

Associations between smoking and adenocarcinomas and squamous cell carcinomas of the uterine cervix (United States)

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Abstract

Objectives: Few studies of smoking and cervical carcinoma have addressed the rare cervical adenocarcinomas or used DNA-based tests to control for human papillomavirus (HPV) infection.

Methods: This multicenter case-control study included 124 adenocarcinoma cases, 307 community controls (matched on age, race, and residence to adenocarcinoma cases), and 139 squamous carcinoma cases (matched on age, diagnosis date, clinic, and disease stage to adenocarcinoma cases). Participants completed risk-factor interviews and volunteered cervical samples for PCR-based HPV testing. Polychotomous logistic regression generated adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for both histologic types.

Results: Eighteen percent of adenocarcinoma cases, 43% of squamous carcinoma cases, and 22% of controls were current smokers. After control for HPV and other questionnaire data, adenocarcinomas were consistently inversely associated with smoking (*e.g.* current: OR = 0.6, 95% CI 0.3–1.1; ≥ 1 pack per day: OR = 0.7, 95% CI 0.4–1.3), while squamous carcinomas were positively associated with smoking (*e.g.* current: OR = 1.6, 95% CI 0.9–2.9; ≥ 1 pack per day: OR = 1.8, 95% CI 1.0–3.3). Results in analyses restricted to HPV-positive controls were similar.

Conclusion: Smoking has opposite associations with cervical adenocarcinomas and squamous carcinomas. Although both histologic types are caused by HPV and arise in the cervix, etiologic co-factors for these tumors may differ.

Introduction

Cervical carcinogenesis requires oncogenic human papillomaviruses (HPV) and other etiologic co-factors [1]. The conclusion that cigarette smoking potentially causes cervical carcinomas [2] was biologically plausible yet based primarily on epidemiologic studies that were conducted before HPV was identified as the sexually transmitted agent responsible for most [3], if not all [4], cervical carcinomas. HPV has been associated with smoking [5] and thus could confound associations between cervical carcinoma and smoking [6].

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For numerous cancer sites, smoking appears to be more strongly associated with squamous cell carcinomas than adenocarcinomas [7]. Although squamous cell carcinomas are the predominant histologic type of cervical carcinomas [8], the relative and absolute incidence of cervical adenocarcinomas has been rising [9]. Adenocarcinomas now account for 10–15% of all cervical carcinomas [10]. Previous epidemiologic studies of smoking and cervical carcinomas have primarily included squamous cell carcinomas or did not distinguish between histologic types, and the few studies that specifically evaluated adenocarcinomas preceded the availability of sensitive and specific DNA-based HPV tests. To appraise smoking as a potential risk factor in cervical adenocarcinoma, we used a newly developed PCR-based HPV test and conducted a case-control study of smoking and other risk factors for cervical adenocarcinomas and squamous carcinomas.

Materials and methods

Study population

We briefly summarize the study methods, which we described elsewhere [11]. Women between the ages of 18 and 69 who were newly diagnosed with *in-situ* or invasive primary adenocarcinoma of the uterine cervix, adeno-squamous carcinoma, or other rare histologic types of cervical carcinoma with glandular involvement at one of six US medical centers between 1992 and 1996 were eligible as adenocarcinoma cases. We retrospectively identified women diagnosed between January 1992 and June 1994 (the date the study began) and prospectively recruited women diagnosed between July 1994 and March 1996. A panel of three pathologists performed a simultaneous microscopic review of adenocarcinomas and provided the study diagnoses. Using random-digit dialing, we generated a random sample of telephone numbers within the telephone exchange of each adenocarcinoma case, enumerated all adult women in households at those numbers, excluded women who reported a hysterectomy, and individually matched healthy controls to adenocarcinoma cases at a 2:1 ratio on age (± 5 years), race, and geographic region (*i.e.* telephone exchange).

