

SMOKING AND ORAL CONTRACEPTIVES AS RISK FACTORS FOR CERVICAL CARCINOMA *IN SITU*

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Human papillomavirus (HPV) is probably a necessary but definitely not a sufficient cause of cervical carcinoma. However, it remains unclear which factors, in addition to HPV, are important for the development of cervical carcinoma and its precursor lesions. To address this issue, we conducted a case-control study nested in a population-based cohort consisting of women participating in cytological screening in one Swedish county, any time during 1969 through 1995. Detailed information on sexual practice, smoking habits and oral contraceptive (OC) use were collected through telephone interviews with 422 case patients diagnosed with cervical carcinoma *in situ* and 422 control subjects. All cytological smears were analyzed for presence of HPV16/18 by a polymerase chain reaction (PCR)-based method. Odds ratios (OR) were used as measures of relative risk. After multivariate adjustment, a 2-fold higher risk was observed among current smokers compared with never smokers [OR 1.94; 95% confidence interval (CI) 1.32–2.85], an association apparently confined to women younger than 45 years. Current use of OCs was associated with a 4-fold increased risk overall (OR 3.64; 95% CI 1.91–6.93) with a monotonic increase with increasing duration of use (p for trend < 0.001). The number of sexual partners was significantly, positively associated with risk among HPV 16/18-negative (p for trend < 0.005) but not among HPV 16/18-positive women. Our data confirm the association between smoking and cervical carcinoma *in situ*, which might be age-dependent. Our results further indicate a relation with OC use and the risk for cervical carcinoma *in situ*. *Int. J. Cancer* 81:357–365, 1999.

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Being the most common cancer among women in developing countries and the second among women worldwide, cervical carcinoma is an important public health problem (Parkin *et al.*, 1993). During the past 20 years, overwhelming evidence indicates that certain types of human papillomaviruses (HPV) play a fundamental role in the etiology of cervical neoplasia (IARC, 1995). HPV infection is common, especially among sexually active young women. Compared with an estimated 79% lifetime risk for HPV infection, cervical cancer is a rare event (Syrjänen, 1996). Hence, because only a minority of HPV-infected women—and possibly some women without HPV infection—develop cervical carcinoma, other risk factors are required in cervical carcinogenesis.

Previous epidemiological studies, however, have yielded conflicting results regarding the role of other putative factors (Brinton, 1992). Some studies showed an increased risk for cervical carcinoma *in situ* related to smoking (Becker *et al.*, 1994b; Brisson *et al.*, 1994; Brock *et al.*, 1989; Kjer *et al.*, 1996; La Vecchia *et al.*, 1986) and oral contraceptives (OCs) (Brisson *et al.*, 1994; Kjaer *et al.*, 1993), whereas others failed to support such association (Liaw *et al.*, 1995; Morrison *et al.*, 1991; Muñoz *et al.*, 1993; Schiffman *et al.*, 1993). High parity (Muñoz *et al.*, 1993; Schiffman *et al.*, 1993) as well as infections with Chlamydia (Muñoz *et al.*, 1993) and herpes simplex virus 2 (Becker *et al.*, 1994a) are other reported risk factors. Small sample size, performance among selected groups (such as sexually transmitted disease clinic attendees) and the use of insensitive methods for HPV detection may explain these

conflicting results (Muñoz *et al.*, 1988). Of particular concern is that most studies have not been able to adjust for HPV status properly (Franco, 1991; Schiffman and Schatzkin, 1994). Consequently, it remains unclear which factors, in addition to HPV, are important for the development of cervical carcinoma and its precursor lesions.

To address these important issues, in particular the role of smoking and OC use, we used data from a case-control study, nested in a large population-based screening program in Sweden, with detailed information on lifestyle factors and repeated polymerase chain reaction (PCR)-based measurements of HPV status.

SUBJECTS AND METHODS

Setting

Our nested case-control study was based on a study population comprising all women resident in Uppsala county, with a total population of approximately 281,000 individuals, any time from 1969 through 1995. Health care in Sweden is socialized, giving equal access to medical care for all citizens. Screening for cervical cancer started on a limited scale in 1961, and an organized program was introduced in Uppsala county in November 1967. At the start of the program, all women aged 30–49 years (later 25–49 years) were invited to attend every 3–4 years. As a complement, large numbers of Papanicolaou (Pap) smears have been taken as opportunistic screening outside the organized program, as described in detail elsewhere (Gustafsson *et al.*, 1995). All information from the organized and opportunistic screening in Uppsala county have been computerized in a cytology register and the smears stored since 1969 at the Department of Pathology, University Hospital in Uppsala. A total of 732,287 smears from 146,889 women were registered from 1969 through 1995.

Subjects

Using the cytology register, we defined a cohort comprising all women who had at least one smear registered during 1969 to 1995, provided that: 1. their first registered smear was normal (Pap = 1); 2. they were born in Sweden; and 3. they were younger than 50 years old at entry into the cohort. The time of the first registered smear defined the entry into the cohort. Eligible for the study were those women in the cohort who were alive and available for personal interview at the start of the study (January 1, 1996).

Incident cases of cervical carcinoma *in situ* were identified through computerized linkage between the study cohort and the

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National Cancer Registry from 1969 through 1995. This registry, established in 1958, is now considered almost 100% complete (Socialstyrelsen, 1995). Notification to the registry is mandatory not only for invasive cancer of the cervix, but also for precancerous lesions classified as cancer *in situ* of the uterine cervix. For each case, 5 potential controls, individually matched by date of entry into the cohort (± 90 days) and by year of birth, were selected randomly from the study cohort. Eligible controls had no history of prior *in situ* or invasive cervical carcinoma and had not undergone hysterectomy before the date of diagnosis of their corresponding case.

