Relationship Between Cigarette Smoking and Human Papilloma Virus Types 16 and 18 DNA Load

Long Fu Xi,[1,2] Laura A. Koutsky,[2] Philip E. Castle,[3] Zoe R. Edelstein,[2] Craig Meyers,[4] Jesse Ho,[1] and Mark Schiffman[3]

[1]Department of Pathology, School of Medicine, and [2]Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington; [3]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; and [4]Department of Microbiology and Immunology, The Pennsylvania State College of Medicine, Hershey, Pennsylvania

Abstract

Background: **Although cigarette smoking has been associated with increased human papilloma virus (HPV) detection, its impact on HPV DNA load is unknown.**
Methods: **The study subjects were women who were positive for HPV16 and/or HPV18 at enrollment into the Atypical Squamous Cells of Undetermined Significance–Low-grade Squamous Intraepithelial Lesion Triage Study. Assessments of exposure to smoke and sexual behavior were based on self-report. Viral genome copies per nanogram of cellular DNA were measured by multiplex real-time PCR. Linear or logistic regression models were used to assess the relationship between cigarette smoking and baseline viral load.**
Results: **Of the 1,050 women (752 with HPV16, 258 with HPV18, and 40 with both HPV16 and HPV18), 452 (43.0%) were current smokers and 101 (9.6%) were former smokers at enrollment. The baseline viral load was statistically significantly greater for current compared with never smokers (P = 0.03 for HPV16; P = 0.02 for HPV18) but not for former smokers. Among current smokers, neither HPV16 nor HPV18 DNA load seemed to vary appreciably by age at smoking initiation, smoking intensity, or smoking duration. The results remained similar when the analysis of smoking-related HPV16 DNA load was restricted to women without detectable cervical abnormality.**
Conclusion: **Higher baseline HPV16 and HPV18 DNA load was associated with status as a current but not former smoker. A lack of dose-response relationship between cigarette smoking and viral load may indicate a low threshold for the effect of smoking on HPV DNA load.** (Cancer Epidemiol Biomarkers Prev 2009;18(12):3490–6)

Introduction

Cigarette smoking has been found to increase the risk of cervical cancer and its immediate precursor, cervical intraepithelial neoplasia grade 3, among women infected with oncogenic human papilloma virus (HPV) compared with women who do not smoke (1-9). A population-based case-control study in Sweden has shown a synergistic effect between cigarette smoking, and both HPV16 positivity and HPV16 DNA load on the development of cervical carcinoma *in situ* (10). It is generally thought that in addition to the carcinogenic effects of cigarette smoke on cervical tissue, such as genotoxic DNA adducts (11) and the presence of mutagens in cervical mucus (12), the excess risk may be mediated by its effects on the immunologic control of HPV infections (13).

Studies of the impact of cigarette smoking on the early natural history of HPV infections have focused on the humoral immune response to HPV (14, 15), and on the prevalence (16-21), incidence (22-26), and persistence of HPV infections (27-33). Most previous studies showed some degree of association between exposure to cigarette smoke and these measurable events in the natural course of HPV infections, although it might be that women who smoke have sexual behaviors that increase the risk of infection. Population data on the effects of cigarette smoking on viral load, an important attribute of HPV infections, have been so far unavailable. A recent *in vitro* study by Alam et al. (34) showed that exposure of cervical cells to benzo(*a*)pyrene, a major carcinogen in cigarette smoke, stimulated higher levels of virion synthesis in HPV-infected cell lines. Because benzo(*a*)pyrene can be detected in the cervical mucus of smokers (35), cigarette smoke could increase the viral load of HPV infections.

We therefore evaluated the relationship between cigarette smoking, and HPV16 and HPV18 DNA load among women infected with at least one of these two important genotypes, using the baseline data from women who participated in the Atypical Squamous Cells of Undetermined Significance–Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS). The ALTS was a 2-year multicenter randomized clinical trial that was designed to evaluate strategies for triaging women with mildly abnormal Pap smears.

Materials and Methods

Study Subjects. The subjects eligible for the present study were ALTS participants who had HPV16 and/or

Received 7/31/09; revised 8/24/09; accepted 9/18/09; published online 12/3/09.
Grant support: Public Health Service grant CA 84396 (L.F. Xi).
Note: This study was part of the project ancillary to the ALTS clinical trial but does not represent the ALTS Group.
Requests for reprints: Long Fu Xi, Suite 510, Northgate Executive Center 1 Building B, 155 NE 100th Street, Seattle, WA 98125. Phone: 206-616-9787; Fax: 206-616-9788. E-mail: longfu@u.washington.edu

doi:10.1158/1055-9965.EPI-09-0763

HPV18 DNA detected in their enrollment cervical samples (baseline infections) by PCR-based reverse line blot assay (36, 37). All ALTS participants had a referral Pap smear interpretation of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesions. At enrollment, women underwent the same pelvic examination, with collection of cervical specimens for cytology and HPV testing. Information on demographics, sexual behavior, lifestyle, and medical history was collected by personal interview at enrollment and during 2-year follow-up. A detailed description of the ALTS design and the characteristics of the participants are available elsewhere (38, 39). The data and cervical specimens used in this study were those collected at trial enrollment.

