Male Sexual Behavior and Human Papillomavirus DNA: Key Risk Factors for Cervical Cancer in Spain

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Background: **It is now established that certain types of human papillomaviruses (HPVs) are the sexually transmitted agents etiologically linked to cervical cancer. Studies assessing the contribution of the male's sexual behavior and genital HPV DNA status to the risk of development of cervical neoplasia in sexual partners have yielded inconsistent results. *Purpose:* This study evaluates the role of men's sexual behavior and the presence of HPV DNA in the penis on the development of cervical cancer in their sexual partners in Spain, a low-risk area for cervical neoplasia. *Methods:* Husbands (n = 633) of women participating in two case–control studies of cervical neoplasia were interviewed to obtain information on lifestyle habits, including sexual practices. Cytologic samples were taken from the distal urethra and the surface of the glans penis of 183 husbands of case women and of 171 husbands of control women. These samples were analyzed by a polymerase chain reaction-based system using a generic probe and 25 type-specific probes for the detection and typing of HPV DNA. Serologic specimens were also obtained and analyzed for antibodies to *Chlamydia trachomatis*, *Treponema pallidum*, herpes simplex virus type II, and *Neisseria gonorrhoeae*. *Results:* The presence of HPV DNA in the husbands' penis conveyed a fivefold risk of cervical cancer to their wives (adjusted odds ratio [OR] for HPV DNA positivity = 4.9; 95% confidence interval [CI] = 1.9-12.6). The risk of cervical cancer was strongly related to HPV type (adjusted OR for HPV type 16 = 9.0; 95% CI = 1.1-77.5), to the husbands' number of extramarital partners (adjusted OR = 11.0; 95% CI = 3.0-40.0; for ≥21 women versus one), and to the number of prostitutes as extramarital sexual partners (adjusted OR = 8.0; 95% CI = 2.9-22.2; for ≥10 women versus none). Presence of antibodies to *C. trachomatis* (adjusted OR = 2.6; 95% CI = 1.4-4.6) and an early age at first sexual intercourse of the husband (adjusted OR = 3.2; 95% CI = 1.7-5.9; for ≤15 years versus ≥21 years) were also associated with cervical neoplasia in the wife. After adjustment for these variables and for the wife's pack-years of smoking, the husband's smoking was moderately associated with cervical cancer in his wife (adjusted OR = 2.5; 95% CI = 1.4-4.4; for ≥26.2 pack-years versus none). *Conclusions:* The study supports the role of men as vectors of the HPV types that are related to cervical cancer. Lifetime number of female sexual partners, number of female prostitutes as sexual partners, and detection of HPV DNA in the penis of husbands are all surrogate markers of exposure to HPV during marriage. *Implications:* Men who report multiple sexual partners or who are carriers of HPV DNA may be vectors of high-risk HPV types and may place their wives at high risk of developing cervical cancer. Prostitutes are an important reservoir of high-risk HPVs. [J Natl Cancer Inst 1996;88:1060-7]**

Epidemiologic studies (*1-5*) that have involved the use of DNA hybridization methods have helped to demonstrate that certain types of human papillomaviruses (HPVs) are the sexually transmitted agents etiologically linked to cervical cancer. They have also shown that sexual intercourse is the predominant mode of acquiring the viral infection (*6-10*) and that the existence of multiple sexual partners is a surrogate marker for the presence of HPV DNA (*4,11-13*).

Several lines of evidence have suggested that the sexual behavior of males can contribute to the risk of cervical cancer in their sexual partners. Correlation studies (*14-16*) have shown geographic clustering of female genital and male penile cancers. Case–control studies have shown an increased risk of cervical cancer among wives of men with cancer of the penis (*17-19*), among second wives of men whose previous wife had died of cervical cancer (*20*), and among wives of men who traveled frequently (*21*).

The association of the number of sexual partners of the husband and cervical cancer in his wife was first reported in a study among Jewish women (*22*). A decade later, Buckley et al. (*23*) reported that, among self-reported monogamous women, the risk of cervical cancer increased eightfold in relation to the number of sexual partners their husbands had had. Although several subsequent studies (*24-26*) confirmed this association, one study (*27*) did not, and the role of contacts with prostitutes was not statistically significant in any of the relevant studies (*23,25-27*). Finally, two studies in which the early HPV detection assays—filter in situ (*25*) and Virapap (Digene Diagnostics Inc., Silver Spring, MD) (*27*)—were used failed to demonstrate any association between the presence of HPV DNA in the penis and the development of cervical cancer.

