Sexual Behavior, Sexually Transmitted Diseases, and Risk of Cervical Cancer

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To explore sexually transmitted diseases and sexual behavior as risk factors for cervical cancer, we analyzed data from a population-based case-control study of breast and cervical cancer in Costa Rica. Data from 415 cases of cervical carcinoma *in situ*, 149 cases of invasive cervical cancer, and 764 controls were included in the analysis. Multivariate analysis showed that lifetime number of sex partners, first intercourse before age 15 years, number of livebirths, herpes simplex virus type 2 seropositivity, and serologic evidence of previous chlamydial infection were predictors of carcinoma *in situ*. Serologic evidence of previous syphilis was not associated with carcinoma *in situ*. Predictors for invasive cervical cancer included lifetime number of sex partners, first intercourse before age 15 years, number of livebirths, serologic evidence of previous syphilis, herpes simplex type 2 infection, and chlamydial infection. Cigarette smoking, socioeconomic status, self-reported history of sexually transmitted diseases, and douching were not associated with either carcinoma *in situ* or invasive cervical cancer. (Epidemiology 1995;6:409–414)

Keywords: cervical cancer, sexually transmitted diseases, sexual behavior, herpes simplex virus, chlamydia, parity, oral contraceptives.

Cervical cancer is generally regarded as a sexually transmissible condition.[1,2] Factors that have been shown to be associated with increased risk of carcinoma *in situ* (CIS) and invasive cervical cancer include number of sexual partners, young age at first intercourse, nonuse of barrier contraception, certain sexually transmitted infections, cigarette smoking, low socioeconomic status, and lack of Papanicolaou smear screening.[3–6] Specific sexually transmitted agents that have been associated include human papillomavirus (HPV), herpes simplex virus type 2 (HSV-2), *Chlamydia trachomatis*, *Trichomonas vaginalis*, and the organisms that cause bacterial vaginosis.[4,5,7–12] Because these factors are often highly correlated, the relative importance of each is difficult to assess. Few studies have addressed sexual behavior variables and sexually transmitted infections simultaneously.

Along with many other Latin American countries, Costa Rica has one of the highest incidence rates of cervical cancer in the world (36 cases per 100,000 person-years). Costa Rica maintains a national tumor registry and offers comprehensive medical services, including free cervical cancer screening.[13,14] Several studies have shown that risk factors for cervical cancer among Latin women are generally similar to those of U.S. women.[11,15–17] To explore sexually transmitted disease-related risk factors for cervical carcinoma *in situ* and invasive cervical cancer, we analyzed data from a population-based case-control study of cervical and breast cancer.

Subjects and Methods

Methods employed in this study have been reviewed in detail elsewhere.[18–20] Several analyses of these data have been published, including studies on hormonal contra-

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ception as a risk factor for cervical[19,20] and breast cancer[18]; prevalence of sexually transmitted diseases (STDs) in Costa Rican women[21]; and HSV-2 seropositivity,[22] chlamydial infection,[23] human immunodeficiency virus (HIV) infection,[24] human T-lymphotropic virus type I (HTLV-I) infection,[25] and syphilis[26] among control women.

Selection of Cases and Controls

We selected histologically confirmed[27] cases of cervical carcinoma newly diagnosed between January 1, 1982, and March 31, 1984, from the Costa Rican National Tumor Registry. Patients were between 25 and 59 years of age at the time of diagnosis; 583 had CIS, and 293 had invasive cervical cancer. Between September 1984 and February 1985, we identified 983 eligible control women through a national multistage probability household survey. Controls were 25–59 years old at the time of interview and were frequency-matched to the age distribution of cervical and breast cancer cases.