To address potential referral bias and evaluate whether risk factors differ according to tumor histology, we included a sample of women diagnosed with squamous carcinomas. Using identical eligibility criteria, squamous carcinoma cases were individually matched to the adenocarcinoma cases on clinic, age at diagnosis (± 5 years), diagnosis date, and stage of disease at diagnosis (*in-situ* vs. invasive). Institutional review

boards at the National Cancer Institute and each center approved the study.

Interviews

Participants completed personal risk factor interviews with trained staff. Adenocarcinoma and squamous carcinoma cases reported exposures that occurred before a reference date, which was 12 months before their date of diagnosis. Community controls were assigned the reference date of their index adenocarcinoma case and reported only exposures before that date.

HPV DNA testing

With consent, we collected one self-administered and two clinician-administered cervicovaginal samples from cases and controls. Self-administered specimens were collected with Dacron swabs and stored in 1 ml Specimen Transport Medium (STM; Digene Corporation, Silver Spring, MD). Clinician-administered samples were collected during pelvic examinations using two Dacron swabs, each stored in 1 ml STM. For community controls, cases who were sampled before treatment, and cases whose treatment did not include removal of the entire cervix, clinicians collected one specimen from the ectocervix and one sample from the endocervix. For cases sampled after surgical treatment (*i.e.* who no longer had an intact cervix), clinicians obtained both Dacron swab specimens from the vaginal cuff. For sample collection, controls visited the clinic from which their index adenocarcinoma case was recruited. All participants had the option of in-home interviews and sample collections, which included self-administered samples only. A PCR-based reverse line blot detection method [12] that uses the MY09/11 L1 consensus primer system to individually discriminate 27 genotypes determined HPV status, which was grouped according to type [11] after confirming HPV-16 status with a second set of primers [13]. The 90% agreement between the results of the clinician-administered and the self-administered samples (Gravitt PE *et al.*, submitted) allowed us to use data from self-administered samples for women who did not have clinician-administered samples.

Exposure assessment

We classified all smoking exposures relative to the reference date and defined ever smoking as at least 100 cigarettes before the reference date. We asked smokers whether they smoked at their reference date (current smoking), the age at which they first smoked regularly, and how many cigarettes they usually smoked per day or

per week. Current smokers reported whether they had ever stopped for at least one year, and summary measures of total years smoking and pack-years smoked incorporated these non-smoking years.

Study population

Two hundred three women with potential adenocarcinomas were identified, but 27 refused, seven died before they could be enrolled, and 27 could not be enrolled for other reasons. Eighteen of the remaining 142 eligible and interviewed cases were diagnosed with other cervical carcinomas that were not adenocarcinomas and were excluded from this analysis. Cervical samples were available from 116 of the 124 final adenocarcinoma cases. Two hundred fifty-five women with squamous carcinomas were identified, but 38 refused, 25 had died, and 46 could not be enrolled for other reasons. Of these 146 remaining eligible cases, 139 completed the interview, and 129 contributed a cervical sample. Four hundred seventy controls were identified, but 126 refused, and 37 could not be enrolled for other reasons. All of these 307 eligible controls completed the interview, and 255 contributed a cervical sample. The final analytic group included 124 adenocarcinomas (33 *in-situ* and 91 invasive), 139 squamous carcinomas (48 *in-situ* and 91 invasive), and 307 community controls.

Statistical analysis

Polychotomous logistic regression [14] generated odds ratios (ORs) and 95% confidence intervals (CIs) to estimate risks associated with smoking for cervical adenocarcinomas and squamous cell carcinomas relative to the community control group. We evaluated all questionnaire variables (*e.g.* demographic factors, infertility, menarche, anthropometry, sexual history, menopausal factors, other medical conditions, use of oral contraceptives, family history of cancer, parity and pregnancy characteristics, and Pap smear screening) as potential confounders. Variables that were associated with both exposure and outcome, and that altered the parameter estimates for smoking (based on logistic regression models [15] for each case group separately *vs.* all controls) by at least 10% were included parsimoniously in final polychotomous regression models. Therefore, final models retained age (<30, 30–39, 40–49, 50–59, ≥60 years), race/ethnicity (Caucasian-American, other American), HPV status (negative or low-risk types; type 16, 18, 18-like, or other cancer-associated types; unknown), education (<high school, high school graduate, beyond high school), number of Pap smears in the 10 years before the reference date (0, 1–5, 6–9, ≥10),