To evaluate the relationship between male sexual behavior, HPV DNA, and cervical cancer, four case–control studies of cervical cancer were conducted in

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See "Notes" section following "References."

which husbands of case and control women were also invited to participate. Two studies took place in Spain and two took place in Colombia because of the contrasting age-adjusted incidence rates of cervical cancer (6.7 per 100 000 women in Spain and 42.2 per 100 000 women in Colombia). The evaluation of the male role showed some relevant differences by country; the findings for each country are therefore reported separately [*see also* Muñoz et al. (*28*) in this issue of the Journal]. In the present report, we evaluate the findings from Spain. In the accompanying report, Muñoz et al. (*28*) assess the results from Colombia.

Subjects and Methods

Subjects

Husbands or regular sexual partners of women recruited in two case–control studies of cervical neoplasia were invited to participate. The design of the studies and the main results concerning women have been detailed elsewhere (*2,3,11,12,29,30*). In brief, field work was conducted in nine areas in Spain from June 1985 through December 1987. Eligible case subjects were all women with incident, histologically confirmed, invasive squamous cell carcinoma of the cervix (n = 250) or cervical intraepithelial neoplasia (CIN) grade III (CIN III) (n = 249) (*31*) identified among residents (of at least 6 months' standing) in the study areas. Control subjects for the case subjects with invasive squamous cell carcinoma of the cervix were age-stratified, randomly selected women from the general population (n = 238). Control subjects for the CIN III case subjects were women with a normal cytology smear who were matched to the case subjects by age and recruitment center (n = 242). The target ratio was one control subject per case subject.

Current husbands were defined as men having had regular sexual intercourse with the index women for at least 6 months, irrespective of the existence of a formal marriage or a common house. The husbands were interviewed by specially trained male interviewers using a structured questionnaire. The interview usually took place at nearby health facilities and only rarely in the subject's home. On average, the interview was completed in 25 minutes.

Cell and Serum Samples

Two exfoliated cell samples were collected from the husbands at the health care facility by use of two saline-wet, cotton-tipped swabs from the distal urethra and the external surface of the glans and coronal sulcus of the penis. A smear was prepared for cytology reading, and the remaining cells were eluted in phosphate-buffered saline, pelleted, and stored at –20 °C. Cell pellets were often visible to the naked eye; however, these pellets were, on average, smaller than those recovered from the cervix in the cervical neoplasia studies. Serum samples were also obtained and analyzed for antibodies to herpes simplex virus type II (by enzyme-linked immunosorbent assay [ELISA]), *Chlamydia trachomatis* (by indirect immunofluorescence), *Neisseria gonorrhoeae* (by indirect hemagglutination), and *Treponema pallidum* (by ELISA). The details of the different techniques and antigens used as well as the criteria of positivity were described elsewhere (*29*). All protocols were cleared by the International Agency for Research on Cancer and by local ethical committees.

Detection of HPV DNA by Polymerase Chain Reaction

HPV DNA sequences were searched for in the cytologic specimens obtained. A widely used polymerase chain reaction (PCR) DNA amplification-based method was employed for the detection and typing of HPV. L1 consensus primers MY09/MY11 were used but with modifications; most of these modifications have been described by Hildesheim et al. (*32*). Primer HMB01 was added to MY09/MY11 to facilitate amplification of HPV-51; the generic probe (a mixture of HPV-16 and HPV-18) was enlarged to also include HPV-51 and HPV-66; 25 type-specific probes were utilized (*32-34*). Primers for a fragment of β-globin gene served as an internal control to ascertain whether each specimen was sufficient for amplification.

All specimens were prepared and analyzed by standard procedures in the laboratory of K. V. Shah. Positive controls showed that the assay detected fewer than 25 copies of HPV-16 per reaction. None of the negative controls (one human kidney tissue fragment per 25 specimens analyzed) revealed HPV DNA, suggesting that laboratory contamination was not common. Quality-control (internal reproducibility) specimens (one masked, repeated specimen per 25 specimens analyzed) showed perfect agreement, suggesting both reliability and lack of contamination. All utensils were disposable and were discarded immediately after each use (single use for each specimen). Standard methods to avoid and to monitor for contamination were used throughout the laboratory analysis (*35*). PCR assays were performed in a blinded manner with regard to the case or control status of the subjects.