Interviews and Serologic Testing

Trained female interviewers questioned cases and controls about their reproductive, contraceptive, and sexual histories. Cases and controls were interviewed between September 1984 and February 1985; 92.8% of eligible controls, 89.2% of eligible patients with CIS, and 66.9% of eligible patients with invasive cervical cancer completed an interview. After receiving informed consent, a technician collected serum specimens after the interview from 88.1% of interviewed controls, 95.0% of patients with CIS, and 92.3% of patients with invasive cervical cancer. Sera were analyzed for antibodies to HSV-1 and HSV-2 by immunodot assays using purified glycoproteins Gg-1 and Gg-2.[28] Antibodies to C. *trachomatis* were assayed by the simplified microimmunofluorescence (MIF) test[29]; we considered any titer of 1:16 or greater to be positive. Serologic evidence of current or previous syphilis was assayed using the rapid plasma reagin (RPR) card test and the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-Tp)[26,30]; however, only the MHA-Tp was included in our analysis as an indicator of previous syphilis. Serologic tests for genital types of human papillomavirus were not available.

Statistical Analysis

A detailed summary of women excluded from analysis is reported elsewhere.[19] We included only women with squamous cell carcinomas that were histologically confirmed by a panel of three Costa Rican pathologists.[27] To assure that controls were at risk for cervical cancer but had no history of this cancer, we excluded those who reported a previous hysterectomy or cone biopsy. Because interviews were conducted up to 3 years after the date of case diagnosis, and the exposure of interest occurred before diagnosis, we adjusted many variables to an index date.[31] For each case, the index date was her date of diagnosis. For controls, we assigned an index date of February 15, 1983, the midpoint of the 27-month case enrollment period.

We excluded from analysis women who were not 25–58 years of age at the index date. A total of 415 cases of CIS, 149 cases of invasive cervical cancer, and 764 controls were included in the analysis. As a result of frequency matching by age, controls were older than CIS patients and younger than invasive cancer patients. Therefore, we adjusted all analyses for age and included age as a continuous variable in the logistic regression models. We built separate models for CIS and invasive cancer, and we calculated odds ratios and 95% confidence intervals (CI).[32] Variables of interest included self-reported histories of gonorrhea, syphilis, genital warts, genital herpes, chancroid, and treatment for these sexually transmitted diseases; serologic evidence of previous syphilis, HSV-2 infection, and C. *trachomatis* infection; douching (ever); oral contraceptive use (ever); number of pregnancies and livebirths; number of Papanicolaou smears; cigarette smoking (ever); age at first intercourse; and number of lifetime sexual partners. A household possession index as a proxy for socioeconomic status was calculated according to methods previously described.[33] We entered number of sexual partners (up to 6) and number of livebirths as continuous variables; all other variables were categorical.

These variables were used to build a multiple logistic regression model for cervical cancer.[32,34] Initially, we included all of the variables of interest in the model and calculated regression coefficients. We excluded from the model any variable with a *P*-value greater than 0.25. The regression coefficients for the remaining variables in the model were recalculated and compared with the coefficients in the previous model and the full model. If any coefficient changed more than 25%, we put the excluded variable back into the model. At every step, the *P*-values for all of the variables not in the model were also recalculated, and a variable with a *P*-value of less than or equal to 0.25 was included back in the model. This process of exclusion and retention of variables continued until all of the variables not in the model had a *P*-value greater than 0.25 and had no appreciable effect (less than 25%) on the coefficients of variables that remained in the model. If a variable that we judged to be of epidemiologic importance was excluded, we put that variable back into the final model. We also included all two-way interaction terms for number of partners, serologic evidence of sexually transmitted diseases, socioeconomic status, age at first intercourse, oral contraceptive use, and history of sexually transmitted diseases.

Results

Frequencies of any self-reported sexually transmitted disease were low (2–7%) among controls; however, serologic evidence of previous sexually transmitted diseases was common (Table 1). Forty-two per cent of controls had HSV-2 antibody, and 57% had chlamyd-