and lifetime sexual partners (0–1, 2–9, ≥10). Clinic, a matching variable, was dropped from the models because it did not influence the results. Among controls, smoking was also positively associated with a prior induced abortion, a younger age at first sexual intercourse, family history of cancer, infertility, endometriosis, oral contraceptive use, more time since last Pap smear, and increasing weight, and negatively associated with age at first pregnancy, but additional adjustment for these variables had no effect on the risk estimates. The SAS system [16], with SAS-callable SUDAAN for polychotomous regression [17], computed all analyses.

Results

Sample collection for HPV testing preceded surgical treatment for 31% of adenocarcinoma cases and 42% of squamous carcinoma cases. Eighty two percent of adenocarcinoma and 73% of squamous carcinoma samples collected before treatment contained HPV DNA, whereas 38% and 42%, respectively, of samples collected after treatment contained HPV; 19% of the control samples contained HPV. Adenocarcinoma cases were strongly associated with HPV types 18 (age- and ethnicity-adjusted OR = 11.7) and 16 (OR = 5.6), and more strongly associated with types 18 (OR = 104.1) and 16 (OR = 43.2) when we excluded cases sampled after treatment. Squamous carcinomas were also strongly associated with HPV 18 (OR = 5.2) and 16 (OR = 10.0), although less strongly associated with types 18 (OR = 2.3) and more strongly associated with type 16 (OR = 38.0) after exclusion of cases sampled after treatment.

Detailed demographic characteristics have been reported [11]. Eighteen percent of adenocarcinoma cases, 43% of squamous carcinoma cases, and 22% of controls were current smokers at the reference date, while 50%, 66%, and 47%, respectively, were ever smokers (Table 1). Three sets of ORs (ORs adjusted for matching factors age and ethnicity; ORs also adjusted for education, Pap smear screening, and number of sexual partners; and ORs further adjusted for HPV) demonstrate how control for confounding affected the associations between case status and smoking. Age- and ethnicity-adjusted ORs for adenocarcinomas were close to the null, but full adjustment moved the ORs below 1.0. Current smoking (OR = 0.6) and smoking at least one pack per day (OR = 0.7) were inversely associated with adenocarcinomas.

For squamous cell carcinomas, age- and ethnicity-adjusted ORs were significantly above 1.0 for most exposures. Full adjustment for questionnaire data

Table 1. Polychotomous logistic regression: ORs and 95% CI for 124 adenocarcinomas and 139 squamous carcinomas vs. 307 controls

