A Cumulative Case-Control Study of Risk Factor Profiles for Oncogenic and Nononcogenic Cervical Human Papillomavirus Infections[1]

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Abstract

Human papillomaviruses (HPVs) play an essential role in the etiology of cervical cancer, but besides an established role for sexual transmission, little is known about other risk factors for HPV infection. Risk factors for nononcogenic, oncogenic, and HPV 16 cervical infections were investigated using a cumulative case-control approach nested in an ongoing cohort study of low income women from São Paulo, Brazil. HPV DNA was detected and typed by the MY09/11 PCR protocol. Risk factor information was obtained via interviews. In a case-control analysis, we compared women who harbored infections with exclusively nononcogenic types ($n = 123$), exclusively oncogenic types ($n = 94$), and any HPV 16 ($n = 60$) to women remaining HPV-negative ($n = 512$) throughout 1 year of follow-up. A strong negative association was found between age and oncogenic infections, but not with nononcogenic infections. Oral contraceptive use was strongly and exclusively associated with oncogenic and HPV 16 infections. Markers of sexual activity were associated with all types of infections, although with varying strengths. Our results suggest some important differences in the epidemiological correlates of HPV infection according to oncogenicity that may have implications for the planning of specific preventive strategies aiming at reduction of cervical cancer risk.

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Introduction

There is a vast body of literature from experimental and epidemiological studies documenting the etiological role of HPVs[3] in the development of cervical cancer (1). Cervical HPV infections are extremely common among women of reproductive age, but only a small proportion of these infections actually results in cytological abnormalities. To this date, our understanding of the transmission, persistence, and clearance of such infections remains limited.

Epidemiological studies have demonstrated that different HPV types convey varied levels of risk for cervical cancer (2, 3). Recent studies have suggested that the risk factor profile for HPV infection may vary according to oncogenic risk (4–6). Differing associations were found between the prevalence of HPV infection and markers of sexual activity after stratification between nononcogenic and oncogenic HPV types. However, there was limited consistency between the results of these studies. Detection of nononcogenic HPV types was either: (*a*) associated with markers of sexual activity only among younger women (4); (*b*) associated with recent sexual activity exclusively (5); or (*c*) not associated at all with sexual behavior (6). These results provide potentially important information on the natural history and transmission of HPV, but the inconsistencies suggest a need to further investigate the reported differences in risk factor profiles.

We investigated correlates and putative determinants of cervical HPV infection with nononcogenic and oncogenic HPV types in the Ludwig-McGill cohort study (7). We also present our first results on risk factors for infection with HPV 16, the most common type producing genital infections. Because most HPV infections are transient and because a woman could harbor multiple HPV types over time (8), making it difficult to study candidate determinants for grouped HPVs, we used more stringent definitions of controls and cases than did previously published studies. In this report, we compare women persistently HPV-negative to women who harbored exclusively oncogenic or nononcogenic HPV infections during follow-up.

Materials and Methods

Subjects. The study population consisted of a subset of participants in the Ludwig-McGill cohort study, a longitudinal investigation of the natural history of HPV infection and precursor lesions of cervical cancer involving repeated measurements of life-style, nutritional, and behavioral risk factors. The study design and methods have been described elsewhere (7). Participants were women attending a comprehensive maternal and child health program (Maternidade Escola Vila Nova Cachoeirinha) for low-income families in São Paulo, Brazil. Women were eligible to participate if they: (*a*) were aged

[3] The abbreviations used are: HPV, human papillomavirus; OR, odds ratio; CI, confidence interval; OC, oral contraceptive.

between 18 and 60 years; (*b*) were a permanent resident of São Paulo (city); (*c*) were not presently pregnant nor planning to become pregnant for a year; (*d*) had an intact uterus and no present referral for hysterectomy; (*e*) reported no use of vaginal medication in the previous 2 days; and (*f*) reported no treatment for cervical disease in the previous 6 months. In addition, women had to be interested in complying with the scheduled returns, at least for the subsequent 2 years.

The investigation began in November 1993 and is still ongoing. Follow-up consisted of a visit every 4 months for the first year and two visits per year for the next 4 years for a total follow-up of 5 years. Compliance with return visits was encouraged by giving meal tickets to compensate women for their time and transportation costs. An initial cash value of $5 was given at enrollment and increased by $5 at each return visit with a maximum of $20 at the fourth return. At each visit, subjects answered an interviewer-administered structured questionnaire, providing information on sociodemographic, lifestyle, sexual, reproductive, and contraceptive factors. Cervical specimens were also collected at each visit for Pap cytology and HPV testing.