Among women with a current husband, the participation rates in the interview were 86.4% (306 of 354) and 78.8% (327 of 415) for husbands of case women and control women, respectively ($P<.05$). Contribution of cell specimens was 73% (223 of 306) and 66% (217 of 327) among husbands of case women and control women, respectively. Of these, PCR amplification (as assessed by the β-globin amplification test) was successful for 82% (183 of 223) and 79% (171 of 217) of the study participants who contributed cell specimens, respectively. Overall, 183 (59.8%) husbands of case women and 171 (52.3%) husbands of control women ($P = .06$) contributed cytology specimens that were informative with regard to HPV DNA status.

Subjects who contributed cell specimens were systematically compared with the group of noncontributors with regard to the distribution of risk factors. Men in the control group reporting six or more sexual partners contributed cytologic specimens at a lower rate than those reporting one to five sexual partners ($P = .001$). This "specimen contribution bias" could have reduced the proportion of HPV-positive subjects in the control group, leading to an overestimation of the odds ratios (ORs) in relation to HPV. To assess the magnitude of this potential bias, we applied the HPV prevalences observed in each category of number of sexual partners among the specimen contributors to the number of participants who did not contribute specimens (also grouped by their number of sexual partners). The magnitude of the recalculated ORs did not change from the results presented in Table 1 (data not shown).

Statistical Analyses

The association between selected characteristics of the sexual behavior of men and cervical cancer in their wives was evaluated by use of unconditional linear logistic regression models to estimate ORs and 95% confidence intervals (CIs) after controlling for the effects of potential confounders (*36*). Separate analyses were conducted with the use of unconditional and conditional methods for the CIN III matched study. The results of both analyses were very similar (in the conditional analyses, OR point estimates were slightly reduced and their confidence limits were slightly wider); the unconditional analyses are presented.

The two types of cervical cancer studied (i.e., CIN III and invasive squamous cell carcinoma) showed very similar risk patterns and were combined in the presentation of results after study type was added as a regressor variable. Because of their potential confounding effects and the stratified design, age (≤39 years, 40–49 years, and ≥50 years) and study area were also included in all logistic models.

The adjustments used to evaluate number of sexual partners before and during marriage included a term for duration of current marriage, and these variables were adjusted for each other. To evaluate the association of cervical cancer with type-specific HPVs, we grouped the HPVs into high-risk types (types 16, 31, 33, 39, 51, 52, 58, 59, and 66), low-risk types (types 6, 11, and 53), and HPV-X (specimens testing positive by the generic probe and negative by all 25 type-specific probes). Subjects from whom biological specimens were not obtained or from whom the amplification of DNA in their specimens was inadequate (β-globin negative) were included in the analyses as a separate category (unknown HPV status).

Statistical significance was set at the .05 α value, and all P values were derived from two-sided statistical tests.

Results

Table 1 shows the distribution of some selected variables and their association with cervical cancer. Lack of education was associated with cervical cancer. Smoking among men was a risk factor for cervical cancer in their wives and showed a significant dose–response relationship with duration of smoking (data not shown), average number of cigarettes smoked per day (data not shown), and the estimated number of pack-years. Reported history of genital warts, sero-

Table 1. Risk of cervical cancer in women in Spain according to selected characteristics of their husbands