| Controls | | Adenocarcinomas | | | | | Squamous cell carcinomas | | | | |
|--------------------------|------------|-----------------|-----------------|-----------------|-----------------|---------|--------------------------|-----------------|-----------------|-----------------|----------|
| Smoked | Percentage | Percentage | OR ^a | OR ^b | OR ^c | 95% CI | Percentage | OR ^a | OR ^b | OR ^c | 95% CI |
| Never | 53 | 50 | 1.0 | 1.0 | 1.0 | Ref | 34 | 1.0 | 1.0 | 1.0 | Ref |
| Ever | 47 | 50 | 1.1 | 0.9 | 0.8 | 0.5–1.2 | 66 | 2.1 | 1.6 | 1.4 | 0.8–2.3 |
| Former | 25 | 32 | 1.3 | 1.0 | 1.0 | 0.6–1.6 | 22 | 1.4 | 1.2 | 1.1 | 0.6–2.1 |
| Current | 22 | 18 | 0.9 | 0.6 | 0.6 | 0.3–1.1 | 43 | 3.0 | 1.9 | 1.6 | 0.9–2.9 |
| Age first smoked (years) | | | | | | | | | | | |
| 11–15 | 12 | 10 | 0.8 | 0.7 | 0.6 | 0.3–1.4 | 19 | 2.2 | 1.5 | 1.3 | 0.6–2.6 |
| 16–17 | 13 | 15 | 1.2 | 0.9 | 0.8 | 0.4–1.6 | 21 | 2.5 | 1.7 | 1.5 | 0.7–2.9 |
| 18–20 | 13 | 17 | 1.3 | 1.0 | 1.0 | 0.5–1.9 | 15 | 1.6 | 1.4 | 1.3 | 0.6–2.7 |
| 21 or later | 9 | 9 | 1.0 | 0.7 | 0.7 | 0.3–1.6 | 10 | 1.5 | 1.1 | 1.1 | 0.4–2.6 |
| <i>p</i> (trend) | | | | | | 0.51 | | | | | 0.46 |
| Amount (pack/day) | | | | | | | | | | | |
| <1 | 25 | 27 | 1.2 | 0.9 | 0.9 | 0.5–1.5 | 24 | 1.4 | 1.2 | 1.1 | 0.6–1.9 |
| ≥1 | 21 | 23 | 1.0 | 0.8 | 0.7 | 0.4–1.3 | 42 | 3.3 | 2.1 | 1.8 | 1.0–3.3 |
| <i>p</i> (trend) | | | | | | 0.28 | | | | | 0.06 |
| Duration (years) | | | | | | | | | | | |
| ≤10 | 19 | 19 | 1.0 | 0.8 | 0.7 | 0.4–1.4 | 16 | 1.3 | 1.1 | 1.0 | 0.5–2.0 |
| 11–20 | 13 | 14 | 1.2 | 0.9 | 0.9 | 0.5–1.8 | 24 | 2.8 | 2.1 | 1.9 | 0.95–3.9 |
| >20 | 12 | 17 | 1.2 | 0.9 | 0.8 | 0.4–1.6 | 25 | 2.9 | 1.8 | 1.5 | 0.7–2.9 |
| <i>p</i> (trend) | | | | | | 0.50 | | | | | 0.13 |
| Pack-years | | | | | | | | | | | |
| <5 | 17 | 17 | 1.1 | 0.9 | 0.8 | 0.4–1.6 | 15 | 1.3 | 1.2 | 1.2 | 0.6–2.4 |
| 5–14 | 14 | 15 | 1.2 | 1.0 | 0.9 | 0.4–1.7 | 18 | 1.8 | 1.5 | 1.3 | 0.7–2.6 |
| ≥15 | 15 | 18 | 1.2 | 0.9 | 0.8 | 0.4–1.6 | 31 | 3.5 | 2.0 | 1.7 | 0.9–3.2 |
| <i>p</i> (trend) | | | | | | 0.41 | | | | | 0.11 |

^a Adjusted for age and ethnicity.

^b Also adjusted for education, lifetime sexual partners, and number of Pap smears in last 10 years.

^c Also adjusted for HPV; 95% CI is for this OR.

reduced the associations (although some remained significantly elevated), and additional adjustment for HPV further reduced the associations. The associations with smoking at least one pack per day were positive and indicated a linear, albeit not statistically significant ($p = 0.06$), trend. Neither years since first smoked nor years since last smoked (among former smokers) was consistently associated with adenocarcinomas or squamous carcinomas (data not shown).

We used a five-level outcome variable (control, invasive adenocarcinoma, adenocarcinoma *in-situ*, invasive squamous cell carcinoma, and squamous cell carcinoma *in-situ*) to examine associations between smoking and stage of disease at diagnosis. After full adjustment, associations were slightly but not materially stronger for invasive squamous carcinomas (e.g. current smoking, OR = 3.0, 95% CI 1.7–5.3) than squamous carcinoma *in-situ* (OR = 2.6, 95% CI 1.3–5.3). Stage-specific ORs for adenocarcinomas were similar and closer to the null than the ORs based on the combined case group (data not shown).