HPV DNA Analysis. Samples of ectocervical and endocervical cells were collected with an Accelon biosampler (Medscand, Inc.) and immersed in a tube containing Tris-EDTA buffer at pH 7.4. DNA samples were purified by spin column chromatography and tested by the MY09/11 PCR protocol, which targets a conserved 450-bp region of the L1 gene (8, 9). Amplified products were hybridized with generic and type-specific oligonucleotide probes, allowing identification of 27 HPV types. If amplicons hybridized with the generic but with none of the type-specific probes, RFLP analyses of the amplified fragment were performed to extend the number of identifiable HPVs to >40 genital types (10). Amplified products that hybridized with the generic probe only were considered positive for unknown types. Integrity of the DNA specimens was verified by amplification of a 268-bp region of the human β-globin gene (9). All specimens were coded and tested without knowledge of the subjects. Standard precautions were taken to prevent specimen contamination.

HPV types were grouped according to their oncogenic potential. We used a modified version of the classification proposed by Bauer *et al.* (3). Oncogenic HPVs included types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 68. All other HPV types, including unknown types, were classified as nononcogenic.

Study Design. We report results based on a cumulative case-control analysis of a sample of subjects from the Ludwig-McGill cohort, *i.e.*, a subset of the first 1425 women who completed up to four visits and whose specimens have been tested for HPV DNA and resulted informative (*i.e.*, were β-globin positive). The cumulative case-control design has not been previously used to investigate determinants of HPV infection; most studies to date have used cross-sectional data for this purpose. In the cumulative case-control design, controls are defined as "residual noncases" or subjects who have not experienced the outcome at the end of the follow-up period. In this report, the control group included only women who remained persistently HPV-negative in all four specimens collected throughout the first year of follow-up. Women who tested positive for HPV DNA at least once were considered for inclusion as cases. Because most HPV infections are transient, women who had prevalent HPV infection at entry were included in the case groups, assuming that a very low proportion of them would be harboring persistent infections at baseline. Two nonoverlapping case groups were assembled by restricted selection from this group of HPV-positive women: (*a*) those who had only nononcogenic (low-risk) infections and (*b*) those who had only oncogenic (high-risk) infections. Cases of synchronous (same specimen) or metachronous (separate specimens) multiple infection with types that belonged to more than one group were excluded to minimize a possible dilution effect when comparing risk factor profiles. A third case group was not exclusive from the latter and included women who were positive for HPV 16 infection at least once, regardless of whether or not other HPV types were present.

Except for the HPV 16 case group, subjects with β-globin-negative specimens were excluded from the analysis. An average of 2.7% of the specimens per visit were β-globin-negative. A total of 126 subjects were excluded for this reason.

Statistical Analysis. Descriptive statistics were first carried out for the subcohort of 1425 women and for the control group ($n = 512$). The proportion of women HPV positive for any nononcogenic HPV type, any oncogenic HPV type, and for HPV 16 were calculated for all levels of the predictor variables. The OR was used to gauge the magnitude of the statistical association between each predictor variable and the risk of each of the three HPV infection outcomes using the case-control samples described above. Unconditional logistic regression was used to estimate the crude and adjusted ORs and their 95% CIs. Variables with Ps < 0.25 for the univariate models were selected as candidates for the multivariate models. Before using the continuous form of a variable, linearity of the logit was assessed graphically. Final models were determined by backward elimination of candidate variables based on the likelihood ratio tests and goodness-of-fit information. The SPSS v8.0 software was used for all statistical analyses.

Results

The 1425 participants that formed the sampling frame for this analysis represented mostly the subcohort of women admitted early in the study (a total of 2528 women were enrolled in the cohort with a 70% response rate). For these subjects, the total number of cervical specimens from enrollment and follow-up visits that were tested for HPV was 4873, with a mean of 3.4 specimens/subject and an average length of follow-up of 10.6 months. The mean age was 33.3 years (SD = 8.9; median = 33.0). Most of the women were White (66%), and only 17.3% had an education level above elementary school. Forty-four % had one lifetime sexual partner, 35% had two or three lifetime partners, and 21% of the women had four or more lifetime sexual partners. The average number of pregnancies reported was 3.6 (SD = 2.3; median = 3.0).