Review of cytological slides and histological specimens

The first registered smear for the cases and for one matched control for each case (randomly selected from the initial set of 5) were reviewed by a skilled cytotechnician, blinded for case-control status. Cases regarded as not having a normal smear (Pap = 1) were excluded. Controls without a normal first smear were replaced by another control, selected randomly from the other matched controls. The histological specimens (either a small biopsy or a complete cone) from all eligible cases were reviewed by an experienced pathologist (J.P.).

Data collection

We collected data by telephone interviews performed by 2 trained interviewers. All eligible subjects were first approached by a mailed letter of information. Subsequently, informed consent was obtained by telephone. Appointment for a later telephone interview was made with those subjects who agreed to participate. The interviewers were blinded to the case-control status and were not informed about the study hypotheses. We used a comprehensive structured questionnaire to collect information on demographic and socioeconomic characteristics, sexual and reproductive behavior, smoking habits, contraceptive methods and history of gynecological diseases, including sexually transmitted diseases. Detailed information was requested on smoking, sexual practices and OC use. The recall of OC history was aided by a mailed chart with color pictures of all brands marketed in Sweden during the years 1964–1995.

Included cases and controls

Cases with unlisted telephone numbers or without a telephone were excluded. Control women having unlisted telephone numbers or no telephone were replaced by another matched control, randomly selected from the other chosen controls. A total of 504 cases and 504 individually matched controls fulfilled the cytological criteria for being included in the study. For the interviews, 35 cases with unlisted telephone numbers together with their matched controls were excluded. Thus, there were 469 eligible cases and 469 individually matched eligible controls; case-control pairs in the following are referred to as risk sets.

HPV analyses

All available smears taken from the 469 eligible risk sets from the time of their entry into the cohort until the date of diagnosis of carcinoma *in situ* for the cases were analyzed for presence of HPV using a PCR-based detection method. The smears were collected from the Department of Pathology, University Hospital in Uppsala, Sweden, and sent for analyses to the Department of Medical Genetics, Biomedical Center in Uppsala. Before delivery, all smears were sorted according to risk set and coded. Thus the 2 laboratory technicians performing the analyses were blinded to the case-control status. To avoid bias caused by a drift in the technique for analysis over time, all smears in a risk set were analyzed at the same time.

For the DNA extraction from the archival smears we used a modification of an extraction protocol, described in detail elsewhere (Chua and Hjerpe, 1995; Josefsson *et al.*, 1999). Subsequently, the HPV analyses were performed using a highly sensitive PCR-system based on the 5' exonuclease activity of Taq polymerase in a TAQMAN assay (Livak *et al.*, 1995). For this purpose, we

converted our previously developed PCR system for the E1 open reading frame of the HPV genome (Ylitalo *et al.*, 1995), to a detection system using the 5' exonuclease assay (Holland *et al.*, 1991). This assay uses the 5' to 3' exonuclease activity of Taq polymerase to cleave a dual-labeled, non-extendible, hybridization probe during the extension phase of the PCR. The probe has one fluorescent dye attached as a reporter at the 5' end, and in the undigested form, the emission from this reporter dye is quenched by a second fluorescent dye, attached to the 3' end. Concomitant with accumulation of the PCR product is a release of reporter dye. The PCR products were hybridized with probes for HPV 16 and 18. In addition, the amplification of a human beta-actin gene fragment was used as a positive control. The sensitivity of the PCR-system for analyses of archival Pap smears is largely dependent on the DNA quality and the potential presence of inhibitors during the PCR-reaction. By adding bovine serum albumin to the PCR-reaction, the inhibition caused by the Pap stain is removed, thus yielding a sensitivity comparable to high-m.w. DNA. The characteristics of this PCR based assay for HPV typing have been described in detail elsewhere (Josefsson *et al.*, 1999).

Statistical analyses

Odds ratios (ORs) and 95% confidence intervals (CIs) were used as measures of relative risk. Because of the matched design, we used a conditional logistic regression to estimate ORs. Crude ORs were calculated without adjustment for variables other than those inherent in the matching variables [age, time (± 3 months) of first smear].

In multivariate analyses, we adjusted for potential confounding by years in school (<7, 7–9, 10–12, >12 years), marital status (married, single, divorced, widowed), smoking (never/ex/current), OC use (never/ex/current), age at menarche (<13, 13, >13 years), age at first sexual intercourse (never, 11–15, 16–17, 18–19, ≥ 20 years), number of sexual partners before diagnosis (0–1, 2–3, 4–9, ≥ 10), parity (nulliparous, 1, 2, 3, ≥ 4) and age at first child (nulliparous, <20, 20–24, 25–29, ≥ 30 years). Multivariate analyses were performed omitting body mass index (BMI) and genital infections from the final models because adjustment for these factors did not influence risk estimates. Analyses of smoking variables were conducted separately among risk sets with cases diagnosed younger than and at or older than 45 years of age.

All conditional logistic regression analyses were performed by likelihood-ratio tests using the PHREG procedure in SAS (The PHREG procedure, 1996). Test for trend in smoking and OC use were performed by assigning an ordinal score (the median) to grouped values and then treating this score as continuous in the regression models.