HPV is considered a necessary cause of cervical carcinoma, and therefore women who are not infected with HPV can be considered not at risk of developing cervical carcinoma. We therefore repeated the analyses after excluding the 206 controls who were HPV-negative and the 52 controls who did not volunteer a cervicovaginal sample (Table 2). For adenocarcinomas, associations (adjusted for age and ethnicity only) were similar to those from the full models that used all controls. Associations with squamous carcinomas were slightly weaker than those based on full models with all controls. Further adjustment for number of sexual partners or Pap smear screening did not change these results.

We employed a number of additional techniques to attempt to control for HPV. First, we repeated the analyses among the controls and cases in whom HPV was detected. After adjustment for age, ethnicity, and HPV type (low-risk types vs. cancer-associated HPV types), adenocarcinomas ($n = 58$) were not associated with ever smoking (OR = 1.0, 95% CI 0.4–2.3) but

Table 2. Polychotomous logistic regression ORs and 95% CIs: all adenocarcinomas and all squamous carcinomas vs. HPV-positive controls

| Characteristic | HPV-positive Controls (No.) ^a | All cases | | | |
|--------------------------|--|-----------------|---------|---------------------|---------|
| | | Adenocarcinomas | | Squamous carcinomas | |
| | | OR ^b | 95% CI | OR ^b | 95% CI |
| Never smoked | 22 | 1.0 | Ref | 1.0 | Ref |
| Smoker | 27 | 0.7 | 0.3–1.5 | 1.5 | 0.7–3.0 |
| Former smoker | 12 | 1.0 | 0.4–2.5 | 1.2 | 0.5–3.1 |
| Current smoker | 15 | 0.5 | 0.2–1.1 | 1.6 | 0.7–3.5 |
| Pack-years smoked | | | | | |
| <5 | 8 | 1.1 | 0.4–2.8 | 1.5 | 0.6–3.9 |
| 5–14 | 8 | 0.8 | 0.3–2.1 | 1.3 | 0.5–3.5 |
| ≥15 | 9 | 0.5 | 0.2–1.6 | 1.5 | 0.5–4.2 |
| <i>p</i> (trend) | | | 0.26 | | 0.39 |
| Amount smoked (pack/day) | | | | | |
| <1 | 11 | 1.2 | 0.5–2.9 | 1.6 | 0.6–3.9 |
| ≥1 | 16 | 0.4 | 0.2–1.0 | 1.3 | 0.6–3.0 |
| <i>p</i> (trend) | | | 0.10 | | 0.49 |
| Years smoked | | | | | |
| ≤10 | 11 | 0.9 | 0.4–2.1 | 1.2 | 0.5–3.0 |
| 11–20 | 5 | 1.3 | 0.4–4.0 | 3.2 | 1.0–9.7 |
| >20 | 10 | 0.4 | 0.1–1.2 | 0.8 | 0.3–2.7 |
| <i>p</i> (trend) | | | 0.25 | | 0.57 |

^a Excludes controls for whom HPV status was not known.

^b Adjusted for age and ethnicity.

were negatively associated with smoking at least one pack per day (OR = 0.6, 95% CI 0.2–1.6) or for more than 20 years (OR = 0.6, 95% CI 0.2–2.2). Squamous carcinomas (*n* = 70) were positively associated with ever smoking (OR = 1.7, 95% CI 0.8–4.0) and smoking at least one pack per day (OR = 1.5, 95% CI 0.6–4.0).