Variable	Husbands of case subjects* No.	%	Husbands of control subjects† No.	%	OR‡ (95% confidence interval)
Education§					
Secondary or higher	96	31.8	116	35.6	1.0 (referent)
Primary	155	51.3	173	53.1	1.1 (0.8-1.6)
None	51	16.9	37	11.3	**1.8 (1.0-3.1)**
Unknown	4		1		
					P for trend = .08
Smoking					
Never	34	11.1	86	26.3	1.0 (referent)
Ever	272	88.9	241	73.7	**2.6 (1.7-4.0)**
Status					
Ex-smoker	47	15.4	54	16.5	**1.9 (1.0-3.3)**
Current	225	73.5	187	57.2	**2.8 (1.8-4.4)**
					P for trend <.00001
Pack-years					
0.1-13.2	54	18.1	75	23.3	1.6 (0.9-2.7)
13.3-26.1	97	32.4	80	24.8	**2.7 (1.7-4.6)**
≥26.2	114	38.1	81	25.2	**3.3 (2.0-5.5)**
Unknown	7		5		
					P for trend <.00001
History of genital warts					
Never	283	92.8	319	97.9	1.0 (referent)
Ever	22	7.2	7	2.1	**3.7 (1.5-8.9)**
Unknown	1		1		
Antibodies to *Chlamydia trachomatis*					
Negative	205	79.5	257	92.1	1.0 (referent)
Positive	53	20.5	22	7.9	**2.9 (1.7-5.0)**
Unknown	48		48		
HPV DNA typing by polymerase chain reaction					
Status					
Negative	151	82.5	165	96.5	1.0 (referent)
Positive	32	17.5	6	3.5	**6.5 (2.6-16.2)**
Unknown	123		156		
Type specific					
Low-risk types‖	2	1.1	2	1.2	1.2 (0.2-8.7)
High-risk types¶	25	13.7	4	2.3	**7.5 (2.5-22.4)**
HPV-X	5	2.7	0	0.0	**334.0 (0.1-∞)**
Type 16 only	9	4.9	1	0.6	**11.7 (1.5-92.6)**

*The total number of husbands was 306. Their mean age ± standard deviation was 44.7 years ± 12.5 years.

†The total number of husbands was 327. Their mean age ± standard deviation was 45.4 years ± 12.7 years.

‡Odds ratio (OR) adjusted for age group (<39 years, 40-49 years, and ≥50 years), study type (cervical intraepithelial neoplasia grade III or invasive squamous cell carcinoma of the cervix), and study area. Boldface numbers indicate statistical significance. *P* for trend tests were two-sided.

§Primary education includes any schooling received up to approximately 10 years of age; secondary or higher education is any additional schooling received beyond primary education.

‖Includes HPV types 6, 11, and 53.

¶Includes HPV types 16, 31, 33, 39, 51, 52, 58, 59, and 66. OR for high-risk types and HPV-X combined: 9.2 (95% confidence interval = 3.2-27.0).

positivity to *C. trachomatis*, and HPV DNA were all associated with cervical cancer risk. No association was found for other related variables of men, including the following: presence of antibodies to herpes simplex virus type II, *N. gonorrhoeae*, and *T. pallidum*; years since last sexual intercourse; genital hygiene; retractile foreskin; sex during menses; condom use; and homosexuality (data not shown).

The prevalence of HPV DNA was 17.5% and 3.5% among husbands of case women and control women, respectively (overall prevalence: 10.7%). Penile HPV DNA prevalence was significantly related to the reported number of female sexual partners (prevalence: 3.2%, 15.4%, and 18.7% for one to five, six to 19, and ≥20 sexual partners, respectively; *P* for trend <.0001) but did not differ by age (prevalence: 10.4%, 12.7%, and 8.8% for age groups ≤37 years, 38-50 years, and ≥51 years, respectively; *P* for trend = .79).

The HPV type-specific distribution among the 38 men found to be HPV positive was as follows: HPV-16 (10 subjects), HPV types 31, 33, and 51 (four subjects each), HPV-58 (three subjects), HPV-53 (two subjects), HPV-6 and/or HPV-11 (two subjects), and HPV types 39, 52, 59, and 66 (one subject each). Five subjects (13.2%) were positive by the generic probe only and were designated as being positive for HPV-X. Five case subjects had multiple infections involving HPV types 16 and 31, 16 and 53, 31 and 66, 31 and 56, and 16, 51, and 53; these infections were con-

sidered as single infections by the predominant HPV type (first in each list for the subsequent analyses). No specimens were positive for HPV types 18, 26, 35, 40, 45, 54, 55, 68, W13B, PAP155, and PAP238A.

The OR (95% CI) estimates for positivity were 6.5 (2.6-16.2) for any HPV type, 9.2 (3.2-27.0) for high-risk HPV types (including HPV-X), and 11.7 (1.5-92.6) for HPV-16. These risk estimates did not vary across age groups. The corresponding attributable fractions were 16.2% for HPV positivity and 5.9% for HPV-16 positivity.