RESULTS

Among the 469 cases and 469 controls approached by a letter and subsequently contacted by telephone, an equal percentage (90%) of cases and controls (422/469 cases and 422/469 controls) coincidentally agreed to participate. Reasons for not participating were as follows: 75 refused, 9 could not be reached, 8 were diseased and 2 died after enrollment. Eight control women had to be excluded because of hysterectomy before the carcinoma *in situ* diagnosis of their corresponding case, leaving 422 cases and 414 controls. Only risk sets for which information from both the case and the control had been obtained contributed to the conditional logistic regression analyses. Thus, a total of 373 risk sets (373 cases and 373 matched controls) were included in the matched analyses. Selected characteristics of participating subjects are summarized in Table 1.

Smoking history

A 2-fold higher risk for cervical carcinoma *in situ* was observed among ever smokers compared with never smokers. Women who were current smokers had a crude OR of 2.28 (95% CI 1.62–3.21) compared with non-smokers, and ex-smokers were at intermediary

TABLE I – CHARACTERISTICS OF 746 PARTICIPANTS IN A NESTED CASE-CONTROL STUDY OF CERVICAL CARCINOMA *IN SITU* IN SWEDEN, 1969–1995

Characteristic	Cases (N = 373)	Controls (N = 373)
	Number (%)	Number (%)
Age at interview (years)		
<30	8 (2.14)	7 (1.88)
30–34	23 (6.17)	30 (8.04)
35–39	65 (17.43)	55 (14.75)
40–44	59 (15.82)	65 (17.43)
≥45	218 (58.45)	216 (57.91)
Age at diagnosis (years)		
<30	88 (23.59)	83 (22.25)
30–34	106 (28.42)	111 (29.76)
35–39	87 (23.32)	88 (23.59)
40–44	45 (12.06)	44 (11.80)
≥45	47 (12.60)	47 (12.60)
Body mass index at diagnosis (kg/m ²)		
<20	89 (23.92)	78 (20.91)
20.00–21.99	83 (22.31)	83 (22.25)
22.00–23.99	60 (16.13)	58 (15.55)
24.00–25.99	54 (14.52)	61 (16.35)
≥26.00	86 (23.12)	93 (24.93)
Missing	1	0
Age at menarche (years)		
<13	128 (34.41)	109 (29.30)
13	95 (25.54)	119 (31.99)
>13	149 (40.05)	144 (38.71)
Missing	1	1
Parity (number of live and still births)		
Nulliparous	65 (17.43)	71 (19.03)
1	67 (17.96)	67 (17.96)
2	158 (42.36)	151 (40.48)
3	61 (16.35)	60 (16.09)
≥4	22 (5.90)	24 (6.43)
Smoking status		
Never	105 (28.15)	168 (45.04)
Ever	268 (71.85)	205 (54.96)
Use of oral contraceptives ¹		
Never	48 (12.90)	84 (22.52)
Ever	324 (87.10)	289 (77.48)
Missing	1	0
Education (years)		
6–9	106 (28.42)	94 (25.41)
10–12	105 (28.15)	108 (29.19)
≥13	162 (43.43)	168 (45.41)
Marital status		
Married	277 (74.26)	290 (77.75)
Unmarried	42 (11.26)	41 (10.99)
Divorced	41 (10.99)	34 (9.12)
Widowed	13 (3.49)	8 (2.14)

¹Combined estrogen-progestin compounds only.

risk, with an OR of 1.67 (95% CI 1.08–2.56) (Table II). No significant trend was observed with age at start of smoking. An increased risk was found with increasing tobacco consumption (duration, intensity, pack-years) with a crude OR 2.22 (95% CI 1.53–3.22) for 10–19 years smoke duration and an OR of 2.73 (95% CI 1.72–4.34) for smoking 10–14 cigarettes per day, when compared with non-smokers. The risk increased steadily from low to moderate smokers. However, heavy smokers, having smoked more than 8 pack-years, had a lower risk for cervical carcinoma *in situ* than moderate smokers but remained at an almost 2-fold increased risk compared with non-smokers. Analyses of time since start smoking and time since stop smoking revealed a highest risk for those who started smoking 10–19 years before diagnosis of carcinoma *in situ* and among current smokers. When adjustments for potential confounding were made in the multivariate analyses, the risks were slightly reduced but remained significant for current smoking, increasing smoke duration and for smoking intensity up to 15 cigarettes per day and up to a cumulative tobacco consumption of 8 pack-years (Table II).