Most of the cases who were classified as HPV-negative were recruited after they received treatment for their disease, and thus the infected cervical tissue was removed before it could be sampled for this study. Analyses restricted to the 34 prospectively recruited adenocarcinoma cases or the 45 prospectively recruited squamous carcinomas generated ORs that were nearly identical to those using only the HPV-positive controls. For both case groups, ORs based on the retrospectively identified cases only were slightly closer to the null for both adenocarcinomas and squamous carcinomas, but in the same direction as those based on all cases (data not shown).

Because adenocarcinomas may share epidemiologic characteristics with endometrial cancer, for which increasing weight is a major risk factor, we stratified the associations by median body mass index (BMI, calculated as kg/m²) among controls. Smoking was inconsistently associated with adenocarcinomas among the 51 cases and 156 controls with BMI < 24 (Table 3). However, among the 73 cases and 142 controls (BMI

was missing for nine controls) with BMI ≥ 24, smoking was inversely associated with adenocarcinomas; the association with smoking at least one pack per day was statistically significant. Stratification by the median weight among controls (141 pounds) yielded similar results. BMI and weight were both positively associated with adenocarcinomas but not associated with squamous carcinomas; stratification by either did not change the results for squamous carcinomas (data not shown).

We addressed whether associations differed according to menopausal status, but few women (44 controls, 21 adenocarcinoma cases, and 22 squamous carcinoma cases) were postmenopausal at the reference date. Restricting the analyses to premenopausal women only or postmenopausal women only produced similar results (data not shown). The adenocarcinoma case group included 25 women classified as having adenosquamous carcinomas, but associations with smoking in this group were similar to those in the rest of the adenocarcinoma cases (data not shown).

Discussion

These data reveal positive associations between smoking and cervical squamous cell carcinoma and inverse

Table 3. Polychotomous logistic regression ORs and 95% CI for cases vs. all controls, by body mass index (BMI; kg/m²)

| Characteristic | BMI < 24 | | | | BMI ≥ 24 | | | |
|--------------------------|-------------------|-----------------|-----------------|---------|-------------------|-----------------|-----------------|---------|
| | Controls (No.) | Adenocarcinomas | | | Controls (No.) | Adenocarcinomas | | |
| | | No. | OR ^a | CI | | No. | OR ^a | CI |
| Never smoked | 87 | 24 | 1.0 | Ref | 69 | 38 | 1.0 | Ref |
| Smoker | 69 | 27 | 1.1 | 0.5–2.4 | 73 | 35 | 0.5 | 0.3–1.1 |
| Former smoker | 36 | 19 | 1.6 | 0.7–3.6 | 39 | 20 | 0.6 | 0.3–1.2 |
| Current smoker | 33 | 8 | 0.6 | 0.2–1.7 | 34 | 15 | 0.5 | 0.2–1.2 |
| Pack-years smoked | | | | | | | | |
| <5 | 26 | 8 | 0.7 | 0.2–2.0 | 26 | 13 | 0.8 | 0.3–1.8 |
| 5–14 | 23 | 9 | 1.4 | 0.5–4.2 | 19 | 10 | 0.6 | 0.2–1.6 |
| ≥15 | 20 | 10 | 2.0 | 0.6–6.4 | 26 | 12 | 0.5 | 0.2–1.1 |
| <i>p</i> (trend) | | | | 0.31 | | | | 0.06 |
| Amount smoked (pack/day) | | | | | | | | |
| <1 pack per day | 38 | 12 | 0.8 | 0.3–2.0 | 40 | 22 | 0.8 | 0.4–1.6 |
| ≥1 pack per day | 31 | 15 | 1.7 | 0.7–4.5 | 32 | 13 | 0.4 | 0.2–0.9 |
| <i>p</i> (trend) | | | | 0.36 | | | | 0.04 |
| Years smoked | | | | | | | | |
| ≤10 | 29 | 9 | 0.7 | 0.2–2.0 | 28 | 15 | 0.6 | 0.3–1.6 |
| 11–20 | 23 | 11 | 1.7 | 0.7–4.5 | 17 | 3 | 0.5 | 0.2–1.4 |
| >20 | 17 | 7 | 1.4 | 0.3–5.8 | 26 | 14 | 0.5 | 0.2–1.3 |
| <i>p</i> (trend) | | | | 0.43 | | | | 0.10 |