Table 2 shows the distribution and risk estimates for the husbands' sexual behavior variables statistically associated

Table 2. *Risk of cervical cancer among women in Spain according to selected sexual behavior characteristics of their husbands*

Variables	Husbands of case subjects		Husbands of control subjects		OR* (95% confidence interval)
	No.	%	No.	%	
No. of female sexual partners					
Lifetime					
1	24	7.9	91	28.1	1.0 (referent)
2-5	58	19.1	112	34.6	**1.9 (1.1-3.4)**
6-10	51	16.8	35	10.8	**5.2 (2.8-9.9)**
11-20	64	21.1	37	11.4	**6.1 (3.3-11.3)**
≥21	106	35.0	49	15.1	**8.3 (4.7-14.7)**
Unknown	3		3		
					P for trend <.00001
During marriage†					
1	137	44.8	214	65.4	1.0 (referent)
2-5	98	32.0	92	28.1	**1.7 (1.1-2.6)**
6-10	28	9.2	14	4.3	**2.1 (1.0-4.5)**
11-20	20	6.5	4	1.2	**6.0 (1.9-18.7)**
≥21	23	7.5	3	0.9	**9.1 (2.6-32.5)**
					P for trend <.00001
Before marriage‡					
1	46	15.2	135	41.7	1.0 (referent)
2-5	60	19.8	78	24.1	**2.1 (1.3-3.6)**
6-10	53	17.5	33	10.2	**4.0 (2.2-7.2)**
11-20	50	16.5	34	10.5	**3.1 (1.7-5.6)**
≥21	94	31.0	44	13.6	**4.3 (2.5-7.4)**
Unknown	3		3		
					P for trend <.00001
No. of female prostitutes as sexual partners					
Never	90	29.7	155	48.0	1.0 (referent)
Ever	213	70.3	168	52.0	**2.1 (1.5-2.9)**
Unknown	3		4		
Lifetime					
1-5	77	25.4	94	29.1	1.4 (0.9-2.0)
6-20	75	24.8	42	13.0	**2.8 (1.8-4.6)**
≥21	61	20.1	32	9.9	**3.5 (2.1-5.8)**
Unknown	3		4		
					P for trend <.00001
During marriage§					
0	209	68.3	287	87.8	1.0 (referent)
1-9	62	20.3	35	10.7	**2.6 (1.6-4.2)**
≥10	35	11.4	5	1.5	**10.9 (4.1-28.6)**
					P for trend <.00001
Age at first sexual intercourse, y					
≥21	47	15.5	125	38.2	1.0 (referent)
16-20	204	67.1	173	52.9	**3.0 (2.0-4.5)**
≤15	53	17.4	29	8.9	**4.6 (2.6-8.3)**
Unknown	2				
					P for trend <.00001
Oral sex					
Never	162	54.0	206	63.4	1.0 (referent)
Ever	138	46.0	119	36.6	**1.7 (1.2-2.5)**
Unknown	6		2		
Anal sex					
Never	240	80.0	291	89.5	1.0 (referent)
Ever	60	20.0	34	10.5	**2.2 (1.4-3.6)**
Unknown	6		2		

*Odds ratio (OR) adjusted for age group, study type (cervical intraepithelial neoplasia grade III or invasive squamous cell carcinoma of the cervix) and study area. Boldface numbers indicate statistical significance. *P* for trend tests were two-sided.

†Further adjusted by years of current marriage and number of sexual partners before current marriage.

‡Further adjusted by years of current marriage and number of sexual partners during current marriage.

§Further adjusted by years of current marriage.

with cervical cancer. The magnitude of the ORs related to the number of female sexual partners and number of contacts with female prostitutes during marriage were all consistently higher than the corresponding estimates for number of female sexual partners during a lifetime and before marriage; these differences, however, were not statistically significant.