TABLE II – OR AND 95% CI OF CERVICAL CARCINOMA *IN SITU* IN RELATION TO SMOKING HABITS

Variable	Number of cases/controls	Crude OR ¹	95% CI	Adjusted OR ²	95% CI
Smoking status					
Never	105/168	1	—	1	—
Ex-smoker	67/61	1.67	1.08–2.56	1.47	0.92–2.34
Current smoker	201/144	2.28	1.62–3.21	1.94	1.32–2.85
			$p < 0.001^4$		$p < 0.005^4$
Age at start (years)					
Never	105/168	1	—	1	—
12–15	108/69	2.60	1.72–3.94	2.13	1.33–3.42
16–17	62/64	1.58	1.02–2.43	1.38	0.85–2.22
18–19	45/34	2.11	1.26–3.54	1.95	1.11–3.42
≥20	53/38	1.99	1.25–3.18	1.70	1.03–2.80
			$p = 0.48^5$		$p = 0.67^5$
Duration (years)					
Never	105/168	1	—	1	—
1–9	68/53	2.01	1.28–3.16	1.73	1.06–2.80
10–19	144/106	2.22	1.53–3.22	1.78	1.18–2.69
≥20	56/46	1.84	1.12–3.03	1.77	1.02–3.08
			$p = 0.82^5$		$p = 0.93^5$
Intensity (cig/day)					
Never	105/168	1	—	1	—
1–4	55/47	1.73	1.08–2.77	1.43	0.86–2.38
5–9	105/78	2.24	1.49–3.37	2.08	1.33–3.24
10–14	75/45	2.73	1.72–4.34	2.13	1.28–3.57
≥15	33/35	1.47	0.83–2.59	1.26	0.68–2.35
			$p = 0.73^{5,6}$		$p = 0.69^{5,6}$
Pack-years ³					
Never	105/168	1	—	1	—
0–<2	38/37	1.52	0.89–2.59	1.29	0.72–2.30
2–<4	53/33	2.55	1.53–4.25	2.22	1.28–3.84
4–<6	53/35	2.61	1.54–4.42	2.11	1.20–3.73
6–<8	45/28	2.91	1.67–5.07	2.59	1.43–4.69
8–<12	37/34	1.82	1.07–3.08	1.52	0.85–2.72
≥12.00	42/38	1.77	1.05–3.00	1.47	0.82–2.64
			$p = 0.55^{5,6}$		$p = 0.51^{5,6}$
Time since start (years)					
Never	105/168	1	—	1	—
1–9	26/27	1.53	0.76–3.08	1.25	0.58–2.67
10–14	62/40	2.76	1.60–4.77	2.44	1.35–4.42
15–19	87/59	2.62	1.65–4.16	2.11	1.27–3.50
≥20	93/79	1.68	1.10–2.58	1.50	0.94–2.40
			$p = 0.65^5$		$p = 0.78^5$
Time since stop (years)					
Never	105/168	1	—	1	—
≥10	33/22	2.31	1.27–4.22	1.93	1.03–3.65
1–9	49/46	1.62	1.01–2.61	1.46	0.86–2.46
0	186/137	2.21	1.56–3.12	1.85	1.25–2.73
			$p = 0.99^5$		$p = 0.97^5$

OR, odds ratio; CI, confidence interval.—¹Univariate odds ratios.—²Multivariate odds ratios adjusted for years in school (<7, 7–9, 10–12, >12), marital status (married, single, divorced, widowed), age at first sexual intercourse (never, 11–15, 16–17, 18–19, ≥20), number of sexual partners (0–1, 2–3, 4–9, ≥10), age at menarche (<13, 13, >13), parity (nulliparous, 1, 2, 3, ≥4), and oral contraceptive use (never, ex, current).—³One pack-year is equivalent to the consumption of 20 cigarettes per day for 1 year.—⁴Test for homogeneity.—⁵Test for trend only among users.—⁶Test for linearity was rejected.

Analyses stratified according to age at diagnosis (<45 years, ≥45 years) showed higher risk estimates for women aged <45 years at diagnosis, crude OR 2.61 (95% CI 1.80–3.78) for current

TABLE III – OR AND 95% CI OF CERVICAL CARCINOMA *IN SITU* IN RELATION TO SMOKING HABITS, BY AGE AT DIAGNOSIS (<45 YEARS, ≥45 YEARS)

Variable	Age at diagnosis <45 years			Age at diagnosis ≥45 years		
	Number of cases/controls	Crude OR ¹ (95% CI)	Adjusted OR ² (95% CI)	Number of cases/controls	Crude OR ¹ (95% CI)	Adjusted OR ² (95% CI)
Smoking status						
Never	83/147	1	1	22/21	1	1
Ex smoker	56/51	1.83 (1.13–2.95)	1.66 (0.99–2.79)	11/10	1.03 (0.39–2.72)	0.66 (0.14–3.13)
Current smoker	187/128	2.61 (1.80–3.78)	2.23 (1.48–3.37)	14/16	0.81 (0.30–2.21)	0.58 (0.10–3.29)
		$p < 0.001^3$	$p < 0.001^3$		$p = 0.89^3$	$p = 0.81^3$
Duration (years)						
Never	83/147	1	1	22/21	1	1
1–9	66/50	2.26 (1.41–3.62)	2.09 (1.26–3.47)	2/3	0.65 (0.11–4.08)	0.16 (0.01–3.88)
10–19	137/99	2.48 (1.67–3.69)	1.98 (1.28–3.06)	7/7	0.98 (0.30–3.25)	0.88 (0.16–4.69)
≥20	40/30	2.22 (1.23–4.02)	2.11 (1.11–4.02)	16/16	0.95 (0.37–2.47)	0.46 (0.07–3.04)
		$p = 0.96^4$	$p = 0.98^4$		$p = 0.78^4$	$p = 0.76^4$
Pack-years						
Never	83/147	1	1	22/21	1	1
0.15–3.95	86/64	2.28 (1.48–3.52)	1.97 (1.24–3.13)	5/6	0.83 (0.24–2.85)	0.62 (0.11–3.34)
4.00–7.95	93/56	3.27 (2.03–5.27)	2.69 (1.62–4.47)	5/7	0.68 (0.18–2.61)	0.61 (0.07–5.36)
≥8.00	64/59	1.89 (1.20–2.98)	1.64 (0.99–2.72)	15/13	1.12 (0.41–3.05)	0.65 (0.11–4.09)
		$p = 0.29^4$	$p = 0.34^4$		$p = 0.55^4$	$p = 0.93^4$

OR, odds ratio; CI, confidence interval. ¹Univariate odds ratios. ²Multivariate odds ratios adjusted for years in school (<7, 7–9, 10–12, >12), marital status (married, single), oral contraceptive use (never, ever), age at first sexual intercourse (11–17, 18–19, ≥20), number of sexual partners (0–1, 2–3, 4–9, ≥10), age at menarche (<13, 13, >13), and parity (nulliparous, >0). ³Test for homogeneity. ⁴Test for trend only among users.

smokers compared with non-smokers, whereas among women aged 45 years or older, no positive association with smoking was found (Table III).

