Oral Contraceptives as Risk Factors for Cervical Adenocarcinomas and Squamous Cell Carcinomas

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Abstract

To assess the hypothesis that oral contraceptives (OCs) increase the risk of cervical adenocarcinomas, we conducted a six-center case-control study of 124 patients with adenocarcinomas, 139 with squamous cell carcinomas, and 307 population controls. Women between the ages of 18 and 69 who were newly diagnosed with cervical adenocarcinomas between 1992 and 1996 were eligible. Healthy female controls and a second case group of incident cervical squamous cell carcinomas were matched to the adenocarcinoma cases. All participants were interviewed regarding OCs, other risk factors for cervical carcinoma, and utilization of cytological screening, and a PCR-based test determined HPV genotype of cervical samples for both case groups and controls. Use of OCs was positively and significantly associated with adenocarcinomas and positively but weakly associated with squamous cell carcinomas. Associations between OCs and invasive adenocarcinomas ($n = 91$), squamous cell carcinoma *in situ* ($n = 48$), and invasive squamous cell carcinomas ($n = 91$) disappeared after accounting for HPV infection, sexual history, and cytological screening, but a positive association remained between current use of OCs and cervical adenocarcinoma *in situ* ($n = 33$). This association persisted after stratification by screening and sexual history and after restriction according to HPV status, but small numbers made it difficult to exclude detection bias, selection bias, or residual confounding by HPV as potential explanations. Current OC use was associated with cervical adenocarcinomas *in situ*, but we saw no other evidence that OCs independently increase the risk of cervical carcinomas.

Introduction

Decreased incidence rates for cervical carcinomas since the 1970s combine declining rates of squamous cell carcinomas (1) with rising rates of adenocarcinomas, especially among younger women (2–5). Effective screening programs contribute to decreasing incidence rates of squamous cell carcinomas (6), but neither improved surveillance and classification of adenocarcinomas (7) nor a birth cohort phenomenon (8) appears to account for all of the increase in adenocarcinomas. The clinical heterogeneity of cervical adenocarcinomas makes etiological investigations challenging (9), but a hypothesized association with use of OCs[2] might explain recent trends (4, 7, 10).

OCs are highly correlated both with sexual behaviors that increase exposure to the HPVs that cause cervical carcinomas and with utilization of Papanicolaou cytological screening (Pap smears; Refs. 11 and 12), which decreases cervical cancer incidence by identifying precursor lesions such as carcinoma *in situ*. Distinguishing causal pathways from spurious associations attributable to residual confounding has therefore been challenging (13–15). Several (16–18) but not all (19–22) studies found elevated risks of adenocarcinomas and elevated risks of squamous cell carcinomas (23–25) among OC users, but complete control of confounding has been difficult (26, 27). Accurate classification of HPV infection in epidemiological studies has only recently become feasible (28).

To evaluate the hypothesis that OCs increase the risk of cervical adenocarcinomas, we conducted a multicentered case-control study involving patients with adenocarcinomas, patients with squamous cell carcinomas, and community controls. Extensive risk factor information, including sexual behavior, screening patterns, and type-specific HPV genotype allowed us to control for multiple potential confounding variables.

Materials and Methods

This study included women who were diagnosed at one of six medical centers (George Washington University Medical Center, Washington, DC; Georgetown University Medical Center, Washington, DC; Graduate Hospital, Philadelphia, PA; Hershey Medical Center, Hershey, PA; University of Maryland Hospital, Baltimore, MD; and Yale/New Haven Hospital, New Haven, CT) in the eastern United States. Institutional review boards at the National Cancer Institute and each institution approved the study. Women between the ages of 18 and 69 who

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[2] The abbreviations used are: OC, oral contraceptive; HPV, human papillomavirus; STM, specimen transport medium; OR, odds ratio; CI, confidence interval.