Table 3 shows the association between selected male characteristics and cervical cancer in a multivariate analysis and the equivalent results restricted to husbands of monogamous women. In both sets of results, the magnitude of the ORs was not significantly different from the results of the univariate analyses. Further adjustment for the presence of HPV DNA in the cervical cells of the women did not change the results. For example, the ORs for husbands' lifetime number of female sexual partners adjusted for penile HPV DNA in sexual partners of monogamous women were 1.0, 3.5, and 5.0 for one to five, six to 20, and 21 or more sexual partners, respectively; the corresponding ORs further adjusted by wives' HPV DNA positivity were 1.0, 3.7, and 5.3, respectively. The association with husband's smoking remained statistically significant after adjustment for the wife's pack-years of smoking.

Table 4 shows the risk estimates for husband's number of female sexual partners and HPV DNA stratified by age. Although there were no statistically significant differences, the point estimates of the ORs for each category of number of partners tended to decrease with age.

Discussion

The underlying hypothesis of this study was that men could be vectors of HPV types typically found in cervical cancer (i.e., HPV types 16, 18, 31, and 33, among others). HPV infection would occur through sexual contacts, notably with prostitutes, and the virus would then be transmitted to the wives or to other subsequent partners.

Confirming the hypothesis, our study is the first to demonstrate a strong association between penile HPV DNA in husbands and cervical cancer in their wives. It also confirms that sexual contacts with women other than the current wife and contacts with female prostitutes during current marriage are strong determinants of the risk of cervical neoplasia, suggesting that the HPV vector capacity of men may be of short duration following exposure.

Penile HPV DNA and Cervical Cancer

The association between HPV DNA in the penile swab and cervical cancer was indeed very strong (i.e., ORs >5). The odds of cervical cancer among monogamous women increased up to 9.5-fold in relation to the presence of high-risk HPV types in the penis of their husbands. The excess risk associated with HPV type 16 was sixfold to ninefold. In our study, the prevalence of HPV DNA in the penis showed a trend with increasing number of sexual partners and with the number of sexual partners who were prostitutes.

The highest point estimates of the ORs for both number of partners and HPV DNA detection were observed in the youngest age groups (Table 4). Thus, one can speculate that self-reported number of partners is as good a marker of ever exposure to HPV as is HPV DNA detection itself. Questionnaires have the additional advantage of describing the exposure to different sexual partners over time, thus providing a more interpretable sequence of events in terms of HPV transmission and latency.

Number of Sexual Partners of the Husband and Cervical Cancer

In this population from Spain, monogamy was reported by 90% of the control women and by 28% of their husbands. The percentage of husbands of control women reporting contacts with prostitutes was 52%. During marriage, there was a strong correlation between husbands' number of sexual partners and husbands' number of prostitutes as sexual partners (correlation coefficient, $R^2 = .95$; $P<.0001$). This pattern of sexual behavior is broadly consistent with the epidemiologic model proposed for a country with a population at low risk of developing cervical cancer (*37*).

One recognized limitation of studies of cervical cancer and male sexual behavior is that, despite the existence of multiple lifetime partners, only the current partner is available for interview and collection of biological specimens. Therefore, in terms of HPV exposure and cervical cancer induction, the sexual behavior of some of the current husbands interviewed may be irrelevant.

To overcome such difficulty, some studies (*23,25,27*) investigated only monogamous women. Although attractive, this approach introduces a strong selection in the participant groups, and it is subject to misclassification of self-reported monogamy among women. It is reassuring that, in our analyses of monogamous women, the pattern and risk estimates linked to husband's behavior were similar to the results of the non-selected groups (Table 3). However, in countries where women report a higher number of sexual partners, studies of monogamous women may be difficult to interpret, and their results may not be applicable to the population at large [*see also* Muñoz et al. (*28*) in this issue of the Journal].

A less often explored option involves evaluation of male sexual behavior during marriage. The results presented in Tables 2 and 3 are consistent in showing that all risk estimates are higher for number of sexual partners during marriage than for number of sexual partners before marriage or over a lifetime. These analyses support the role of men as HPV vectors and further suggest that the HPV carrier status in males may be of relatively short duration.

Ever contacts with prostitutes by men, particularly if occurring during marriage, roughly doubled the risk of cervical cancer in their wives. Contacts by men with 10 or more prostitutes during marriage increased the odds of cervical cancer in their wives to 11-fold (Table 2). This excess risk was slightly reduced after adjustment for the presence of penile HPV DNA and other variables (Table 3), suggesting that the increase in risk is mediated by HPV.