TABLE 1. Distribution of Study Subjects by Selected Characteristics

Characteristic	Cases		Controls (N = 764)
	CIS (N = 415)	Invasive (N = 149)	
Age at index date (years)			
25–29	92	11	145
30–39	213	49	278
40–49	89	44	212
50–58	21	45	129
Socioeconomic status index			
Low	216	99	349
Medium	119	30	220
High	80	20	195
Number of lifetime partners*			
0	1	0	43
1	202	62	494
2–3	151	55	187
4–5	37	17	27
≥6	20	12	7
Age at first intercourse* (years)			
<15	47	19	56
15–18	200	25	262
19–22	117	33	210
≥23	49	11	189
Cigarette smoking (ever)	106	39	159
Douching (ever)	157	47	227
Number of livebirths			
0	13	2	77
1	33	6	82
2–3	158	24	264
4–6	135	51	183
≥7	75	66	157
Oral contraceptive use (ever)	268	49	316
Serologic evidence of sexually transmitted disease			
Syphilis, HSV-2, or chlamydia	336	118	468
Syphilis	36	24	44
HSV-2	227	86	279
Chlamydial infection	273	100	384
History of any sexually transmitted disease†	27	9	17

* Six per cent of controls and 0.2% of CIS cases never had intercourse.
† Self-reported gonorrhea, syphilis, chancroid, genital herpes, or genital warts.

ial antibody. Reactive MHA-Tp assays were found in 7% of controls; however, only 0.7% of controls reported a history of syphilis. The three sexually transmitted disease serology variables were not strongly correlated among cases or controls (all correlation coefficients ≤0.24).

Carcinoma in Situ

Table 2 shows univariate associations between CIS and the variables (adjusted for age) used in building multiple logistic regression models, as well as the regression coefficients for the variables in final multiple logistic regression model. Univariate analyses showed strong associations between CIS and number of lifetime sexual partners, ever-use of oral contraceptives, number of livebirths, antibody to HSV-2, history of syphilis, and history of gonorrhea; and moderate associations with chlamydial antibody, serologic evidence of previous syphilis, and history of genital warts. In the final multivariate model, the rate ratio (RR) for acquiring the disease increased from 1.4 (95% CI = 1.2–1.6) for women with one sex partner to 6.0 (95% CI = 2.7–13.2) for women with six or more sex partners. The rate ratio increased from 1.1 (95% CI = 1.1–1.2) for women who had one livebirth to 3.2 (95% CI = 1.7–5.9) for women with 10 or more livebirths. Rate ratios for CIS were 1.8 (95% CI = 1.1–3.0) for women who had first intercourse before age 15 years, 1.5 (95% CI = 1.1–2.1) for women seropositive for HSV-2, 1.9 (95% CI = 1.4–2.5) for women who had ever used oral contraceptives, and 1.3 (95% CI = 1.0–1.8) for those with chlamydial antibody. Syphilis antibody (MHA-Tp) (RR = 0.9; 95% CI = 0.5–1.5) was also included in the model. When forced into the model, cigarette smoking had no effect on the other variables in the model.

Invasive Cancer

Table 3 shows univariate associations between invasive cervical cancer and the variables (adjusted for age) used in building multiple logistic regression models, as well as the regression coefficients for the variables in final multiple logistic regression model. Univariate analyses showed strong associations between invasive cancer and number of lifetime sex partners, number of livebirths, serologic evidence of previous syphilis, HSV-2 antibody, history of gonorrhea, history of syphilis, and low socioeconomic status; and moderate associations with chlamydial antibody and history of genital herpes. In the final multivariate model (Table 3), the rate ratio for invasive cancer increased from 1.5 (95% CI = 1.3–1.8) for women with one sex partner to 12.8 (95% CI = 4.2–39.2) for women with six or more sex partners. The rate ratio increased from 1.3 (95% CI = 1.2–1.4) for women with one livebirth to 10.0 (95% CI = 4.4–22.7) for those with 10 or more livebirths. Rate ratios were 2.5 (95% CI = 1.2–5.4) for women who had first intercourse before age 15 years and 1.9 (95% CI = 1.1–3.5) for women with serologic evidence of previous syphilis (MHA-Tp). Use of oral contraceptives, HSV-2 antibody, and chlamydial antibody were also included in the final model. We also evaluated the associations of syphilis, HSV-2, and chlamydial antibodies separately, adjusted for other variables