Use of OCs

We found positive associations between OC use and the risk for cervical carcinoma *in situ* (Table IV). Current users of combined estrogen-progestin OCs had an almost 4-fold increased risk compared with non-users, crude OR 3.78 (95% CI 2.09–6.85). For ex-users, the corresponding OR was 2.22 (95% CI 1.38–3.56). There was a clear trend of increasing risk for cervical carcinoma *in situ* with increasing duration of use: OR was 5.85 (95% CI 2.48–13.76) for more than 14 years of use compared with never (p for trend < 0.001) (Table IV). We examined the importance of time since start and time since stop in relation to risk for *in situ* cervical carcinoma. The highest risks were noticed among those who started using OCs more than 15 years before the diagnosis (OR 4.32; 95% CI 2.33–8.01) and among current users (OR 3.86; 95% CI 2.13–7.01), compared with non-users. Use of low-dose progestin did not increase risk, but rather slightly decreased risk (OR 0.59; 95% CI 0.37–0.96) for ex-users compared with never users. Exposure to this type of OC was disregarded in the subsequent multivariate analyses. After adjusting for potential confounding factors, the risks associated with ever use and duration of OC use became weaker but remained significant (Table IV).

Sexual and reproductive factors

Both early and late menarche were associated with a slightly increased risk, yet not significantly so, for cervical carcinoma *in situ* (Table V). Age at first sexual intercourse, parity (number of live births), or age at first child were not significantly associated with the risk for cervical carcinoma *in situ*. Risk increased with number of sexual partners (OR 2.67; 95% CI 1.51–4.72) for those reporting a total of 10 or more partners before the date of diagnosis compared with those having had 0 or 1 sexual partner (p for trend < 0.005) (Table V). After multivariate adjustment, the risk estimates were reduced, with only the association with increasing number of sexual partners remaining equally strong and significant (Table V).

Body mass index and weight change

Neither BMI at age 20, BMI at age of diagnosis, nor a weight change from age 20 affected risk for cervical carcinoma *in situ* (data not shown).

Gynecological infections

Women reporting ever having had a genital infection had an increased risk for cervical carcinoma *in situ* (OR 1.67; 95% CI 1.19–2.36), compared with those reporting never having had such infection (data not shown). We found no association between self reported episodes of specific genital infections, except for trichomoniasis with crude OR 3.56 (95% CI 1.70–7.45) and cervical carcinoma *in situ* risk.

Histological classification

On reviewing the histological specimens for the 373 case women included in the matched analyses, 32 specimens initially classified as carcinoma *in situ* were regarded as slight dysplasia and 2 patients were found to have invasive carcinoma. Further, 36 specimens could not be found. When omitting the 34 cases with specimens not confirming a diagnosis of carcinoma *in situ* from the analyses, the risk estimates changed only marginally (data not shown).

HPV typing

The total number of smears analyzed for HPV16/18 by PCR were 1,959 for the 373 case patients and 1,313 for the 373 matched control subjects. These smears had been taken during a certain time period for each case-control pair, depending on when they had their first registered smear and when the cases were diagnosed with carcinoma *in situ*. When we restricted the analyses to the smear taken nearest to diagnosis (and the corresponding date among the control women), the risk for cervical carcinoma *in situ* was highly increased among HPV16-positive women with OR 15.79 (95% CI 8.04–30.95), compared with HPV16-negative women. The risk conferred by HPV18 positivity was 2.33 (95% CI 1.07–5.10) (data not shown).

In a stratified analysis, we divided the matched pairs into 2 groups according to the HPV16/18 status of the case patient's most recent smear. We included only risk sets in which cases had a smear taken within 3 years before diagnosis, thus excluding 51 case-control pairs. Risks associated with smoking variables, measures of OC use, parity and age at first sexual intercourse were largely similar in the analyses restricted to HPV16/18-positive and -negative cases (Table VI). However, whereas the total number of sexual partners before the time of diagnosis was only modestly related to the risk for carcinoma *in situ* in the group with HPV16/18-positive