Limitations of the Study

If transmission of HPV DNA by men is the key biological feature, adjustment of the risk estimates for sexual behavior variables by the HPV DNA status of men and women should reduce or eliminate any additional association. In spite of using a well-characterized PCR system, however, we found a residual effect of lack of education, smoking, number of

Table 3. Multivariate assessment of male-associated risk factors for cervical cancer in Spain: all husbands and husbands of monogamous women

Variable	All husbands:* OR† (95% confidence interval)	Husbands of monogamous women	
		No. of husbands of case/control subjects	OR† (95% confidence interval)
Total		218/294	
Education‡			
Secondary or higher	1.0 (referent)	54/98	1.0 (referent)
Primary	1.01 (0.7-1.5)	121/162	1.2 (0.8-2.0)
None	1.57 (0.8-2.9)	40/33	**2.0 (1.0-4.0)**
Unknown		3/1	
	P for trend = .26		*P* for trend = .07
Smoking, pack-years§			
0	1.0 (referent)	24/83	1.0 (referent)
0.1-13.2	1.5 (0.8-2.7)	34/62	1.8 (0.8-3.7)
13.3-26.1	**2.0 (1.1-3.5)**	69/72	**2.1 (1.1-4.1)**
≥26.2	**2.5 (1.4-4.4)**	86/72	**2.5 (1.3-4.8)**
Unknown		5/5	
	P for trend = .001		*P* for trend = .005
No. of female sexual partners during marriage‖			
1	1.0 (referent)	86/192	1.0 (referent)
2-5	**1.6 (1.0-2.4)**	73/83	1.5 (0.9-2.4)
6-10	**2.1 (1.0-4.6)**	23/13	2.0 (0.8-4.6)
11-20	**5.9 (1.7-19.4)**	17/3	**7.2 (1.7-30.0)**
≥21	**11.0 (3.0-40.0)**	19/3	**9.8 (2.6-36.9)**
	P for trend <.00001		*P* for trend <.00001
No. of female prostitutes as sexual partners during marriage‖			
0	1.0 (referent)	136/257	1.0 (referent)
1-9	**2.0 (1.2-3.3)**	51/32	**2.2 (1.2-3.7)**
≥10	**8.0 (2.9-22.2)**	31/5	**7.7 (2.7-21.9)**
	P for trend <.00001		*P* for trend <.00001
Age at first sexual intercourse, y			
≥21	1.0 (referent)	32/115	1.0 (referent)
16-20	**2.4 (1.5-3.7)**	144/152	**2.5 (1.5-4.2)**
≤15	**3.2 (1.7-5.9)**	41/27	**3.5 (1.7-7.1)**
Unknown		1/10	
	P for trend <.00001		*P* for trend = .0001
Anal sex			
Never	1.0 (referent)	184/264	1.0 (referent)
Ever	**2.0 (1.2-3.4)**	31/28	1.3 (0.7-2.5)
Unknown		3/2	
Antibodies to *Chlamydia trachomatis*			
Negative	1.0 (referent)	148/230	1.0 (referent)
Positive	**2.6 (1.4-4.6)**	33/19	**2.1 (1.1-4.2)**
Unknown		37/45	
HPV DNA typing by polymerase chain reaction			
Status			
Negative	1.0 (referent)	108/149	1.0 (referent)
Positive	**4.9 (1.9-12.6)**	22/5	**5.3 (1.8-15.6)**
Unknown		88/140	
Type specific¶			
Low-risk types	0.4 (0.1-4.1)	1/2	0.3 (0.02-3.5)
High-risk types and HPV-X	**7.4 (2.4-22.6)**	21/3	**9.5 (2.6-34.4)**
Type 16 only	**9.0 (1.1-77.5)**	5/1	6.2 (0.6-60.7)

*The numbers of husbands of case and control subjects for each category are given in Tables 1 and 2.

†Odds ratio (OR) adjusted for age group, study type (cervical intraepithelial neoplasia grade III or invasive squamous cell carcinoma of the cervix), and study area, plus all variables in the table except education. Boldface numbers indicate statistical significance. *P* for trend tests were two-sided.

‡Primary education includes any schooling received up to approximately 10 years of age. Secondary or higher education is any additional schooling received beyond primary education.