Fig. 1 shows the strategy for selecting the cases and controls included in the analysis of risk determinants. The control group included 512 women who tested consistently negative for HPV DNA throughout the first four visits, with a minimum of 12 months of follow-up. The 356 women who had harbored an HPV infection at least once either at enrollment or during follow-up constituted the potential case group. We then restricted this group to (*a*) 123 women whose cervical specimens had shown the presence of exclusively nononcogenic HPV types during the full first year of follow-up (on one or more occasions) and (*b*) 94 women who harbored only oncogenic HPV types for the same period (on one or more occasions). We also performed an analysis for women who had harbored HPV 16 at any point during follow-up ($n = 60$), among which 24 were also part of the exclusively oncogenic case group. Restricting the analysis to women who had tested

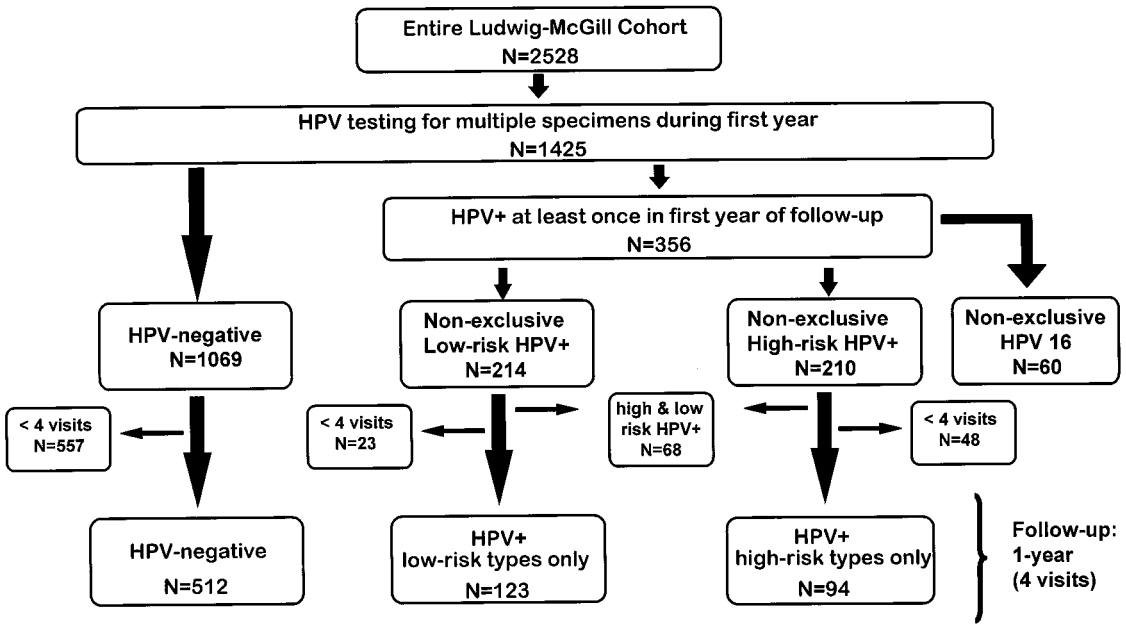

Fig. 1. Sampling scheme for the analysis of determinants of cumulative HPV history using information from up to four visits. Women who remained HPV-negative throughout the first year of follow-up were used as controls ($n = 512$). The three case groups included: (*a*) 123 women who tested positive exclusively for nononcogenic (low-risk) HPV types during the first year of follow-up; (*b*) 94 women who harbored exclusively oncogenic (high-risk) HPV types during the first year of follow-up; and (*c*) 60 women who harbored HPV 16 during follow-up (without excluding women who harbored other types).

positive only to HPV 16 (and no other type) during the first year of follow-up would have resulted in a case group too small ($n = 21$) for identification of risk determinants.