were diagnosed between January 1, 1992 and March 1, 1996 with incident invasive primary adenocarcinoma or adenocarcinoma *in situ* of the uterine cervix, adenosquamous carcinoma, or other rare histological types of cervical carcinoma with glandular involvement (ICD-O 180.0, 180.1, 180.8, or 180.9) were eligible. Enrollment began in July 1994, and therefore we retrospectively ascertained incident cases diagnosed between January 1992 and June 1994. Prospective ascertainment of cases between July 1994 and March 1996 occurred at the three largest clinical centers (Georgetown, Hershey, and Yale). Women diagnosed with endometrial carcinomas, sarcomas, fibromas, myomas, or lymphomas were ineligible. A three-pathologist panel reviewed pathology reports and histological specimens from 88% of the adenocarcinoma cases and confirmed the original histological diagnosis of these cases.

A sample of patients with squamous cell cervical carcinomas formed a second case group. Using the same eligibility criteria, we matched these women on clinic, age at diagnosis ($\pm$5 years), diagnosis date ($\pm$3 months), and stage of disease at diagnosis (carcinoma *in situ versus* invasive carcinoma) to women with adenocarcinomas at a 1:1 ratio. Diagnosis date or age-matching criteria were relaxed if no matching squamous case could be found.

Modified random digit dialing identified population controls, who were individually matched 2:1 to the adenocarcinoma cases on age ($\pm$5 years), clinic, race/ethnicity, and telephone exchange. We generated a random sample of telephone numbers within each adenocarcinoma case's exchange and called all households to enumerate adult women by age and ethnicity; the response to this phase was 79%. We then called two age- and ethnicity-matched potential controls for each case (three for cases older than 45, to account for increasing prevalence of hysterectomy) to determine hysterectomy status, which was reported by 75% of respondents. After exclusion of women with a hysterectomy (and random exclusion of one control if all three were eligible), eligible controls were invited to participate. We matched other eligible controls from the initial enumerated lists or relaxed the matching criteria (*e.g.*, 6-year age intervals) when two controls could not be matched. Refusals were replaced but included in the response denominators.

All consenting and eligible participants completed personal risk factor interviews with trained staff. To minimize the effect of diagnosis on exposures and to exclude recent Pap smears that led to the diagnosis of cervical cancer, both case groups reported exposures that occurred before a reference date, which was 12 months before their diagnosis. Controls were assigned the reference date of their index adenocarcinoma case. A calendar approach captured recency, latency, current use, and duration of OC use relative to the reference date.

We sought consent from all participants to collect cervicovaginal cells for HPV DNA testing using both self-administered and clinician-administered samples. The self-administered specimen was collected with a Dacron swab and stored in 1 ml of STM (Digene Corp., Silver Spring, MD). Clinician-administered samples were collected during pelvic examinations using two Dacron swabs, each stored in 1 ml of STM. For population controls, cases who were sampled before treatment and cases whose treatment did not include removal of the cervix, clinicians collected one specimen from the ectocervix and one from the endocervix. For cases sampled after surgical treatment who no longer had an intact cervix, both clinician-administered Dacron swab specimens were obtained from the vaginal cuff. All specimens were frozen, stored at the clinics, and regularly shipped on dry ice to the National Cancer Institute repository for storage. Controls were invited to visit the clinic from which their index case was recruited to complete the data collection. All participants had the option of an in-home interview and sample collection, but home visits included only the self-administered samples.

A PCR-based reverse line blot detection method that uses the MY09/11 L1 consensus primer system and individually discriminates 27 HPV genotypes determined HPV DNA genotype (29). After testing, we learned that some of the STM had been contaminated with HPV 16 DNA-containing plasmids during manufacture. To distinguish true HPV 16 infections from contamination, we retested all HPV 16-positive samples with a second set of primers that do not amplify the segment of HPV 16 DNA contained in the contaminating plasmids (30). Only the specimens that tested positive using both sets of primers were considered HPV 16 positive. Positive samples were grouped hierarchically according to type: type 18, type 16, type 18-related (types 39, 45, 59, or 68), other cancer-associated types (types 26, 31, 33, 35, 51, 52, 55, 56, 58, W13B, Pap 291, or Pap 238A), or "low-risk" types (types 6, 11, 40, 42, 53, 54, 57, 66, or Pap 155). Comparison of the clinician-administered and self-administered HPV results revealed good agreement (90% agreement; Kappa coefficient, 0.76). We therefore used the results of the self-administered samples for women who did not have clinician-administered samples.