TABLE IV – OR AND 95% CI OF CERVICAL CARCINOMA *IN SITU* IN RELATION TO OC USE

Variable	Number of cases/controls	Crude OR ¹	95% CI	Adjusted OR ²	95% CI
Progestin (low dose)					
Never	332/310	1	—	1	—
Ex-user	30/49	0.59	0.37–0.96	0.60	0.36–1.01
Current user	7/10	0.66	0.25–1.74	0.80	0.28–2.26
Missing	4/4				
		$p = 0.08^3$		$p = 0.15^3$	
Combined estrogen-progestin					
Never	48/84	1	—	1	—
Ex-user	241/239	2.22	1.38–3.56	1.98	1.17–3.33
Current user	77/48	3.78	2.09–6.85	3.64	1.91–6.93
Missing	7/2				
		$p < 0.001^3$		$p < 0.001^3$	
Combined estrogen-progestin					
Age at start (years)					
Never	48/84	1	—	1	—
11–15	51/48	2.95	1.52–5.73	2.25	1.04–4.87
16–17	86/64	3.60	1.95–6.64	2.94	1.49–5.79
18–19	56/67	2.13	1.16–3.91	1.96	1.01–3.79
20–24	89/64	3.20	1.83–5.62	2.97	1.62–5.43
≥25	42/46	1.72	0.95–3.11	1.71	0.89–3.29
Missing	1/0				
		$p = 0.10^4$		$p = 0.42^4$	
Duration (years)					
Never	48/84	1	—	1	—
<1	41/55	1.54	0.86–2.73	1.27	0.67–2.41
1–<2	28/32	1.86	0.92–3.75	1.66	0.78–3.57
2–<5	76/68	2.55	1.45–4.48	2.29	1.22–4.28
5–<10	97/86	2.52	1.46–4.35	2.37	1.30–4.33
10–<15	48/37	3.20	1.67–6.13	2.93	1.44–5.98
≥15	28/9	5.85	2.48–13.76	5.46	2.14–13.92
Missing	7/2				
		$p < 0.001^{5,7}$		$p < 0.001^{5,7}$	
Time since start (years)					
Never	48/84	1	—	1	—
1–9	72/63	2.15	1.18–3.91	1.94	1.02–3.69
10–14	94/106	1.81	1.05–3.15	1.75	0.96–3.20
15–19	100/64	4.32	2.33–8.01	3.89	1.99–7.59
≥20	58/56	2.42	1.15–5.10	2.28	0.99–5.27
Missing	1/0				
		$p < 0.001^{3,6}$		$p < 0.01^{3,6}$	
Time since stop (years)					
Never	48/84	1	—	1	—
≥15	48/45	2.46	1.24–4.89	2.11	0.99–4.51
5–14	117/134	1.91	1.16–3.13	1.67	0.96–2.89
1–4	76/60	2.97	1.68–5.26	2.73	1.46–5.11
0	77/48	3.86	2.13–7.01	3.74	1.95–7.15
Missing	7/2				
		$p = 0.04^{4,8}$		$p = 0.02^{4,9}$	

OR, odds ratio; CI, confidence interval; OC, oral contraceptive.—¹Univariate odds ratios.—²Multivariate odds ratios adjusted for years in school (<7, 7–9, 10–12, >12), marital status (married, single, divorced, widowed), age at first sexual intercourse (never, 11–15, 16–17, 18–19, ≥20), number of sexual partners (0–1, 2–3, 4–9, ≥10), age at menarche (<13, 13, >13), parity (nulliparous, 1, 2, 3, ≥4), and smoking (never, ex, current).—³Test for homogeneity.—⁴Test for trend only among users.—⁵Test for trend among all.—⁶Test for linearity among users was rejected.—⁷Log-scale estimate = 0.08.—⁸Log-scale estimate = –0.03.—⁹Log-scale estimate = –0.04.

cases, the association with number of partners among HPV16/18-negative cases was strong, and increased steadily with number of partners, giving OR 8.03 (95% CI 2.26–28.55) for 10 or more partners vs. 0–1 partner.

DISCUSSION

Our case-control study has the advantage of being population-based, having an equally high participation rate among case and control subjects and repeated measurements of HPV status for cases and controls up to several years before the time of diagnosis of cervical carcinoma *in situ*. We found a strong age-dependent

association between smoking and cervical carcinoma *in situ*. Furthermore, our data support a positive association between OC use and cervical carcinoma *in situ*.

Infection with certain HPV types has been associated with remarkably high risk for cervical carcinoma in most studies performed during the last decade (IARC, 1995). In our study, we found a 16-fold increased risk when having an HPV16 infection diagnosed in the period 0–3 years prior to the diagnosis of carcinoma *in situ*. The strong and consistent association between HPV and cervical neoplasia fulfills standard epidemiological criteria for causality (IARC, 1995). However, the discrepancy between HPV prevalence and the incidence of cervical carcinoma

TABLE V – OR AND 95% CI OF CERVICAL CARCINOMA *IN SITU* IN RELATION TO SEXUAL AND REPRODUCTIVE FACTORS

Variable	Number of cases/controls	Crude OR ¹	95% CI	Adjusted OR ²	95% CI
Age at menarche (years)					
<13	128/109	1.47	1.01–2.13	1.42	0.94–2.14
13	95/119	1	—	1	—
>13	149/144	1.30	0.91–1.86	1.16	0.78–1.73
Missing	1/1				
		$p = 0.12^4$		$p = 0.25^4$	
Age at first sexual intercourse (years)					
Never	1/1	1.00	0.06–15.99	1.50	0.04–64.90
≥20	38/48	1	—	1	—
18–19	86/94	1.16	0.67–2.02	0.70	0.35–1.42
16–17	148/138	1.34	0.82–2.22	0.79	0.43–1.45
11–15	99/91	1.42	0.81–2.51	0.86	0.46–1.61
Missing	1/1				
		$p = 0.15^6$		$p = 0.33^6$	
Number of sexual partners before age 20					
0	39/49	0.90	0.53–1.52	0.97 ³	0.52–1.80
1	113/124	1	—	1	—
2–3	130/123	1.22	0.86–1.75	1.09	0.73–1.64
4–9	67/62	1.22	0.78–1.91	1.10	0.65–1.85
≥10	22/12	2.07	0.98–4.40	1.71	0.72–4.02
Missing	2/3				
		$p = 0.05^5$		$p = 0.29^5$	
Number of sexual partners before diagnosis					
0–1	36/70	1	—	1	—
2–3	100/105	1.87	1.15–3.04	1.68	0.98–2.88
4–9	170/145	2.48	1.53–4.02	2.29	1.30–4.03
≥10	66/52	2.67	1.51–4.72	2.83	1.45–5.51
Missing	1/1				
		$p < 0.005^5$	Estimat. = 0.06	$p = 0.01^5$	Estimat. = 0.07
Age at first child (years)					
Nulliparous	65/71	0.85	0.55–1.31	0.77	0.41–1.43
<20	70/57	1.17	0.75–1.83	1.07	0.64–1.78
20–24	136/128	1	—	1	—
25–29	78/84	0.86	0.57–1.28	0.88	0.55–1.43
≥30	24/33	0.65	0.35–1.19	0.66	0.32–1.37
		$p = 0.06^7$		$p = 0.25^7$	
Parity (number live and still births)					
Nulliparous	65/71	0.91	0.56–1.47	0.77	0.41–1.43
1	67/67	1	—	1	—
2	158/151	1.05	0.70–1.58	1.10	0.68–1.79
3	61/60	1.03	0.62–1.73	0.94	0.51–1.76
≥4	22/24	0.92	0.45–1.87	0.81	0.36–1.85
		$p = 0.75^5$		$p = 0.87^5$	