^a Adjusted for age, ethnicity, lifetime sexual partners, education, number of Pap smears in past 10 years, and HPV.

associations between smoking and cervical adenocarcinoma, and thus indicate that smoking may have different effects on the histologic subtypes of cervical carcinoma. Adenocarcinoma cases smoked significantly less than the matched squamous carcinoma cases and the matched community controls. When based on the same controls, the fully adjusted polychotomous regression models uncovered opposite risks associated with smoking: smokers had decreased risks of adenocarcinomas but elevated risks of squamous carcinomas.

Adjustment for confounding decreased all initial ORs but moved the ORs for the two tumor types in opposite directions relative to the null. For adenocarcinomas, adjustment for the questionnaire-based risk factors transformed null associations to inverse associations, and the addition of HPV data generated even stronger inverse associations. For squamous carcinomas, strong positive associations decreased after adjustment for questionnaire data, and decreased further when HPV was included. A major strength of this study was the ability to control for HPV, but we recognize the imperfections of our HPV assessment. We could not obtain pretreatment samples from all cases, we only tested for 27 HPV genotypes (although all known oncogenic types were included), and the resolution of suspected plasmid contamination of some samples required use of a second primer set with lower sensitivity

[11]. The rarity of adenocarcinomas necessitated retrospective ascertainment of some cases, whose prior surgical treatment removed the infected cervical tissue before it could be sampled. We therefore employed several approaches to account for HPV. First, we adjusted for measured HPV status and the number of lifetime sexual partners, which remains an important confounder when HPV is misclassified [18]. Second, we compared all cases (who are assumed to be HPV-positive [4]) to just those controls who tested HPV-positive. Third, we repeated the analyses using only the HPV-positive cases and the HPV-positive controls. Each approach produced coherent and consistent associations for both case groups, which suggests that the observed results are real.

Most of the earlier studies that addressed the suspected causal association between smoking and cervical carcinoma (reviewed in ref. 2) relied on surrogate variables (such as number of sexual partners or age at first intercourse) to adjust for the suspected sexually transmitted etiologic agents, which were later identified as HPV. Controlling for confounding in this way cannot guarantee unbiased results [19], and smoking can be correlated with HPV independent of these surrogates [5]. Recent studies that directly tackled confounding by HPV through evaluation of HPV-positive cases and controls suggest that confounding alone does not

explain the increased risks of squamous carcinomas associated with smoking. Smokers were at significantly increased risk of developing high-grade precursor lesions (Hildesheim *et al.*, submitted) [20] and cervical intraepithelial neoplasia III (CIN III) [21], the recognized precursors to invasive squamous carcinomas. However, other similar study designs that observed elevated unadjusted risks but no increased risks of invasive squamous carcinomas [22], CIN [23], or CIN III [24, 25] after control for HPV concluded that confounding may explain the association.

Although much fewer in number, previous studies of adenocarcinomas found no association with smoking. A US study of 40 adenocarcinomas and 23 adenosquamous carcinomas reported an OR of 1.0 for smoking over 30 cigarettes per day [26], and neither current smoking (OR = 1.0) nor former smoking (OR = 1.2) was associated among 195 cases and 386 controls in Los Angeles [27]. A multicenter international case-control study of 271 adenocarcinomas and 106 adenosquamous carcinomas observed a null association [28], as did hospital-based case-control studies in Italy [29] and Latin America [30]. None of these studies controlled for HPV; therefore null associations may reflect residual confounding by HPV [5] for adenocarcinomas: women who smoke are more likely to be infected with HPV but less likely to develop adenocarcinomas. Control for this negative confounding (*i.e.* toward the null) would tend to generate true relative risks that are below 1.0. However, a case-case comparison, in which confounding by HPV may be less of a concern, using British registry data, revealed no difference in smoking exposure among 704 adenocarcinomas and 4599 squamous carcinomas [31].