In total, 1,071 women met the eligibility criteria, including 759 with baseline HPV16 infections, 258 with baseline HPV18 infections, and 54 with both HPV16 and 18 types (813 with HPV16 and 312 with HPV18). We excluded the following women: (*a*) 19 (11 positive for HPV16 and 8 positive for HPV18) whose enrollment samples were unavailable for viral DNA quantification, (*b*) 1 whose enrollment sample was positive for HPV18 but negative for cellular DNA by quantitative assay, and (*c*) 15 (10 positive for HPV16 and 5 positive for HPV18) who self-reported as current ($n = 13$) or former smokers ($n = 2$) but had provided no information on number of cigarettes per day. As a result, there were 792 women (97.4%) with baseline HPV16 infections and 298 women (95.5%) with baseline HPV18 infections in the analysis (40 with both types). The protocol for this study was approved by the Institutional Review Boards at the University of Washington and the National Cancer Institute.

Assessment of Exposure to Cigarettes. Smoking status at enrollment and sexual behavior were ascertained based on self-reported data from the enrollment questionnaire. Women who had ever smoked cigarettes on a regular basis for ≥6 mo by the time of enrollment were categorized as ever smokers and those otherwise as never smokers. Among ever smokers, status as a current or former smoker was self-defined by ALTS participants. No information was collected from former smokers about the time of smoking cessation. The intensity and duration of cigarette smoking were dichotomized according to self-reported age at smoking initiation (<16 versus ≥16 y), number of years smoked (<6 versus ≥6 y), and number of cigarettes per day (<20 versus ≥20 cigarettes/d). The number of pack-years of exposure (<4 versus ≥4 pack-years) that takes into account both the intensity and duration of smoking was estimated by the number of years smoked multiplied by the number of cigarettes per day and then divided by 20. Additionally, we subcategorized the number of cigarettes per day, stratifying by the number of years smoked. The lifetime number of male sex partners and number of male sex partners in the year before study entry were categorized as 0 to 4, 5 to 9, or ≥10, and 0 to 1 versus ≥2, respectively.

Quantification of HPV16 and HPV18 DNA Load. Cervical swab samples preserved in specimen transport medium (Digene Corporation, Silver Spring, MD) were obtained from the NIH biorepository through the ALTS committee. DNAs were extracted from an aliquot of 100 μL specimen transport medium samples with the use of a procedure described previously (40). The DNA extracts were suspended in 30 μL TE (10 mmol/L Tris, pH 8.0; 1 mmol/L EDTA) and stored at −20°C until the assay. These samples were initially tested for characterization of HPV16 and HPV18 variants. The remainder was used for viral load quantification.

The number of HPV16 and HPV18 E7 copies, and the amount of cellular DNA in the enrollment cervical swab samples were measured by multiplex real-time PCR, as described previously (41, 42). Each sample was assayed in triplicate on the same plate. The viral load in each of the triplicate measurements was individually normalized to the input amount of cellular DNA and expressed as E7 copy number/1 ng of cellular DNA.

DNA extracts underwent a thaw-freeze step at least once before the measurement of viral load. Some underwent the process ≥3 times if they were positive for both HPV16 and HPV18, or were repeatedly tested for quality control. To examine whether the estimate of viral load would be affected by the limited number of thaw-freeze steps, we compared the amount of cellular DNA between samples positive for both HPV16 and HPV18, and those positive for HPV16 or HPV18 alone, and between initial tests and retests. No meaningful difference was observed (data not shown).

Viral E7 DNA was undetectable by real-time PCR in 61 samples previously positive for HPV16 in the reverse line blot assay (33 from never smokers, 25 from current smokers, and 3 from former smokers) and 21 samples previously positive for HPV18 (11 from never smokers, 8 from current smokers, and 2 from former smokers). It is possible that the amount of viral DNA in these samples might have been too small to be detected. We therefore assigned a value of 1 viral copy/1 ng of cellular DNA to each of these samples for analyses. Considering that the negative result could also be caused by factors such as deletion or integration, we did parallel analyses with these samples excluded. The results were similar; for simplicity, they were not presented.