Two hundred three women with potential adenocarcinomas were identified, but 27 chose not to participate, 2 were too ill to participate, 11 could not be located, 7 died before they could be enrolled, and 10 could not be enrolled for other reasons. Four of the 146 eligible cases could not be interviewed, and 18 interviewed cases were diagnosed with other cervical carcinomas that were not adenocarcinomas. Cervical samples were available from 116 of the final 124 adenocarcinoma cases. Two hundred fifty-five women with squamous cell carcinomas were identified, but 38 chose not to participate, 3 were too ill, 29 could not be located, 25 had died, 12 could not be enrolled for other reasons, and 2 did not speak English. Of these 146 remaining eligible cases, 139 completed the interview, and 129 contributed a cervical sample.

Four hundred seventy controls were identified, but 126 chose not to participate, 1 was too ill, 15 could not be located, and 21 could not be enrolled for other reasons. All of these 307 eligible controls completed the interview, and 255 contributed a cervical sample. Demographics, patterns of sexual behavior, and use of OCs for 25 women with adenosquamous tumors and 13 with other rare histological types of cervical carcinomas with glandular involvement resembled those for the 86 adenocarcinomas, and therefore the analysis combined these three groups. The final analytic group included 124 adenocarcinomas (33 adenocarcinoma *in situ* and 91 invasive tumors), 139 squamous cell carcinomas (48 carcinoma *in situ* and 91 invasive tumors), and 307 community controls.

To avoid loss of cases without a matched control and because controls were individually matched to adenocarcinoma cases but not to squamous cell carcinoma cases, unconditional logistic regression was used in favor of conditional logistic regression (31). ORs and 95% CIs estimated relative risks. Final regression models retained a parsimonious combination of matching and confounding variables that altered parameter estimates for OCs by at least 10%: age ($<$30, 30–39, 40–49, 50–59, and $\geq$60), ethnicity (Caucasian *versus* non-Caucasian), household income ($<$\$30,000, \$30,000-\$49,999, and $\geq$\$50,000), HPV genotype (negative or low-risk; types 16, 18, 18-related, or other cancer-associated types; unknown), number of Pap smears in the previous 10 years ($<$10 *versus* $\geq$10), and

number of lifetime sexual partners (<4 *versus* ≥4). The addition of other variables (*e.g.*, smoking) to this combination did not change the OC parameter estimates. Clinic was dropped from final models because it had no impact on OC associations. Tests for trend reflect two-sided *P*s from models that included ordinal variables for categorical exposures. The SAS system (32) computed all analyses.

Results

Sample collection preceded treatment for 31% of adenocarcinoma cases and 42% of squamous cell carcinoma cases. Eighty-two % of adenocarcinoma and 73% of squamous cell carcinoma samples collected before treatment contained HPV DNA, whereas 38 and 42%, respectively, of those collected after treatment contained HPV; 19% of the control samples contained HPV. Adenocarcinomas were significantly associated with HPV types 18 and 16 (age- and ethnicity-adjusted ORs for all cases *versus* controls: OR, 11.7 and OR, 5.6, respectively; cases sampled before treatment: OR, 104.1 and OR, 43.2, respectively). Squamous cell carcinomas were more strongly associated with HPV type 16 than type 18 (all cases: OR, 10.0 and OR, 5.2, respectively; cases sampled before treatment: OR, 38.0 and OR, 2.3, respectively).