Two recent case-control studies addressed risk factors for both adenocarcinomas and squamous carcinomas using hospital-based controls, a different (but valid) primer system for the PCR-based HPV tests, and similar methods of statistical adjustment in regression models. Both studies reported increased risks of borderline significance for squamous carcinomas, whereas two exposed cases out of 33 generated a positive, non-significant association with adenocarcinomas in the Philippines [32] while there was no association with 39 adenocarcinoma cases in Thailand [33]. Instability of estimates based on these small numbers, which make control for confounding more difficult, likely explains the differences between these studies and our, also imprecise, results. Larger studies are needed.

Our results raise questions about the mechanisms proposed to explain a role for smoking in cervical carcinogenesis. Smoking may impair cellular immunity [34] and thus facilitate persistent HPV infection [35],

which is probably necessary for progression to carcinoma [24]. HPV persistence, however, has not been consistently associated with smoking [36, 37]. Higher DNA adduct levels associated with smoking among women with normal [38] and abnormal [39] cytology imply that tobacco-related carcinogens act on cervical keratinocytes. Cervical mucus from smokers with both normal cytology [40] and CIN [41] contains higher levels of nicotine and cotinine and higher levels of tobacco-specific carcinogens [42] than does cervical mucus from nonsmokers or passive smokers. Fundamental changes such as impaired immunity, DNA adducts, and mucosal carcinogens would be expected to increase risk for all cervical carcinomas, and therefore additional experimental work should address the histologic differences in the epidemiologic data.

Inverse associations with adenocarcinomas support the hypothesis that cervical adenocarcinomas are etiologically related to adenocarcinoma of the endometrium [43]. Smoking, through a hypothesized anti-estrogenic action [44], decreases the risk of endometrial adenocarcinoma [45], which arises from estrogen excess [46]. The stronger inverse association with smoking among heavier women in our data is also seen for endometrial cancer [47, 48], especially among postmenopausal women. Our study included primarily premenopausal women, and few women in our study are considered obese (BMI > 30). Although the similarities to endometrial cancer are provocative, not all risk factors for cervical adenocarcinomas behaved as they do for endometrial carcinomas (note equivocal associations between cervical adenocarcinomas and oral contraceptives [11] and menopausal estrogens [49]), and the role of estrogen itself in cervical adenocarcinoma etiology is unclear.

Our panel of three pathologists reviewed histologic slides from potential cases to reduce diagnostic misclassification of adenocarcinomas [50], a rare and clinically heterogeneous tumor [51]. Control selection methods should have decreased referral bias, but bias due to nonresponse was possible if smoking habits differed in 21% of eligible adenocarcinoma cases, 26% of squamous carcinoma cases, and 27% of controls who did not participate. The mean age at diagnosis in both case groups (40 years) is approximately 10 years younger than the mean age at diagnosis for most US cervical carcinomas [52]. If the association between squamous carcinomas and smoking differs by age, the generalizability of our results may decrease, but the internal validity is unaffected.

Our results might underestimate the true risk associated with smoking if squamous carcinoma cases underreported their smoking, and biased reporting by controls could move the estimates in either direction. Recall bias

could fully account for the unadjusted null (or adjusted inverse) associations with smoking if adenocarcinoma cases systematically underreported their true smoking history. However, the estimated expected proportion [53] of female smokers misclassified as nonsmokers in our study population is likely too small to account for the inverse associations we observed. In addition, the opposite associations for the two histologic types indicate that recall bias – which is presumed to operate on the presence of disease, rather than on the presence of a particular subtype of disease – alone cannot explain these results.

In conclusion, smoking may introduce opposite risks of adenocarcinomas and squamous carcinomas. Although both histologic types are caused by HPV and arise in the cervix, etiologic co-factors for these tumors may differ.

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