Statistical Analyses. The normalized baseline HPV16 and HPV18 DNA load was individually $\log_{10}$ transformed; the mean value of the triplicate measurements was used for analyses. We used Student's *t* test to compare viral load by race (White versus non-White), current use of oral contraceptives (yes or no), number of male sex partners in the past year, parity (nulliparous versus parous), number of Pap tests per year in the last 5 y (<1 versus ≥1 tests/y), referral Pap (atypical squamous cells of undetermined significance versus low-grade squamous intraepithelial lesions), HPV variant (European versus non-European), and coinfection with other types (yes or no). Differences in baseline viral load by age at enrollment (18-19, 20-24, 25-29, or ≥30 y) and lifetime number of male sex partners were tested by one-way ANOVA. The proportions of having ≥2 recent male sex partners by smoking status were compared by χ^2 test. Statistical tests were two-sided at the 5% significance level.

Linear regression models (43) were used to examine the relationship between baseline viral load and self-reported smoking status at enrollment (current versus noncurrent, and former or current versus never). The models were adjusted for age at enrollment, race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types. The linear regression models were also used to compare baseline viral load by a variety of

measures of smoking intensity and smoking duration. Each measure of cigarette smoking was modeled independently of one another because of their strong correlation with each other.

To explore whether or not the relationship of interest would be altered by other factors, we evaluated the association of baseline viral load with smoking status overall, and separately by race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types with the use of polynomial logistic regression (44). Odds ratios (OR) and 95% confidence intervals (95% CI) for the overall association were adjusted for age at enrollment, race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types. In this model, the viral load, a continuous variable, was treated as a covariate. Thus, the magnitude of OR indicated risk of having a status of current or former smoker as per 1 $\log_{10}$ unit change of baseline viral load. We noted that most study subjects had a cytologic diagnosis of atypical squamous cells of undetermined significance or worse, and/or a histologic diagnosis of cervical intraepithelial neoplasia grade 1 or worse at enrollment. To avoid potential disturbance by these concurrent cervical abnormalities of the association of baseline viral load with cigarette smoking, the analysis of baseline HPV16 DNA load was repeated, restricted to women without any detectable cervical lesion.

Results

Of the 1,050 ALTS participants (752 with HPV16, 258 with HPV18, and 40 with both HPV16 and HPV18) included in this study, 452 (43.0%) were current smokers by self-report, and 101 (9.6%) were former smokers at enrollment. Of the 452 current smokers, 39.8% began smoking before 16 years of age, 55.8% smoked ≥6 years, 38.1% smoked ≥20 cigarettes/d, and 48.5% reported ≥4 pack-years. The mean values of the $\log_{10}$-transformed HPV16 E7 copy number/1 ng of cellular DNA were 2.65 (95% CI, 2.50-2.79), 2.87 (95% CI, 2.62-3.11), and 2.92 (95% CI, 2.78-3.06) for never, former, and current smokers, respectively. The corresponding values for women with baseline HPV18 infections were 3.73 (95% CI, 3.49-3.98), 3.25 (95% CI, 2.38-4.13), and 4.13 (95% CI, 3.82-4.43), respectively. Among never smokers, baseline HPV16 DNA load was related to race, parity, and referral Pap; baseline HPV18 DNA load was marginally related to coinfection with other HPV types (Table 1).

Table 1. Baseline HPV16 and HPV18 DNA load by characteristics among never smokers