Ethnicity, education, and household income varied slightly between case groups and the community controls (Table 1). Height, weight, age at which menstrual periods became regular, and age at first pregnancy were associated with adenocarcinomas but did not confound the OC relationships, whereas adult weight gain, smoking, parity, infertility, age at which regular douching began, number of years of regular douching, and number of sexual partners in the past 5 years or in the 10 years after first intercourse were associated with both histological types but also did not confound the OC associations (data not shown). Most cases but fewer than half of the controls reported at least four sexual partners. Controls were more likely than both case groups to have had a Pap smear in the past year. Squamous cell carcinoma cases reported the fewest, and adenocarcinoma cases the most, Pap smears in the previous 10 years.

Table 1 Percent distribution of demographic factors, sexual behavior, and Pap smear screening for 124 women with adenocarcinomas, 139 with squamous cell carcinomas, and 307 community controls

	Adenocarcinomas	Squamous cell carcinomas	Controls
Age at reference date			
<30 years	21.8	20.4	21.8
30–39 years	31.5	37.4	34.9
40–49 years	32.3	26.6	27.4
50–59 years	4.8	7.2	11.7
≥60 years	9.7	8.6	4.6
Ethnicity			
Caucasian American	90.3	80.6	86.5
African American	6.5	13.0	9.2
Non-Caucasian, non-African American	3.2	6.5	4.3
Education			
Less than high school	11.3	13.1	9.2
High school graduate	25.8	44.5	25.5
Beyond high school	62.9	42.3	65.4
Household income			
<$30,000	35.0	48.6	27.4
$30,000–$50,000	26.8	30.4	31.4
>$50,000	38.2	21.0	41.4
Smoking			
Never	50.0	35.0	53.6
≤10 years	19.4	16.1	19.1
11–20 years	13.7	24.1	13.2
>20 years	16.9	24.8	14.1
No. of sexual partners in lifetime			
0–1	20.5	13.8	33.8
2–3	17.2	21.1	25.3
4 or more	62.3	65.2	41.0
No. of Pap smears in 10 years before diagnosis[a]			
<5	29.3	42.4	33.3
6–9	13.0	16.1	17.7
10 or more	57.7	41.6	49.0
Months since most recent Pap smear[a]			
12–23	51.6	47.5	65.2
24 or more	41.1	46.0	27.0
Unknown	7.3	6.5	7.8

[a] Pap smears <12 months before the date of diagnosis (or the reference date for controls) were considered diagnostic Paps and were excluded.

OC Use among Adenocarcinoma and Squamous Cell Carcinoma Patients. Each measure of OC was positively associated with adenocarcinomas, with stronger associations for longer and more recent exposures (Table 2). Adjustment for income, number of Pap smears, sexual partners, and HPV infection attenuated all of the associations. Sexual partners and HPV were the most influential confounders, and none of the results differed after excluding the 25 women with adenosquamous carcinomas or the 13 women with other rare adenocarcinomas. Initial ORs for squamous cell carcinoma were >1.0, but full adjustment generated null associations.

OC Use by Stage of Disease at Diagnosis. Women diagnosed with carcinoma *in situ* and invasive carcinomas were compared separately to all controls (Table 3). Years since first and last use are not presented because they were highly correlated with duration of use and age at first use. Current and longer use were significantly associated with adenocarcinoma *in situ*. The association between adenocarcinoma *in situ* and first use before age 17 likely reflected a duration effect, because 10 of those 13 women had used OCs for at least 6 years. Adjustment produced null associations for invasive adenocarcinomas, invasive squamous cell carcinomas, and squamous cell carcinoma *in situ*.

To explore residual confounding by Pap smear screening, we stratified according to recent Pap smears (<12 months before the reference date) or annual Pap smears in the 10 years before the reference date. Current use was associated with adenocarcinoma *in situ*, regardless of annual Pap smears (OR, 11.9 among women with annual Pap smears; OR, 4.6 among women without) or recent Pap smears (OR, 8.4 among women with a recent Pap smear; OR, 6.9 among women without). OCs for >6 years was associated with adenocarcinoma *in situ* among annually screened women (OR, 4.7) and among women without a recent Pap smear (OR, 6.4) but not among those without annual Pap smears (OR, 1.0) or those who were recently screened (OR, 0.8). Stratification did not change the null associations for invasive adenocarcinomas (data not shown).

