	4	1.6	1.0 (0.34 to 3.2)
9–68	0	587	15	2.6	1.0 (referent)
	1–2	566	15	2.6	1.0 (0.51 to 2.1)
	⩾3	214	2	0.93	0.37 (0.08 to 1.6)
69–122	0	298	4	1.3	1.0 (referent)
	1–2	352	1	0.28	0.21 (0.02 to 1.9)
	⩾3	154	1	0.65	0.48 (0.05 to 4.3)
9–122	0	602	19	3.2	1.0 (referent)
	1–2	584	16	2.7	0.87 (0.45 to 1.7)
	⩾3	220	3	1.4	0.43 (0.13 to 1.5)
0–122	0	810	31	3.8	1.0 (referent)
	1–2	718	29	4.0	1.1 (0.64 to 1.7)
	⩾3	261	7	2.7	0.70 (0.31 to 1.6)

*CIN3 = cervical intraepithelial neoplasia grade 3.

†As reported on the enrollment questionnaire.

‡The number of women seen represents counts of women from the analytic subcohort (n = 1812) who were screened by Kaiser Permanente in the defined follow-up interval. Thus, a summation of the numbers in the 0–8 month, 9–68 month, and 69–122 month intervals will exceed 1812 because many women visited during more than one interval.

first 2 years of follow-up and who had had at least one intervening Pap smear between enrollment and diagnosis (i.e., a total of three or more Pap smears during the study) and the 107 matching control subjects. In this subanalysis, the risk estimates for CIN3 or cervical cancer were increased but were less stable because of the small number of case patients for former smokers (OR = 2.5, 95% CI = 0.57 to 11; n = 5), women who smoked less than one pack per day (OR = 7.9, 95% CI = 1.6 to 39; n = 7), and women who smoked one or more packs pack per day (OR = 12, 95% CI = 0.82 to 170; n = 1) compared with

Table 4. Absolute risks and relative risks (RRs) of CIN3 or cervical cancer (with corresponding 95% confidence intervals [CIs]) associated with current oral contraceptive (OC) use in each time interval of follow-up*

Time interval, mo	Current OC use†	No. of women seen‡	No. of women diagnosed with CIN3 or cancer	Absolute risk, %	RR (95% CI)
0–8	No	1129	17	1.5	1.0 (referent)
	Yes	522	7	1.3	0.89 (0.37 to 2.1)
9–68	No	896	24	2.7	1.0 (referent)
	Yes	391	8	2.1	0.76 (0.35 to 1.7)
69–122	No	576	3	0.52	1.0 (referent)
	Yes	187	2	1.1	2.1 (0.35 to 12)
9–122	No	919	27	2.9	1.0 (referent)
	Yes	402	10	2.5	0.85 (0.41 to 1.7)
0–122	No	1149	44	3.8	1.0 (referent)
	Yes	526	17	3.2	0.84 (0.49 to 1.5)

*CIN3 = cervical intraepithelial neoplasia grade 3.

†As reported on the enrollment questionnaire.

‡The number of women seen represents counts of women from the analytic subcohort (n = 1812) who were screened by Kaiser Permanente in the defined follow-up interval. Thus, a summation of the numbers in the 0–8 month, 9–68 month, and 69–122 month intervals will exceed 1812 because many women visited during more than one interval.

Table 5. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for CIN3 or cervical cancer associated with smoking habits, current oral contraceptive (OC) use, and parity from a conditional logistic model that matched case patients and controls subjects on cytologic interpretation of baseline Pap smears, age, and screening behavior*

	CIN3 or cervical cancer (n = 68)	
Covariate	No of cases†	OR (95% CI)
Smoking		
Never	23	1.0 (referent)
Former smoker	17	3.3 (1.6 to 6.7)
Current smoker, <1 pack/day	15	2.9 (1.4 to 6.1)
Current smoker, ⩾1 pack/day	13	4.3 (2.0 to 9.3)
Current OC use		
No	44	1.0 (referent)
Yes	17	0.61 (0.32 to 1.1)
No. of live births		
0	31	1.0 (referent)
1–2	29	1.2 (0.67 to 2.1)
⩾3	7	0.67 (0.24 to 1.9)

*Estimates for each covariate were not adjusted for the other covariates. CIN3 = cervical intraepithelial neoplasia grade 3; OC = oral contraceptive.

†The number of cases for the analysis of OC use and number of live births were less than 68 because of missing data for these covariates.

those for non-smokers (n = 6). The risk for CIN3 or cervical cancer increased monotonically with increasing level of smoking exposure (P_{trend} = .005; data not shown).

Discussion

We present prospective data that strongly implicate smoking as a statistically significant risk factor for CIN3 and cancer among women who are infected with oncogenic HPV. Our results provide some of the strongest evidence to date of the elevated risk of CIN3 and cervical cancer among smokers, because we prospectively studied approximately 2000 women who were infected with oncogenic HPV. A recent nested case–control study also found that, among women infected with oncogenic HPV, former smokers and women who smoked one or more packs of cigarettes per day were more likely (approximately twofold increased likelihood for the former group and approximately threefold increased likelihood for the latter group) to have CIN3 or cervical cancer than were women who never smoked *(8)*.

One limitation of our study is that there was only a single baseline measurement of smoking and HPV DNA positivity. In addition, respondents to the questionnaire were at higher risk of CIN3 or cervical cancer than were nonresponders, which may limit the generalizability of the smoking association reported in this study. However, our finding does not represent an information bias (e.g.., selection into the study was not based on smoking habits) and therefore study comparisons are internally consistent. Shorter follow-up times for smokers compared with those for nonsmokers in this study may have resulted in a screening/detection bias and an underestimation of the impact of smoking.