Characteristic	$\log_{10}$ HPV16 E7 copies/1 ng of cellular DNA			$\log_{10}$ HPV18 E7 copies/1 ng of cellular DNA		
	No.	Mean (SD)	*P*	No.	Mean (SD)	*P*
Age at study entry (y)						
18-19	54	2.92 (1.23)	0.19	19	3.31 (1.89)	0.67
20-24	172	2.61 (1.35)		81	3.77 (1.48)	
25-29	79	2.72 (1.30)		28	3.83 (1.47)	
≥30	48	2.35 (1.60)		33	3.80 (1.78)	
Race*						
White	201	2.77 (1.28)	0.05	72	3.55 (1.61)	0.18
Non-White	151	2.48 (1.46)		89	3.89 (1.56)	
Current use of oral contraceptives						
No	171	2.57 (1.44)	0.33	91	3.88 (1.64)	0.17
Yes	182	2.71 (1.29)		70	3.54 (1.50)	
Lifetime no. of male sex partners†						
0-4	155	2.63 (1.42)	0.94	79	3.70 (1.45)	0.90
5-9	119	2.56 (1.36)		46	3.73 (1.69)	
≥10	75	2.83 (1.22)		35	3.85 (1.78)	
No. of male sex partners in the past year‡						
0-1	237	2.64 (1.40)	0.96	104	3.68 (1.70)	0.53
≥2	116	2.65 (1.29)		56	3.85 (1.38)	
Parity						
Nulliparous	179	2.82 (1.27)	0.01	64	3.76 (1.45)	0.88
Parous	174	2.47 (1.43)		97	3.72 (1.67)	
No. of Pap tests/y in the last 5 y§						
<1	214	2.68 (1.40)	0.67	70	3.69 (1.53)	0.76
≥1	138	2.61 (1.29)		91	3.77 (1.63)	
Referral Pap						
ASC-US	224	2.53 (1.44)	0.03	74	3.52 (1.76)	0.12
LSIL	129	2.85 (1.18)		87	3.91 (1.41)	
Coinfection with other HPV types						
No	92	2.75 (1.28)	0.41	41	4.11 (1.52)	0.08
Yes	261	2.61 (1.39)		120	3.60 (1.59)	
HPV variant‖						
European	250	2.85 (1.26)	0.58	50	4.27 (1.29)	0.20
Non-European	77	2.76 (1.04)		86	3.95 (1.41)	

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion.
*Excluded was one HPV16-positive woman who did not provide race information. The category non-White includes African-American, American-Indian/Alaskan, or Asian/Pacific Islander women.
†Excluded were four HPV16-positive women and one HPV18-positive woman who did not provide information on number of lifetime male sex partners.
‡Excluded was one HPV18-positive woman who, at enrollment, did not provide information on number of male sex partners in the past year.
§Excluded was one HPV16-positive woman who did not provide information on Pap history. The category <1 Pap test includes women who had no Pap test in the past 5 y (n = 13 for those positive for HPV16; n = 6 for those positive for HPV18).
‖Excluded were 23 HPV16-positive women and 25 HPV18-positive women whose enrollment samples were insufficient for variant characterization.

Table 2. Correlation between baseline HPV16 and HPV18 DNA load and self-reported smoking status

	Log_{10} HPV16 E7 copies/1 ng of cellular DNA				Log_{10} HPV18 E7 copies/1 ng of cellular DNA			
	No.	Mean (SD)	*P*	Adjusted *P*-value*	No.	Mean (SD)	*P*	Adjusted *P*-value*
Current smoker								
No	438	2.69 (1.32)			179	3.69 (1.60)		
Yes	354	2.92 (1.34)	0.01	0.04	119	4.13 (1.68)	0.02	0.005
Smoking history								
Never	353	2.65 (1.36)			161	3.73 (1.58)		
Former	85	2.87 (1.15)	0.17	0.35	18	3.25 (1.76)	0.24	0.23
Current	354	2.92 (1.34)	0.005	0.03	119	4.13 (1.68)	0.05	0.02

*Adjusted for age at enrollment, race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types.

As shown in Table 2, after adjustment for age at enrollment, race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types, baseline HPV16 DNA load was statistically significantly higher among current smokers ($P = 0.03$) but not former smokers ($P = 0.35$) compared with women who never smoked. Likewise, HPV18 DNA load was statistically significantly higher among current smokers ($P = 0.02$) but not former smokers ($P = 0.23$) compared with women who never smoked. The proportion of having ≥2 male sex partners in the past year was highest among current smokers and lowest among never smokers ($P = 0.001$). Additional adjustment for the number of recent male sex partners did not appreciably alter the relationship between baseline viral load and smoking status (data not shown).

To determine whether there was a dose-response relationship between viral load and cigarette smoking, the smoking status was subclassified according to age at smoking initiation, number of years smoked, number of cigarettes per day, number of cigarette pack-years, and the combination of the years smoked and cigarettes per day. Among current smokers, neither HPV16 nor HPV18 DNA load seemed to vary appreciably by age at smoking initiation, smoking intensity, or smoking duration (Table 3). The results remained the same when similar analyses were done among former smokers (data not shown).

Overall, status as a current smoker at enrollment was statistically significantly associated with baseline viral load (HPV16: $OR_{adjusted}$, 1.15; 95% CI, 1.02-1.30; HPV18: $OR_{adjusted}$, 1.23; 95% CI, 1.04-1.45). To examine whether other determinants played a role in the association of interest, data were stratified according to race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types. As shown in Table 4, although the associations with current smoking were not statistically significant in all subgroups, a trend of positive association was the same across the strata. We did not observe an association between viral load and status as a former smoker compared with never smokers overall or in similar stratified analyses (data not shown).