OCs and HPV Status. Because HPV infection is considered a necessary cause of cervical cancers, women who are not infected with HPV are not at risk for developing cervical cancers and thus should be excluded from the analyses. We repeated the analyses after excluding the controls in whom HPV was not detected or for whom a cervical sample was not available (Table 4). Current OCs and OCs for >6 years were significantly associated with adenocarcinoma *in situ* but not with invasive adenocarcinomas or squamous cell carcinoma *in situ*.

Table 2 OC use among adenocarcinoma cases, squamous cell carcinoma cases, and community controls: Adjusted ORs and 95% CIs

	Controls	Adenocarcinomas			Squamous cell carcinomas		
	n^a	n^a	OR[b]	OR[c] (95% CI)	n^a	OR[b]	OR[c] (95% CI)
Never used OCs	76	23	1.0[d]	1.0[d]	34	1.0[d]	1.0[d]
Ever used OCs	231	101	1.6	1.0 (0.5–1.9)	105	1.1	0.8 (0.4–1.5)
Former use	187	70	1.3	0.9 (0.5–1.7)	85	1.1	0.9 (0.5–1.7)
Current use[e]	41	28	3.0[f]	1.8 (0.7–4.3)	19	1.3	0.8 (0.3–2.0)
Duration of use							
2 years or less	70	27	1.4	0.9 (0.4–2.0)	33	1.2	0.8 (0.4–1.7)
2–6 years	81	28	1.2	0.7 (0.3–1.5)	33	1.0	0.7 (0.4–1.5)
>6 years	77	43	2.1[f]	1.2 (0.6–2.6)	38	1.2	0.9 (0.4–2.0)
				P (trend) = 0.58			*P* (trend) = 0.88
Age at first OC use							
Before 17	58	29	2.2[f]	1.0 (0.5–2.3)	31	1.5	0.9 (0.4–2.1)
18 or 19	56	25	1.8	1.1 (0.5–2.4)	32	1.6	1.2 (0.5–2.6)
20–22	58	26	1.7	1.0 (0.4–2.2)	26	1.2	0.8 (0.4–1.8)
After 22	56	18	1.2	0.9 (0.4–1.9)	15	0.7	0.6 (0.2–1.3)
				P (trend) = 0.69			*P* (trend) = 0.22
Years since first use							
10 or fewer	59	25	2.0	1.3 (0.5–3.2)	21	1.1	0.7 (0.3–1.9)
10–15	39	19	2.1	1.0 (0.4–2.6)	32	2.4[f]	1.8 (0.7–4.4)
15–20	50	21	1.7	1.2 (0.5–2.9)	20	1.1	1.0 (0.4–2.4)
>20	80	33	1.3	0.7 (0.3–1.6)	31	0.9	0.6 (0.3–1.3)
				P (trend) = 0.52			*P* (trend) = 0.36
Years since last use							
2 or fewer	59	31	2.6[f]	1.3 (0.5–3.1)	31	1.4	0.9 (0.4–2.1)
2–10	59	21	1.6	0.8 (0.4–2.0)	26	1.1	0.7 (0.3–1.6)
10–18	65	32	1.8	1.2 (0.6–2.5)	25	0.9	0.8 (0.4–1.8)
>18	45	14	1.0	0.6 (0.2–1.5)	22	1.2	0.9 (0.4–2.0)
				P (trend) = 0.46			*P* (trend) = 0.63

[a] Excludes missing responses.
[b] Adjusted for age (<30, 30–39, 40–49, 50–59, and ≥60 years) and ethnicity (Caucasian *versus* non-Caucasian).
[c] Adjusted for age, ethnicity, income (<$30,000, $30,000–$50,000, and >$50,000), HPV (negative or low-risk; types 16, 18, 18-related, or other cancer-associated types; unknown), lifetime number of sexual partners (<4 *versus* ≥4), and number of Pap smears in last 10 years (<10 *versus* ≥10).
[d] Referent group for the column.
[e] Defined as OC use 12 months before diagnosis for cases and at reference date for controls.
[f] 95% CI excludes 1.0.