OR, odds ratio; CI, confidence interval.¹Univariate odds ratios.²Multivariate odds ratios adjusted (when applicable) for years in school (<7, 7–9, 10–12, >12), marital status (married, single, divorced, widowed), smoking (never, ex, current), oral contraceptive use (never, ex, current), age at first sexual intercourse (11–15, 16–17, 18–19, ≥20), number of sexual partners (0–1, 2–3, 4–9, ≥10), age at menarche (<13, 13, >13), age at first child (nulliparous, <20, 20–24, 25–29, ≥30), and parity (nulliparous, 1, 2, 3, ≥4).³Not adjusted for number of sexual partners before diagnosis.⁴Test for homogeneity.⁵Test for trend.⁶Test for trend only among women with sexual debut.⁷Test for trend only among parous women.

in most populations indicates that HPV is not a sufficient cause for the development of cervical neoplasia. Indeed, cofactors that interact with potentially oncogenic HPV types appear crucial for malignant transformation of the cervical epithelium. For such other factors, published results, however, are far from consistent. Despite intensive research, it remains unclear which cofactors are important for the development of cervical carcinoma (Brinton, 1992). Confusion decreased when better PCR-based methods for detection of HPV were applied. Most, or perhaps all, increased risk related to high number of sexual partners and early sexual intercourse may be mediated by sexually transmitted HPV infection. This could be confirmed in our analyses stratified according to HPV 16/18 status, in which no significantly increased risk related to a high number of

sexual partners was found in the HPV16/18-positive case group. In the HPV16/18-negative case group, however, a highly increased risk was found among women with multiple partners. This increased risk might very well diminish or disappear if we could adjust for other HPV types in this group.

The possible association between cigarette smoking and cervical cancer has been debated during the past 20 years. In his 1990 review of the epidemiological publications on smoking and cervical carcinoma, Winkelstein (1990) noted that almost all studies found a positive association with smoking, chiefly among current and heavy smokers. He concluded that there was evidence to support the idea of a causal association between cigarette smoking

TABLE VI – MULTIVARIATE ANALYSES OF CERVICAL CARCINOMA *IN SITU* IN RELATION WITH SELECTED RISK FACTORS, BY HPV 16/18 STATUS OF CASES

Variable	HPV 16/18 positive cases before diagnosis and their matched controls 178 cases/178 controls		HPV 16/18 negative cases before diagnosis and their matched controls 138 cases/138 controls	
	Adjusted OR ¹	95% CI	Adjusted OR ¹	95% CI
Smoking status				
Never	1	—	1	—
Ex-smoker	2.12	1.04–4.32	1.49	0.66–3.36
Current smoker	2.34	1.28–4.27	1.82	0.93–3.58
	$p = 0.01^2$		$p = 0.19^2$	
Smoke duration (years)				
Never	1	—	1	—
1–9	2.34	1.06–5.16	1.54	0.70–3.37
10–19	2.49	1.33–4.66	1.74	0.85–3.53
≥20	1.79	0.80–4.05	1.99	0.68–5.88
	$p = 0.62^4$		$p = 0.66^4$	
Pack-years				
Never	1	—	1	—
0.15–3.95	2.32	1.13–4.78	1.42	0.70–2.88
4.00–7.95	3.42	1.61–7.25	2.73	1.17–6.35
≥8.00	1.60	0.79–3.22	1.56	0.69–3.53
	$p = 0.17^4$		$p = 0.95^4$	
OC use				
Never	1	—	1	—
Ex-user	1.54	0.76–3.12	1.53	0.67–3.52
Current user	2.65	1.06–6.67	2.32	0.88–6.08
	$p = 0.12^2$		$p = 0.23^2$	
OC use duration (years)				
Never	1	—	1	—
<2	1.55	0.65–3.70	0.92	0.30–2.81
2–<10	2.23	1.02–4.86	2.90	1.10–7.62
≥10	2.79	1.14–6.87	3.11	0.94–10.32
	$p = 0.03^{3,5}$		$p = 0.01^{3,6}$	
Age at sexual debut (years)				
≥20	1	—	1	—
18–19	0.73	0.29–1.84	0.68	0.21–2.15
16–17	0.47	0.20–1.13	0.99	0.33–2.99
11–15	0.44	0.17–1.18	1.15	0.31–4.33
	$p = 0.05^3$		$p = 0.40^3$	
Number of sexual partners				
0–1	1	—	1	—
2–3	1.90	0.75–4.84	2.45	0.96–6.25
4–9	2.49	0.97–6.39	3.48	1.29–9.39
≥10	2.38	0.84–6.72	8.03	2.26–28.55
	$p = 0.37^3$		$p < 0.005^3$	Estimat. = 0.17
Parity				
Nulliparous	0.54	0.22–1.34	1.41	0.57–3.47
1	1	—	1	—
2	1.03	0.50–2.15	1.51	0.69–3.31
3	1.18	0.49–2.15	0.75	0.28–2.00
≥4	0.81	0.26–2.47	0.43	0.09–2.20
	$p = 0.45^3$		$p = 0.23^3$	