Table 1 shows the frequency distribution of selected explanatory variables in the original subcohort and in the final case and control groups. The percent HPV positivity in the initial subcohort is also shown for nononcogenic, oncogenic HPVs, and HPV 16, including women with multiple infections. HPV positivity for oncogenic types clearly decreased as age increased, ranging from 24.3% in the 18–24-year age group to 8.7% among women aged 45–60 years. By contrast, there was a slight decrease in HPV positivity for nononcogenic HPV types associated with age, which ranged from 18.6% to 14.0% when comparing women aged 18–24 years to those aged 45–60 years. The same pattern was seen with age at first intercourse for both oncogenic and nononcogenic HPV infections in the initial subcohort, an appreciable decline in HPV positivity for oncogenic types as age at first intercourse increased and a slight decrease for nononcogenic HPVs. The proportion of HPV-positive women increased with number of sexual partners for nononcogenic HPV types, oncogenic HPV types, and HPV 16. The rise in HPV positivity was stronger when considering more recent rather than lifetime number of sexual partners for all three categories of infection considered.

Table 2 shows crude and age-adjusted ORs of exclusive infection with nononcogenic and oncogenic viral types according to selected variables using the case-control sampling scheme that excludes infections with types from more than one group. Different patterns of associations emerged based on HPV oncogenicity. Age (P for trend = 0.61) and age at first intercourse were not associated with risk of nononcogenic HPV infections. However, the likelihood of oncogenic HPV infection decreased markedly with both age (P for trend = 0.0001) and age at first intercourse. Concordant with the lack of association between age and nononcogenic infections, the age-adjusted ORs showed little difference from the crude ones. On the other hand, confounding by age was present to varying degrees between predictor variables and oncogenic HPV infections. The age-adjusted associations between number of sexual partners and HPV infection were very similar for both nononcogenic and oncogenic infections. Women with a greater number of sexual partners were more likely to have HPV infections, regardless of oncogenicity-based grouping. Likelihood of infection was more closely influenced by recent history of sexual activity. Women who had taken OCs were more likely to have oncogenic HPV infections than never-users, whereas no association was found with nononcogenic infections. A similar pattern was seen with the number of pregnancies, although none of the 95% CIs excluded unity. Vaginal douching was protective for nononcogenic HPV infections, but was associated with a greater likelihood of oncogenic infection. Race and education were not associated with oncogenic HPV infections, whereas non-White women and women with a higher education level were more likely to have had nononcogenic infections during follow-up. Smoking, both former and present, was associated with an increased likelihood of all HPV infections.

We identified the predictors of HPV infection using multivariate logistic regression models. For nononcogenic infections, age was forced into the model because it has traditionally been considered as one of the classical risk factors. However, because it was not associated with risk of nononcogenic infections, removing it from the models did not change the estimates for the other variables remaining in the models. Table 3 lists the predictors of exclusive nononcogenic HPV infections. Practice of anal intercourse, vaginal douching, and number of previous Pap smears were inversely associated with risk, albeit nonsignificantly at the 5% level, whereas non-White ethnicity, more advanced schooling, more than one recent sexual partner (past

Table 1 Number of subjects in initial subcohort, HPV positivity, and distribution of case-control groups according to selected variables stratified by oncogenic risk