TABLE 2. Risk Factors for Cervical Carcinoma *in Situ* in Costa Rica

Variable	Age-Adjusted OR	Age-Adjusted 95% CI	Adjusted* OR	Adjusted* 95% CI
Number of sex partners (0, 1, 2, 3, 4, 5, ≥6)	1.5	1.3–1.7†	1.4	1.2–1.6†
First intercourse before age 15 years	1.2	0.9–1.8	1.8	1.1–3.0
HSV-2 antibody	2.0	1.6–2.8	1.5	1.1–2.1
Oral contraceptive use	2.3	1.7–3.2	1.9	1.4–2.5
No. of livebirths (0, 1, 2, . . ., ≥10)	1.1	1.1–1.2‡	1.1	1.1–1.2‡
Chlamydial antibody	1.8	1.4–2.3	1.3	1.0–1.8
Syphilis antibody (MHA-Tp)	1.6	1.0–2.9	0.9	0.5–1.5
History of syphilis	5.3	1.9–14.9		
Low socioeconomic status	1.3	1.0–1.7		
Douching	1.4	1.1–1.8		
Cigarette smoking	1.3	1.0–1.7		
Number of previous Papanicolaou smears	1.0	1.0–1.1		
History of gonorrhea	2.6	0.9–7.4		
History of genital warts	1.6	0.5–5.5		
History of genital herpes	0.0			

* Odds ratios adjusted for age and all variables in the model.
† OR for one sex partner.
‡ OR for one livebirth.

in the model. Forcing cigarette smoking into the model did not appreciably change the magnitude of regression coefficients of the variables in the model.

Discussion

Number of lifetime sex partners and age at first intercourse are usually identified as the most important risk factors for cervical dysplasia, CIS, and invasive cervical cancer, and they often substantially confound associations with other variables.[4,5] In our study, both of these factors were associated with CIS and invasive cervical cancer. After number of sex partners was entered into the model for CIS, the associations with most other variables were substantially diminished. Number of sex partners and age at first intercourse may be considered surrogate measures for exposure to carcinogenic sexually transmitted agents. Thus, noncausal associations between cervical cancer and other variables would be expected to diminish after adjustment for direct causal factors that were confounding. Recall and interviewer bias are not likely to have contributed to the observed associations with these two variables, because the questionnaires and interview techniques were standardized, and neither interviewers nor subjects were informed of study hypotheses or previously identified risk factors for cervical cancer. Several studies indicate that the sexual behavior of a woman's partners is a more important predictor of cervical cancer risk than her own behavior.[17,35–37] Detailed information on sex partners was not collected in this study. A multinational study found that risk of cervical cancer among monogamous study subjects in Panama, Bogota, and Mexico City increased with number of husband's partners, but this association was not found among subjects in Costa Rica.[17]

In the past several decades, HSV-2 has been suggested as the causal sexually transmitted agent for cervical cancer. However, interpretation of the numerous epidemiologic studies of HSV-2 and cervical cancer has been seriously hampered by the cross-reactivity between HSV-1 and HSV-2 in serologic assays and/or methodologic flaws in study design, as emphasized in recent reviews.[4,5,12] The assay used in our study is highly specific for HSV-2, and an association with cervical CIS persisted even after adjusting for number of partners and age at first intercourse. However, seropositivity for HSV-2 may represent a marker for sexual activity,[7,38] and therefore exposure to HPV or other sexually transmitted agents.