§Further adjusted for female pack-years of smoking.

‖Further adjusted for years of current marriage. Not adjusted for other "during-marriage" variables.

¶*See* Table 1 footnotes for list of HPV types. ORs for high-risk types excluding HPV-X: 5.6 (95% confidence interval = 1.7-17.7) among all husbands and 7.4 (95% confidence interval = 1.9-28.9) among husbands of monogamous women.

Table 4. Effect of husbands' number of female sexual partners and penile HPV DNA on cervical cancer risk by age group

	Age group					
	≤37 y		38-50 y		≥51 y	
	No. of husbands of case/control subjects	OR* (95% confidence interval)	No. of husbands of case/control subjects	OR* (95% confidence interval)	No. of husbands of case/control subjects	OR* (95% confidence interval)
Lifetime No. of female sexual partners						
1	7/32	1.0 (referent)	7/33	1.0 (referent)	10/26	1.0 (referent)
2-5	23/42	2.65 (1.0-7.02)	14/35	1.80 (0.64-5.04)	21/35	1.47 (0.59-3.67)
6-20	49/25	8.80 (3.37-23.00)	38/28	6.27 (2.41-16.32)	28/19	2.99 (1.14-7.84)
≥21	23/6	19.41 (5.68-66.29)	47/23	9.67 (3.67-25.43)	36/20	4.14 (1.62-10.55)
Unknown	0/1		0/1		3/1	
		P for trend <.00001		P for trend <.00001		P for trend = .0007
HPV-DNA status						
Negative	49/63	1.0 (referent)	48/63	1.0 (referent)	54/39	1.0 (referent)
Positive	11/2	7.81 (1.63-37.49)	13/3	6.57 (1.76-24.56)	8/1	6.06 (0.73-50.52)
Unknown	42/41		45/54		36/61	

*OR = odds ratio. Models adjusted by study type (cervical intraepithelial neoplasia grade III or invasive squamous cell carcinoma of the cervix) and study area. Tests for interactions—between age and number of sexual partners: $P = .53$; between age and HPV-DNA status: $P = .94$. P for trend tests were two-sided.

sexual partners, number of contacts with prostitutes, men's age at first intercourse, anal sex, and seropositivity to *C. trachomatis*.

One likely explanation is that HPV exposure and its associated OR were underestimated, leading to the observed effects of surrogate risk factors. This situation may be partly due to difficulties in sampling of specimens from the male genitalia and partly due to the still low sensitivity of the PCR system employed. A more general limitation of case–control studies is that the estimated HPV DNA prevalence in men may measure relatively recent exposures to HPVs that may be unrelated to the initiation of cervical neoplasia in the wife.

Misclassification of HPV exposure and insufficient adjustment seem to be the two most likely reasons for the increased ORs observed for men's sexual behavior variables and smoking.

The alternative interpretation implies that part of the male role effect is not attributable to the transmission of HPV. Seropositivity for *C. trachomatis* may merely be a surrogate for the presence of HPV or, alternatively, a true risk factor for cervical cancer. According to our studies completed in women showing moderate associations (*29,38*), *C. trachomatis* remains a candidate cofactor deserving further attention. Passive smoking as a risk factor for cervical cancer has also been suggested by other studies, based either on epidemiologic grounds (*39,40*) or on measurements of tobacco derivatives in the cervical mucus and fluids (*41-43*). None of these studies have taken into account the role of HPV DNA in the evaluation of smoking. At present, the claimed association between smoking (active and passive) and cervical cancer remains a research issue. Finally, in the control group, men with the highest number of sexual partners contributed cytologic specimens less often than men with fewer sexual partners. We evaluated the impact of such "specimen contribution bias" on the magnitude of the association between penile HPV DNA and cervical cancer and found it to be minimal (*see* "Subjects and Methods" section for details).

In conclusion, in Spain, a country with a population at low risk for cervical cancer, the presence of HPV DNA in the external genitalia of men conveys a fivefold to ninefold increased risk of cervical cancer to their wives. The risk of cervical cancer increases with the number of the husbands' extramarital sexual contacts, notably contacts with prostitutes. The number of sexual partners before marriage may not be as relevant, suggesting that the HPV DNA carrier state in men may be of relatively short duration.

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Notes

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