Variable	Subcohort	HPV positivity (%) in initial subcohort[a] (N = 1425)			No. subjects in case-control samples			
		Nononcogenic	Oncogenic	HPV 16	Control[b] (N = 512)	Exclusive nononcogenic[c] (N = 123)	Exclusive oncogenic[d] (N = 94)	HPV 16[e] (N = 60)
Age (yr)								
18–24	263	18.6	24.3	4.9	90	22	34	13
25–34	559	15.6	15.7	5.0	214	50	37	28
35–44	431	12.5	10.0	2.8	154	33	17	12
45–60	172	14.0	8.7	4.1	54	18	6	7
Race								
White	936	13.5	14.0	3.8	351	70	63	36
Non-White	487	18.1	16.2	4.9	161	53	31	24
Education								
Elementary	1178	13.8	14.6	4.4	424	91	75	52
High school	246	21.1	15.4	3.3	87	32	19	8
Income (US $/mo)								
<334	679	15.2	16.6	5.6	237	63	45	38
≥334	718	14.5	13.0	3.1	265	57	47	22
Age at first intercourse (yr)								
≤15	399	15.0	18.5	5.8	128	31	41	23
16–17	358	17.0	18.7	6.1	133	29	21	22
18–19	289	14.5	14.5	2.8	109	25	19	8
20–50	379	13.5	7.1	1.8	142	38	13	7
Lifetime sexual partners								
1	625	11.7	10.6	2.7	249	51	35	17
2–3	496	14.3	16.9	4.8	172	39	41	24
≥4	304	23.0	19.7	6.3	91	33	18	19
Sexual partners past 5 yr								
0–1	1110	11.9	10.9	3.1	437	88	69	34
2+	313	26.2	28.4	8.3	75	35	25	26
Sexual partners past yr								
0–1	1337	13.8	13.2	4.0	499	113	82	53
2+	67	40.3	46.3	9.0	11	8	10	6
Anal intercourse								
Never	888	15.5	13.7	3.7	311	83	53	33
Ever	537	14.2	16.4	5.0	201	40	41	27
Yr of OC use								
Never used	191	18.8	16.8	2.1	64	16	7	4
<6 years	710	15.4	14.9	3.9	261	66	56	28
≥6 years	423	12.1	14.4	5.7	149	34	29	24
Pregnancies								
0–1	217	18.4	20.3	4.6	79	19	18	10
2–3	619	14.4	13.2	3.1	232	58	41	19
4–5	331	13.3	14.8	4.8	115	23	19	16
6+	242	14.9	13.6	6.2	82	21	15	15
Previous Pap tests								
0–5	757	15.5	17.0	5.0	257	66	60	38
≥6	653	14.2	11.8	3.2	252	55	33	21
Vaginal douching								
Never/rarely	1287	15.3	14.5	4.0	462	118	83	52
Frequently	138	12.3	16.7	5.8	50	5	11	8
Hygienic tampons								
Never	1260	14.8	14.0	3.7	452	111	76	47
Ever	165	16.4	20.6	7.9	60	12	18	13
Smoking								
Never	701	13.4	13.6	4.3	280	48	42	30
Former	245	16.3	11.0	5.3	76	26	13	13
Present	479	16.7	18.4	3.5	156	49	39	17

[a] Percent positive by HPV infection group in initial subcohort, including women with multiple infections.
[b] Subjects who tested consistently negative in first four visits.
[c] Subjects who harbored exclusively nononcogenic HPV types in first four visits.
[d] Subjects who harbored exclusively oncogenic HPV types in first four visits.
[e] Subjects who harbored HPV 16 in any of first four visits with or without other HPV types.

5 years), and smoking were associated with increased risk. Overall, the magnitude of the associations seen in the multivariate model using extensive mutual adjustment did not differ substantially from the age-adjusted ones (Table 2).

Determinants of exclusive oncogenic HPV infections are shown in Table 4. Age, age at first intercourse, and number of previous smears were inversely associated with risk, whereas higher income, more than one recent partner (past year), and

Table 2 Crude and age-adjusted ORs of HPV infection for selected variables stratified by oncogenic risk

Variable	Nononcogenic HPV		Oncogenic HPV	
	Crude OR	Adjusted OR (95% CI)[a]	Crude OR	Adjusted OR (95% CI)[a]
Age (yr)				
18–24	1.00		1.00	
25–34	0.96		0.46	
35–44	0.88		0.29	
45–60	1.36		0.29	
Race				
White	1.00	1.00	1.00	1.00
Non-White	1.65	1.68 (1.12–2.52)	1.07	1.00 (0.62–1.61)
Education				
Elementary	1.00	1.00	1.00	1.00
High school	1.71	1.76 (1.10–2.82)	1.23	0.99 (0.58–1.76)
Income (per 100 US $/mo)	1.02	1.02 (0.98–1.06)	1.02	1.03 (0.99–1.07)
Age at first intercourse (yr)				
≤15 yr	1.00	1.00	1.00	1.00
16–17	0.90	0.90 (0.51–1.58)	0.49	0.49 (0.27–0.88)
18–19	0.95	0.94 (0.52–1.70)	0.54	0.59 (0.32–1.08)
20–50	1.10	1.09 (0.63–1.89)	0.29	0.38 (0.19–0.77)
Lifetime sexual partners				
1	1.00	1.00	1.00	1.00
2–3	1.11	1.11 (0.70–1.76)	1.70	1.72 (1.05–2.85)
≥4	1.77	1.77 (1.07–2.91)	1.41	1.61 (0.86–3.03)
Sexual partners past 5 yr				
0–1	1.00	1.00	1.00	1.00
2+	2.32	2.43 (1.52–3.90)	2.11	1.77 (1.04–3.03)
Sexual partners past yr				
0–1	1.00	1.00	1.00	1.00
2+	3.21	3.34 (1.30–8.55)	5.53	5.33 (2.14–13.28)
Anal intercourse				
Never	1.00	1.00	1.00	1.00
Ever	0.75	0.75 (0.49–1.14)	1.20	1.20 (0.76–1.89)
Total duration of OC use				
Never used	1.00	1.00	1.00	1.00
<6 yr	1.01	1.02 (0.55–1.87)	1.96	2.46 (1.04–5.85)
≥6 yr	0.91	0.88 (0.45–1.73)	1.78	3.36 (1.30–8.68)
Pregnancies				
0–1	1.00	1.00	1.00	1.00
2–3	1.04	1.01 (0.56–1.82)	0.78	1.08 (0.57–2.05)
4–5	0.83	0.79 (0.39–1.60)	0.73	1.22 (0.57–2.63)
6+	1.06	0.99 (0.47–2.11)	0.80	1.67 (0.72–3.90)
Previous Pap tests	0.98	0.97 (0.93–1.01)	0.92	0.95 (0.90–1.01)
Vaginal douching				
Never/rarely	1.00	1.00	1.00	1.00
Frequently	0.39	0.39 (0.15–0.99)	1.22	1.46 (0.72–2.98)
Use of hygienic tampons				
Never	1.00	1.00	1.00	1.00
Ever	0.81	0.82 (0.43–1.58)	1.78	1.64 (0.91–2.98)
Smoking				
Never	1.00	1.00	1.00	1.00
Former	1.99	2.00 (1.16–3.42)	1.14	1.36 (0.69–2.71)
Present	1.83	1.83 (1.17–2.85)	1.67	1.87 (1.15–3.06)