Associations for invasive squamous cell carcinoma were similar to those based on all controls.

To further address HPV misclassification in cases who were treated before cervical samples were obtained, we repeated the analyses after excluding the 90 adenocarcinoma cases and the 94 squamous cell carcinoma cases from whom samples were obtained after treatment. All six women with adenocarcinoma *in situ* who were sampled before treatment had used OCs, and therefore ORs could not be calculated in this subgroup. For the 28 women with invasive adenocarcinoma, 13 women with squamous cell carcinoma *in situ*, and 32 women with invasive squamous cell carcinomas, results were unchanged (data not shown).

Stratification by number of early (*i.e.*, in the 10 years after first intercourse) or lifetime sexual partners, which served as proxies for infection with HPV, produced similar results. Current use was associated with adenocarcinoma *in situ* among women with fewer than 4 (OR, 10.3) or >4 (OR, 7.4) early partners and among women with fewer than 4 (OR, 16.9) or >4 (OR, 6.6) lifetime partners. OCs for >6 years was associated with adenocarcinoma *in situ* among women with <4 (OR, 4.5) but not >4 (OR, 1.9) early partners and among women with <4 (OR, 14.8) but not >4 (OR, 1.4) lifetime partners. Stratification did not impact associations with invasive adenocarcinomas (data not shown).

We evaluated the interaction between current use and duration of use for adenocarcinomas by collapsing both measures into dichotomous variables: non-current *versus* current, and ≤6 years *versus* >6 years. With women who were not currently using OCs and who had used OCs for ≤6 years as the referent group, current use was positively associated with adenocarcinoma *in situ* among women who used OCs for ≤6 years (OR, 2.1) and significantly associated among women who had used OCs for >6 years (OR, 11.4). OCs for >6 years was not associated with adenocarcinoma *in situ* among non-current users (OR, 0.9). Invasive adenocarcinomas were not associated with OCs (data not shown). Further adjustment for use of barrier contraception or restriction to the women who had used barrier contraception did not change the results (data not shown).

Discussion

Numerous studies have found OC users at increased risk of squamous cell carcinomas (25, 33–36) and even higher risk of invasive adenocarcinomas (16–18, 23, 24), but most studies lacked HPV data or used unreliable detection techniques (37). Recent studies that used more accurate PCR-based techniques confirmed the increased risks for squamous cell carcinomas (38–40) but have not addressed adenocarcinomas. Neither of the two largest studies of adenocarcinomas (16, 17), which both controlled for confounding by sexual history and screening, was able to assess potential confounding by HPV. However, a recent report identified significant associations with OC use among 151 cases of cervical adenocarcinoma *in situ* after controlling for HPV through PCR methods in cases and anti-

Table 3 ORs[a] and 95% CIs for use of OCs among adenocarcinoma cases and squamous cell carcinoma cases, stratified by *in situ versus* invasive tumors