TABLE 3. Risk Factors for Invasive Cervical Cancer in Costa Rica

Variable	Age-Adjusted OR	Age-Adjusted 95% CI	Adjusted* OR	Adjusted* 95% CI	Adjusted† OR	Adjusted† 95% CI
Number of sex partners (0, 1, 2, 3, 4, 5, ≥6)	1.7	1.5–2.1‡	1.5	1.3–1.8‡		
First intercourse before age 15 years	1.0	0.6–1.6	2.5	1.2–5.4		
No. of livebirths (0, 1, 2, 3, . . ., ≥10)	1.3	1.2–1.4§	1.3	1.2–1.4§		
Oral contraceptive use	0.9	0.6–1.3	0.7	0.4–1.1		
Syphilis antibody (MHA-Tp)	2.9	1.7–5.0	1.7	0.9–3.2	1.9	1.1–3.5
HSV-2 antibody	2.3	1.6–3.4	1.4	0.8–2.2	1.6	1.0–2.5
Chlamydial antibody	1.9	1.3–2.9	1.6	1.0–2.7	1.6	1.0–2.5
History of gonorrhea	5.6	1.6–19.3				
History of syphilis	7.4	2.2–25.1				
Low socioeconomic status	2.4	1.6–3.5				
Cigarette smoking	1.3	0.9–1.3				
No. of previous Papanicolaou smears	1.0	1.0–1.1				
Douching	1.1	0.8–1.7				
History of genital herpes	1.8	0.2–17.3				
History of genital warts	0.0					

* Odds ratios adjusted for age and all variables in model.
† Odds ratios adjusted for age and the first 3 variables in table.
‡ OR for one sex partner.
§ OR for one livebirth.

Current evidence points to human papillomavirus as the most important infectious agent involved in cervical carcinogenesis,[4,5] but HPV was not assayed in our study. Cervical HPV infection and HSV-2 infection are both very common in Costa Rican women. In our study, HSV-2 infection was very common. In a multinational study[11] of invasive cervical cancer, 24% of hospital and community controls in Costa Rica had cervical HPV infection detected by deoxyribonucleic acid (DNA) hybridization (William Reeves, personal communication, 1988). Cervical HPV infection was a strong predictor of invasive cervical cancer,[11] and another analysis of the same study suggested a biological interaction between HSV and HPV infection.[39] One hypothesis is that HSV and HPV may act as cocarcinogens, with HSV-2 acting as an initiator of neoplastic change.[40–42] If that hypothesis is correct, an association between HSV-2 and invasive cervical cancer, as well as with CIS, would be expected. In our study, HSV-2 was weakly associated with invasive cervical cancer.

Our study is likely to have underestimated the true association between genital chlamydial infection and cervical cancer, owing to cross-reactivity of the serologic assay for C. *trachomatis*. A sizable proportion of chlamydial seropositivity may have resulted from respiratory infection with *Chlamydia pneumoniae* (TWAR strain) rather than sexually transmitted chlamydial infection.[23] Such resultant misclassification is likely to be nondifferential, and therefore, it would lower the odds ratio. Although MHA-Tp seropositivity may result from pinta and yaws, syphilis is a more likely explanation, as discussed in detail elsewhere.[26] The lack of an association between self-reported sexually transmitted diseases and cervical cancer is not surprising. Self-reports and nonserologic diagnostic tools generally are insensitive measures of previous sexually transmitted infections. Even in populations with higher prevalence of self-reported specific sexually transmitted diseases, associations with cervical cancer have not been observed.[3,6,9]

An association between number of livebirths and invasive cervical cancer in Latin American women has been reported.[16] Because Costa Rican women have high fertility (the mean number of livebirths among controls was 4), such an association would be found more readily than in the U.S. and other less fecund populations. This association is biologically plausible, since childbirth may influence cervical tissue through trauma or pregnancy-induced immunosuppression, hormonal changes, or folacin deficiency.[16]

An association between oral contraceptive use and cervical cancer has not been consistently observed in case-control studies.[4,43] We found a weak inverse association between invasive cervical cancer and oral contraceptive use. The positive association with CIS may be readily explained by detection bias, as has been discussed in detail elsewhere,[19] since carcinoma *in situ* is usually asymptomatic and detectable only by Papanicolaou smear. Women with CIS who had used oral contraceptives were more likely to have had a Papanicolaou smear, to have been referred for a diagnostic biopsy, and to have been enrolled in this study than women with CIS who had never used oral contraceptives.

Although smoking has been identified as an independent risk factor for cervical cancer in some case-control studies, and its effects are biologically plausible,[4,5,44] it was not a risk factor in our study nor in another Latin American study.[45] In both studies, the lower prevalence and daily amount of smoking by women in Latin America,[46] compared with the U.S.,[47] may have precluded the ability to detect an association with cervical cancer.

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Appendix

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