[a] ORs adjusted for age: as a continuous variable for nononcogenic infections and as a categorical variable as shown for oncogenic infections.

longer duration of OC use were strong predictors of increased risk. The association with OC use was substantially affected by the net confounding effect of the variables included in the model. It became one of the strongest predictors upon multivariate adjustment, although the crude estimates were not significantly different from unity.

We also studied risk factors for any infection with HPV 16. The crude age-adjusted and fully adjusted ORs for the association between the potential predictors and HPV 16 infection are presented in Table 5. The age-adjusted ORs were generally in the same direction and magnitude as those for the oncogenic case group. Upon multivariate adjustment, the risk of HPV 16 infection increased with number of sexual partners in the past 5 years, use of hygienic tampons, number of pregnancies, and OC use. Risk decreased with age and number of previous Pap tests.

Discussion

Our results suggest that risk factor profiles for HPV infection vary according to oncogenic risk. The most striking difference was observed with age. Age is recognized as one of the main

Table 3 Risk factors for exclusive nononcogenic HPV infections

Variables in final model	Contrast	OR (95% CI)[a]
Age	Each yearly increment	1.03 (1.00–1.06)
Race	Non-White *vs.* white	1.58 (1.03–2.42)
Education	High school *vs.* elementary	1.95 (1.19–3.20)
Sexual partners in past 5 yr	2+ *vs.* 0–1	2.64 (1.60–4.35)
Anal intercourse	Ever *vs.* never	0.66 (0.42–1.03)
Vaginal douching	Ever *vs.* never	0.42 (0.16–1.09)
Previous Pap tests	Each additional test	0.96 (0.91–1.00)
Smoking	Former *vs.* never	2.18 (1.24–3.86)
	Present *vs.* never	1.98 (1.24–3.17)

[a] ORs are mutually adjusted for all variables in the table.

Table 4 Risk factors for exclusive oncogenic HPV infections

Contrast in variables in final model	OR (95% CI)[a]
Age	
25–34 *vs.* 18–24	0.50 (0.26–0.95)
35–60 *vs.* 18–24	0.39 (0.18–0.86)
Income	
Each US $100/mo	1.04 (1.00–1.08)
Age at first intercourse	
16–17 *vs.* ≤15	0.47 (0.25–0.88)
18–19 *vs.* ≤15	0.69 (0.36–1.32)
20–50 *vs.* ≤15	0.36 (0.17–0.76)
Sexual partners in past yr	
2+ *vs.* 0–1	5.26 (1.94–14.31)
Previous Pap tests	
Each additional test	0.93 (0.87–0.98)
Total duration of OC use	
<6 yr *vs.* never	3.32 (1.23–8.99)
≥6 yr *vs.* never	4.55 (1.55–13.38)

[a] ORs are mutually adjusted for all variables in the table.

risk factors for HPV infection (3, 11, 12), older age being associated with decreased risk. We observed the expected protective association between age and oncogenic HPV infection. However, age was not associated with nononcogenic HPV infection. It is difficult at this point to speculate on the biological meaning of these results. They could reflect differences between oncogenic and nononcogenic types with respect to immunological response, transmission, or effect of other risk behaviors. Pragmatically, this observation supports the need for stratification based on oncogenicity when one studies the natural history and epidemiology of cervical HPV infection.