	Adenocarcinomas				Squamous cell carcinomas			
	In situ		Invasive		*In situ*		Invasive	
	n^b	OR (95% CI)	n^b	OR (95% CI)	n^b	OR (95% CI)	n^b	OR (95% CI)
Never used OCs	2	1.0 (ref)[c]	21	1.0 (ref)[c]	7	1.0 (ref)[c]	27	1.0 (ref)[c]
Ever used OCs	31	3.4 (0.7–16.0)	70	0.8 (0.4–1.6)	41	1.0 (0.4–2.8)	64	0.8 (0.4–1.7)
Former use	13	2.0 (0.4–9.9)	57	0.8 (0.4–1.5)	32	1.3 (0.5–3.6)	53	0.9 (0.4–1.7)
Current use[d]	18	12.6 (2.5–64.2)	10	0.6 (0.2–2.0)	9	0.9 (0.2–3.3)	10	0.9 (0.3–2.6)
Duration of use								
2 years or less	7	3.2 (0.6–17.2)	20	0.8 (0.3–1.8)	10	0.9 (0.3–3.0)	23	0.9 (0.4–2.0)
2–6 years	7	1.7 (0.3–9.5)	21	0.7 (0.3–1.6)	15	0.9 (0.3–2.8)	18	0.7 (0.3–1.6)
>6 years	17	6.0 (1.2–30.7)	26	0.8 (0.3–1.8)	16	1.2 (0.4–3.9)	22	0.9 (0.4–2.2)
P (trend)		$P = 0.03$		$P = 0.62$		$P = 0.67$		$P = 0.74$
Age at first OC use								
Before 17	13	4.6 (0.9–23.9)	19	0.7 (0.3–1.9)	11	0.7 (0.2–2.6)	21	1.2 (0.4–3.0)
18 or 19	8	4.1 (0.7–22.8)	17	0.8 (0.3–2.1)	16	1.6 (0.5–5.0)	16	1.1 (0.4–2.8)
20–22	5	2.2 (0.4–13.1)	21	0.9 (0.4–2.2)	9	1.0 (0.3–3.4)	17	0.9 (0.3–2.2)
After 22	5	2.9 (0.5–17.1)	13	0.7 (0.3–1.7)	5	0.9 (0.2–3.5)	10	0.5 (0.2–1.4)
P (trend)		$P = 0.86$		$P = 0.59$		$P = 0.86$		$P = 0.19$

[a] Adjusted for age, ethnicity, income, HPV, lifetime number of sexual partners, and number of Pap smears.
[b] Excludes missing responses.
[c] Referent group for the column.
[d] Defined as OC use 12 months before diagnosis for cases and at reference date for controls.

Table 4 ORs[a] for use of OCs among cases and HPV-positive controls

	Controls	Adenocarcinomas		Squamous cell carcinomas	
	n^b	*In situ*	Invasive	*In situ*	Invasive
Never used OC	11	1.0[c]	1.0[c]	1.0[c]	1.0[c]
Ever used OCs	37	5.4 (0.7–43.4)	1.3 (0.4–4.4)	1.7 (0.5–6.2)	1.2 (0.4–3.8)
Former use	27	3.1 (0.4–27.5)	1.3 (0.4–4.1)	1.8 (0.5–6.7)	1.0 (0.3–3.2)
Current use[d]	10	17.1 (1.5–188.2)	2.1 (0.4–11.9)	1.6 (0.3–8.5)	0.7 (0.1–3.6)
Duration of use					
2 years or less	9	4.0 (0.4–44.3)	1.5 (0.3–6.6)	1.4 (0.3–7.2)	1.1 (0.3–4.2)
2–6 years	12	4.8 (0.4–51.9)	1.1 (0.2–5.2)	3.8 (0.7–19.3)	1.9 (0.4–8.4)
>6 years	16	6.2 (0.7–52.7)	1.0 (0.2–4.2)	1.1 (0.3–5.0)	0.9 (0.2–3.7)
P (trend)		$P = 0.12$	$P = 0.88$	$P = 0.85$	$P = 0.99$

[a] Adjusted for age, ethnicity, income, HPV, lifetime number of sexual partners, and number of Pap smears.
[b] Excludes missing responses.
[c] Referent group for the column.
[d] Defined as OC use 12 months before diagnosis for cases and at reference date for controls.

body-based methods in controls (41). Armed with a recently developed PCR technique for identifying HPV in both cases and controls, we designed our analysis to evaluate risk associated with OCs in women with histologically confirmed adenocarcinomas.

Adjustment for HPV, sexual history, and screening eliminated the positive associations between OCs and invasive adenocarcinomas, invasive squamous cell carcinomas, and squamous cell carcinoma *in situ*. Multiple measures of OC use remained associated with adenocarcinoma *in situ*, and current use appeared to drive these results. Simultaneous assessment of current use and duration revealed duration associations only among current users. Current use was consistently associated with adenocarcinoma *in situ*, but duration was associated only among recently or annually screened women or among women with fewer lifetime or early partners.