The elevated risk of CIN3 and cervical cancer among smokers persisted even after the women no longer smoked (i.e., among former smokers). Smoking results in systemic exposure to genotoxic compounds that increase the risk of cancer in various organs and are known to cause genomic damage in squamous epithelial cells. Specifically, the metabolites produced by smoking, such as the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) have been measured in cervical mucus *(25)*. Our finding that former smokers have an increased risk of CIN3 or cervical cancer compared with nonsmokers may also suggest that the relative timing of HPV infection with respect to smoking-induced genotoxicity may not be critical for the development of CIN3 and cancer. Another possible explanation for the increased risk in former (and current) smokers is that smoking might be a surrogate for new sexual partners; new sexual partners could lead to additional HPV infections during follow-up. However, our observation of a consistently elevated risk of CIN3 and cervical cancer among smokers during follow-up does not support this explanation.

Conditional logistic models were used to control for factors that may influence our risk estimates. Indeed, the differences between crude RR estimates and OR estimates from the conditional logistic regression analysis appear to be related primarily to negative confounding by the number of Pap smears the study subjects had during follow-up (as a statistical note, calculation of crude OR in place of crude RR in the model shifted the values

by only 0.1). There was a strong positive association between the number of Pap smears and a diagnosis of CIN3 or cervical cancer and a weak negative association between the number of Pap smears and smoking. Thus, matching case patients with control subjects on the number of Pap smears they had during follow-up in the conditional logistic model probably provided a more accurate, albeit higher, estimate of the true risk of CIN3 or cervical cancer that was associated with smoking. Simultaneous adjustment for the other *a priori* hypothesized HPV cofactors—oral contraceptive use at enrollment and history of one or more live births—further strengthened the association between smoking and CIN3 or cervical cancer but did not alter the estimates associated with oral contraceptive use or parity. These adjustments were not presented as the best estimates of risk, because we were concerned about the effects of sparse data on risk estimates derived from conditional logistic regression *(26)*.

Our finding that HPV-infected women who reported using oral contraceptives at enrollment did not have a higher risk of CIN3 or cervical cancer than women who did not use oral contraceptives was consistent with results from some studies *(5,8,12,27)* but not with results from others *(6,10,11,13)*. The size and the prospective nature of our study lend credence to this null finding. Other case–control studies (i.e., those without a prospective study design) are limited by uncertainty about whether the use of oral contraceptives may have increased the likelihood that cases of CIN3 or cervical cancer would be detected at health clinics where oral contraceptives were distributed, how the control subjects were selected, and proper adjustment for HPV infection.

However, our study has several limitations that warrant caution about the conclusion for oral contraceptive use. First, we used only one measurement of oral contraceptive use, obtained at baseline (i.e., enrollment), which did not account for the possible discontinuation or initiation of oral contraceptive use by some women during the course of the study. Second, oral contraceptive users had shorter follow-up times than nonusers, which may have resulted in a censoring bias among the users. Third, among the control subjects, oral contraceptive users were statistically significantly more likely to be diagnosed with CIN1 and CIN2 during follow-up than nonusers (data not shown). This differential detection of milder cervical abnormalities among the control subjects could be related to the statistically significantly higher viral loads in women who used oral contraceptives than in nonusers (data not shown); viral load has been shown to be strongly associated with prevalent cytologic abnormalities *(28)*. Thus, oral contraceptive use may have resulted in increased detection and treatment of women in the control group who might have otherwise progressed to CIN3 or cervical cancer. However, the oral contraceptive users in our study were not screened more frequently than the nonusers. In the multivariable models, case patients and control subjects were matched on the number of Pap smears they had during follow-up, the date of their prediagnosis Pap smears, and the duration of follow-up to minimize the confounding influence of differential screening. Nevertheless, the relationship between oral contraceptive use and cervical neoplasia of all grades must be interpreted carefully because of the potential for screening bias. Finally, we had no information regarding the duration of oral contraceptive use. A recent case–control study *(15)* reported that the risk of CIN3 or cervical cancer associated with oral contraceptive use was elevated only in women who had used oral contraceptives for 5 years or longer. Importantly, we can conclude reassuringly that oral contraceptive use did not increase the risk of CIN3 or cervical cancer in a well-screened population.

A history of live births was also not associated with the risk of CIN3 or cervical cancer among the women in our study cohort. However, the group of women we studied was a low-parity population compared with women who were enrolled in an international study *(14)* that found that multiparity is an HPV cofactor. The elevated risk of CIN3 or cervical cancer associated with parity reported in other studies *(5,10–14)* may be restricted to women who had had more births than the women in our study. We also did not have follow-up information on the number of live births that occurred during the 10-year follow-up period of the study, which may be more relevant to the prospective risk of CIN3 or cervical cancer than the number of live births that occurred prior to enrollment. In cross-sectional case–control studies, which also lack information on the timing of births relative to HPV infection, multiparity may be a surrogate for a birth that occurs during active HPV infection. However, in our study, pregnancy at enrollment was not associated with future risk of high-grade cervical disease (data not shown).

In conclusion, we found that smoking is associated with the future risk of high-grade lesions of the cervix. This association may provide important etiologic clues about multistage carcinogenesis at the cervix. Future studies should examine whether biomarkers associated with smoking, such as smoking-induced DNA adducts and genomic damage, are present within cervical tissue and whether such biomarkers are detectable before the development of CIN3 or cervical cancer. Although anti-smoking campaigns in the United States may reduce the impact of this secondary factor on the incidence of cervical precancer and cancer, we anticipate that smoking may be contributing to increasing rates of cervical cancer in resource-poor regions of the world where cigarette smoking is now on the rise *(29)* and where Pap smear screening programs are suboptimal *(30)*.

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NOTES

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