We also found minor differences in the strength of the association with markers of sexual activity according to oncogenicity. Two of the main sexual markers (age at first intercourse and number of sexual partners in the past year) were strong determinants of oncogenic HPV infections. Only the number of sexual partners in the past 5 years was associated with nononcogenic HPV infections and with a lower magnitude than for oncogenic infections. Although there may be a slight difference in the magnitude of risk and in the relevant period of sexual activity according to oncogenic risk, our results suggest that both oncogenic and nononcogenic HPV infections are associated with number of sexual partners, which is indicative of sexual transmission. It is highly plausible that the transmission of the virus, although mediated by the same route, also depends on cofactors that vary with oncogenicity.

OC use was associated both with oncogenic and HPV 16 infections, but not with infections by nononcogenic HPV types.

Table 5 ORs for the association between selected variables and cervical HPV 16 infection

Variable	Crude OR	Age-adjusted OR (95% CI)[a]	Multivariate OR (95% CI)[b]
Age (yr)			
18–24	1.00		
25–34	0.91		
35–44	0.54		
45–60	0.90		
Race			
White	1.00	1.00	
Non-White	1.45	1.39 (0.80–2.41)	
Education			
Elementary	1.00	1.00	
High school or more	0.75	0.65 (0.29–1.43)	
Income (per US $100/mo)	0.98	0.99 (0.93–1.05)	
Age at first intercourse			
≤15	1.00	1.00	
16–17	0.92	0.91 (0.48–1.72)	
18–19	0.41	0.43 (0.18–0.99)	
20–50	0.27	0.30 (0.12–0.74)	
Lifetime sexual partners			
1	1.00	1.00	
2–3	2.04	2.01 (1.05–3.87)	
≥4	3.06	3.13 (1.55–6.30)	
Sexual partners past 5 yr			
0–1	1.00	1.00	1.00
2+	4.46	4.19 (2.35–7.47)	5.12 (2.73–9.62)
Sexual partners in past yr			
0–1	1.00	1.00	
2+	5.14	4.94 (1.75–14.00)	
Anal intercourse			
Never	1.00	1.00	
Ever	1.27	1.22 (0.71–2.10)	
Total duration of OC use			
Never used	1.00	1.00	1.00
<6 yr	1.72	1.76 (0.59–5.23)	1.65 (0.53–5.10)
≥6 yr	2.58	3.45 (1.11–10.76)	4.20 (1.28–13.79)
No. of pregnancies	1.06	1.15 (1.03–1.30)	1.22 (1.07–1.39)
Previous Pap tests	0.93	0.94 (0.88–1.00)	0.92 (0.86–0.98)
Vaginal douching			
Never/rarely	1.00	1.00	
Frequently	1.42	1.57 (0.70–3.53)	
Use of hygienic tampons			
Never	1.00	1.00	1.00
Ever	2.08	1.92 (0.98–3.79)	2.42 (1.16–5.07)
Smoking			
Never	1.00	1.00	
Former	1.60	1.66 (0.82–3.36)	
Present	1.02	1.04 (0.55–1.95)	

[a] Age used as a continuous variable.
[b] ORs mutually adjusted for all variables retained in full model, including age (continuous).

Previous studies have suggested a moderate increase in overall HPV infection prevalence among OC users (11, 13) and in HPV infection prevalence for both oncogenic and nononcogenic types (5). To our knowledge, no study to date has shown different associations between HPV infection and OC use according to viral oncogenicity. However, it is plausible that a hormonally induced change in immunity against HPV or increased expression of the virus genome could differ according to oncogenic risk. Moreover, it has been proposed that OCs might promote the carcinogenic properties of HPV (14). These combined results suggest that OCs could be an important co-

factor to consider in the cascade of events leading to carcinogenesis.