Four noncausal factors could account for the positive association with adenocarcinoma *in situ*: small numbers, detection bias, selection bias, or misclassification of HPV status. Small numbers offer an attractive explanation; only two women with adenocarcinoma *in situ* had never used OC, but including short-term OC users in the referent group produced similar results. Detection bias is equally tempting, because cervical carcinoma *in situ* is generally asymptomatic (42) and detected through regular Pap smear screening (15). Although OCs and screening were positively correlated in our data, associations remained within the strata of recently or annually screened women.

Our pathology panel reviewed information from potential cases to reduce misclassification (9). However, selection bias was possible if the 21% of eligible adenocarcinoma cases and 26% of squamous cell carcinoma cases who had died, were in poor health, or chose not to participate differentially used OCs and had an unequal distribution of disease stage at diagnosis. Identification of controls from the same geographic region decreased referral bias.

Sexual behavior continued to confound OC associations, presumably because of HPV misclassification (43). Our PCR-based technique identified infection by 27 HPV genotypes but failed to detect HPV in all women with cervical carcinomas. These false-negative results could result from a swab missing the infected area, infection with a HPV genotype that was not

among these 27, and treatment that preceded sampling for most cases, which meant that tissue in which HPV would have been detected was unavailable. The line blot detection method lacks a generic probe and therefore does not detect "uncharacterized" HPV genotypes. The anticontamination primer we used to rule out contamination by HPV 16 is less sensitive than the MY09/11 primer system and could also account for some of the false-negative results. Sexual history and the availability of up to three samples (*i.e.*, two clinician-administered and one self-administered, which was more likely to be HPV positive and may have detected vaginal or vulvar infections) from controls might explain their high prevalence of HPV DNA. We assessed misclassification by excluding HPV-negative controls, excluding cases sampled after treatment, and stratifying on sexual partners, but each approach produced similar results. The MY09/11 primer system is valid (44) but detected HPV in fewer cases with adenocarcinoma *in situ* (50% of samples collected before treatment) than cases with invasive adenocarcinoma (88%), squamous cell carcinoma *in situ* (69%), or invasive squamous cell carcinoma (73%). This difference between the six adenocarcinoma *in situ* and 16 invasive adenocarcinoma samples approached statistical significance (Fisher's exact test, $P = 0.06$). Sensitivity was therefore lowest for adenocarcinoma *in situ*, and residual confounding by HPV may explain why statistical adjustment did not eliminate the OC associations in this group.

Invasive adenocarcinomas arise from adenocarcinoma *in situ* (45, 46), and therefore an association restricted to adenocarcinoma *in situ*, if true, contradicts the current cervical carcinogenesis model (47) by suggesting that adenocarcinoma *in situ* does not always precede invasive adenocarcinomas. This implies that the majority of invasive adenocarcinomas progress through a pathway that does not involve OCs, and that other (*i.e.*, in addition to HPV) risk factors that are not associated with OCs cause invasive adenocarcinomas. Other associations between OCs and preinvasive carcinoma have been attributed to reversible OC effects (27, 33, 36, 48, 49) or to the possibility that OCs promote existing lesions or lesions that arise at critical times (18, 39, 50). Adenocarcinoma *in situ* might also represent a glandular response to particular HPV infections or a phase through which some invasive tumors rapidly pass.

In conclusion, current use of OCs was associated with cervical adenocarcinoma *in situ*, but OCs were not associated with invasive adenocarcinomas or with squamous cell carcinomas from the same population. Noncausal factors appear to explain the previously reported 2-fold increased risk of adenocarcinomas among OC users. Whether the association between OCs and adenocarcinoma *in situ* reflects detection bias, selection bias, residual confounding by HPV, or a true association awaits resolution through additional research.

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