HPV, human papillomavirus; OR, odds ratio; CI, confidence interval; OC, oral contraceptive.—¹Multivariate odds ratios adjusted (when applicable) for years in school (<7, 7–9, 10–12, >12), marital status (married, single), smoking (never, ex, current), oral contraceptive use (never, ex, current), age at sexual debut (11–17, 18–19, ≥20), number of sexual partners (0–1, 2–3, 4–9, ≥10), age at menarche (<13, 13, >13), and parity (never, ever).—²Test for homogeneity.—³Test for trend.—⁴Test for trend only among users.—⁵Log-scale estimate = 0.07.—⁶Log-scale estimate = 0.10.

and cervical carcinoma, thereby adding this malignancy to the list of smoking-related diseases. However, other researchers in this field have been more skeptical about the postulated causal relationship and raised concern about residual confounding due to inadequate HPV measurements (Phillips and Davey Smith, 1994).

Smoking could increase risk for cervical neoplasia through a number of biological mechanisms. One of the mechanisms, which is highly supported, is an immunosuppressive effect of smoking, which increases persistence of HPV infection. Several investigators have reported a lowered number of Langerhans' cells in the cervical epithelium of smoking women with *in situ* cervical

carcinoma, a finding that might explain an impaired cellular immunity (Barton *et al.*, 1988). More convincingly, high contents of smoke-derived nicotine and cotinine have been found in cervical mucus of smokers (Sasson *et al.*, 1985). Our data, although based on small numbers, also suggest an age-dependent relation between smoking and risk of cervical carcinoma *in situ* that could be explained by an anti-estrogenic effect of cigarette smoking, as described by Baron *et al.* (1990). This hypothesis is supported by findings of a different risk for anogenital cancer development associated with smoking among pre- and post-menopausal women (Daling *et al.*, 1992; Frisch and Melbye, 1995; Frisch *et al.*, 1999).

Studying the relation between OC use and cervical carcinoma risk is fraught with problems, because OC use is highly correlated with sexual and reproductive factors and with screening behaviour. We found an increased risk for cervical carcinoma *in situ* associated with both prior and current use of OCs, a risk pattern unaffected by the HPV16/18 status of the cases. Further studies are needed focusing on a possible interactive effect of HPV and OC use over time, because some data indicate that HPV's activity may be enhanced by hormones (Auborn *et al.*, 1991).

We failed to demonstrate any association between parity and cervical carcinoma *in situ*. An increased risk has been reported mainly in studies from Latin America, in populations where multiparity is common (Muñoz *et al.*, 1993). In the Swedish population, where few women have more than 3 children, increasing parity does not appear to be a risk factor for cervical carcinoma *in situ*. The absence of an association with sexually transmitted diseases other than HPV needs cautious interpretation because we had access only to self-reported data on genital infections without serological confirmation.

Our study has potential limitations. Selection bias was minimized because the control subjects were drawn randomly from the source population and because we managed to obtain a high participation rate among both cases and controls. To reduce the risk for information and measurement bias, both the interviewers, the cyto technician and the laboratory technicians were blinded for case-control status. With regard to recall bias, we have no reason to believe a differential recall by patients and controls. Most of the patients were healthy and had had their carcinoma *in situ* many years before the interview, which makes it unlikely that the disease itself would have affected their answers. However, both patients and controls might have had problems recalling their sexual history, smoking habits and OC usage a long time ago. This non-differential misclassification would have distorted our risk

estimates toward null and thereby lead to an underestimation of the true excess risks.

The smears have been analyzed only for presence of HPV16 and 18, which together account for approximately 65% of all HPV infections detected in cervical tumors (Bosch *et al.*, 1995). As a consequence, we have not been able to control for HPV infection totally, which raises concern about residual confounding. In the analyses stratified according to HPV 16/18 status, similar risk estimates for smoking and OC use were obtained in both HPV-positive and HPV-negative women, whereas risk associated with number of sexual partners clearly differed (Table VI). These results support the assumption that the association with number of sexual partners is likely mediated by HPV infection. In contrast, the increased risk associated with smoking and OC use is not related to HPV16/18 status. When we adjusted for HPV16/18 status in multivariate models, the risk estimates for smoking and OC use were lowered, but remained clearly significant among heavy smokers and long-term OC users (data not shown). HPV 16 and 18 are believed to be the main causative types related to *in situ* and invasive carcinoma of the cervix (IARC, 1995). Thus, if these 2 types did not account for the increased risks among smokers and OC users, it is unlikely that the observed risk associations would be explained by the other, less oncogenic HPV types.

In conclusion, our data confirm the association between smoking and cervical carcinoma and indicate a consistent association with OC use and the risk for cervical carcinoma *in situ*. Whether OCs play a genuine causal role or merely reflect an increased risk for HPV acquisition among OC users remains unsettled. Our data also suggest an age-dependent risk for cervical carcinoma *in situ* in relation to smoking, with strong associations in women younger than 45 years. It is important to further disentangle the effect of smoking in different ages because a causal association or interaction with HPV may have important public health impact.

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