Prior studies on oncogenic and nononcogenic HPVs have suggested that the risk factors for infection may differ according to viral oncogenicity. Our results, just as the ones from these previous studies, suggest that markers of sexual activity are strongly associated with oncogenic HPV infections (4–6). The associations that we observed for age (inverse), number of sexual partners, and duration of OC use with oncogenic HPV infections are consistent with those from the Danish study (5). Published results on determinants of nononcogenic HPV infections are less consistent. Prevalence of nononcogenic HPV types was: (*a*) associated with sexual activity only in younger women (4); (*b*) associated only with recent sexual activity (5); or (*c*) not associated with sexual behavior (6). Our results suggest that nononcogenic infections, similarly to oncogenic ones, are associated with markers of sexual activity, only with a lower magnitude. Interestingly, in our study, age was not associated with nononcogenic HPV infection, corroborating the results of Kjaer *et al.* (5).

We presented here our first results from analyses of risk factors for HPV 16 cervical infections. Their interpretation is limited by the relatively small sample size ($n = 60$) and the resulting impossibility to restrict the case group to women who harbored exclusively HPV 16. The risk factors observed were similar, although not exactly the same as the ones found for oncogenic HPV infection. We repeated the analysis for oncogenic HPV infection after excluding the 24 women with HPV 16 to make sure that the profile observed for all oncogenic types combined was not entirely attributable to subjects with HPV 16. Very similar results were obtained after such restriction (data not shown), which confirmed that these determinants are also associated with oncogenic types other than HPV 16. The predictors found in both oncogenic and HPV 16 determinant profiles were age, number of sexual partners (although with a different reference period: past 5 years for HPV 16 and past year for oncogenic HPV types), previous Pap tests, and duration of OC use. Use of hygienic tampons and total number of pregnancies were two factors also associated with a greater risk of HPV 16 infection. Although the results for use of tampons are difficult to interpret, the increased likelihood of HPV 16 infection with the number of pregnancies is consistent with the association observed with OC use if we hypothesize that hormone-related immune factors are at play.

Our analyses included women who were HPV-positive at entry (prevalent infections). This could have led to an overrepresentation of longer duration (persistent) infections. However, because the majority of HPV infections are transient, this is very unlikely. The analyses were repeated after exclusion of women with prevalent infections at the baseline visit with no substantial change in the results (data not shown) except for decreased precision attributable to the smaller sample size. It is possible that the selection of subjects with longer follow-up times and with exclusively nononcogenic and oncogenic HPV infection may have led to some selection bias. However, there is no indication that losses to follow-up in the original study may have occurred differentially in HPV-negative and -positive women.

In this study, we introduced a new approach to investigate determinants of HPV infection that takes into account the cumulative HPV infection history over a defined period of time. One of the main advantages of using this method over a typical cross-sectional or case-control analysis is that it allows greater confidence in ascertaining the status of HPV infection for a given woman on the basis of persistence and oncogenicity. By taking into account a full year of follow-up (four visits) in determining HPV status, we were able to obtain more stringent definitions of cases and controls, which presumably may have helped us to better differentiate between profiles of risk factors on the basis of oncogenicity grouping. Considering the fact that HPV DNA detection is known to be highly transient, more confidence can be placed in a control group defined on the basis of four successively negative HPV tests rather than of only one. An additional advantage is the use of the same control group for analyses of oncogenic, nononcogenic, and HPV 16 infection, especially when one wants to compare the resulting determinant profiles. The main disadvantage of such a cumulative approach is that restrictions lead necessarily to smaller sample sizes, decreasing the precision of the estimates of association. However, this method seems to be the most suitable to disentangle the risk factors associated with the two groups of HPV infection, as judged from the appreciable differences between epidemiological profiles that were revealed.

In summary, we observed differences in determinant profiles according to oncogenic risk of the HPV infection. The main difference resided in the absence of association between age and nononcogenic HPV infection, whereas a strong inverse association was seen with oncogenic infections. More generally, the risk factor profile for oncogenic HPV infection was characterized by strong predictors, such as age, sexual markers, and hormonal contraception. In addition to an association with number of sexual partners and anal intercourse, the risk factor profile for nononcogenic HPV infection also included sociodemographic and hygiene-related variables. Our results suggest different risk factor profiles based on HPV oncogenicity, underscoring the importance of studying oncogenic and nononcogenic HPV infections separately for the purpose of better tailoring prevention strategies.

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