A total of 667 (84.2%) of the 792 women with baseline HPV16 infections and 246 (82.6%) of the 298 women with baseline HPV18 infections had a cytologic interpretation of atypical squamous cells of undetermined significance or worse, and/or a histologic diagnosis of cervical intraepithelial neoplasia grade 1 or worse at enrollment. To eliminate a potential influence of these concurrent cervical abnormalities, the analysis of smoking-related HPV16 DNA load was restricted to women without any cytologic or histologic abnormality at enrollment. The association between status as a current smoker and baseline HPV16 DNA load remained statistically significant ($OR_{adjusted}$, 1.57; 95% CI, 1.07-2.33). Consistent with the results derived from the overall dataset, no appreciable difference was seen when smoking status was stratified by age at smoking initiation, smoking intensity, or smoking duration (data not shown). We did not do a similar analysis for HPV18 because of the small number of infections.

Discussion

In this study of women with prevalent HPV16 and/or HPV18 infections at the time of enrollment into the ALTS, we found that baseline viral load was statistically significantly higher among current smokers than among never smokers. As shown by an analysis of women with no detectable cytologic and histologic abnormality at enrollment, the association between cigarette smoking and baseline viral load did not seem to be mediated by underlying cervical lesion. In addition, the association was not explained by race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types. Concerns remain that the correlation between baseline viral load and cigarette smoking might exist only among certain subgroups and, thus, adjustment for these factors might not adequately rule out confounding effects. We therefore stratified data by these factors individually. Failure to detect effect modifiers suggests that the association observed was independent of these factors. Further, because the samples used for viral load measurement were collected before biopsy or therapy, the association was not distorted by any of these procedures. In the analyses of the effects of other determinants on baseline viral load in the entire study population, we found that none of those listed in Table 1, except for referral Pap and coinfection with other HPV types, was significantly associated with both HPV16 and HPV18 DNA load (data not shown). The coinfection-related reduction of baseline viral load was reported previously (45).

Before interpreting our results, certain limitations to the present study should be considered. Only samples that tested positive for HPV16 and/or HPV18 by PCR-based reverse line blot assay were used for viral DNA quantification. Thus, samples that contained viral DNA below the detectable threshold were not included in this analysis. If this had occurred more frequently among current smokers than among never smokers, the association between viral load and current smoker status could have been overestimated. It is noteworthy, however, that among women with HPV16 infections, the proportion of samples

that tested positive in PCR-based reverse line blot assay but negative in real-time PCR (presuming the presence of a tiny amount of viral DNA) was 7.1% for current smokers and 9.3% for never smokers. Among women with HPV18 infections, the proportions were similar between current and never smokers (6.7% versus 6.8%). In this study, exposure to cigarette smoke was based on participants' self-reported information. Thus, recall bias could be a concern. However, there was no reason to suspect that women with high, compared with low, levels of baseline viral DNA would be more likely to report inaccurate smoking histories. The interview was conducted before HPV testing; HPV infections are asymptomatic. Data from a meta-analysis (46) indicated that, compared with biochemical assessments of smoking, self-reports of smoking were accurate in most studies. Information on time of smoking cessation was not collected in the ALTS. Thus, we were unable to evaluate its effects on HPV DNA load.

We are aware that although additional adjustment for the number of recent male sex partners did not appreciably alter the relationship between baseline viral load and smoking status, residual confounding effects may remain. Smoking is closely related to sexual behavior. It is possible that the smoking behavior of sex partners, in addition to the number of sex partners, may affect the assessment of risk association. For example, there is no doubt that the extent of passive exposure to smoke is related to the extent a partner smokes. Information on partners' behavior was not collected in the ALTS.

Published data about the relationship between cigarette smoking and HPV DNA load are not available for comparison. Nevertheless, our results are generally consistent with those of other studies showing that cigarette smoking is associated with HPV prevalence (16-19), incidence (22, 23), and persistence (27, 28). Previous studies have shown that the cervical mucus of smokers contains measurable amounts of cigarette constituents and their metabolites, such as benzo(*a*)pyrene (35), nicotine, and nicotine-derived nitrosamine 4-(methylnitrosamino)-I-(3-pyridyl)-1-butanone (47). One possible mechanism for the association between cigarette smoking and HPV DNA load is that smoking may increase cell proliferation and turnover in the transformation zone of the cervix. For example, smoking has been associated with Ki-67 staining, a marker for proliferation and metaplasia (9). HPV uses the host cell DNA replication machinery for its own replication. It is possible that a smoking-related increase in host cell proliferation leads to an increase in replication of HPV16 and HPV18 DNA, and/or production of infectious virus. Another possible mechanism is that smoking may increase viral load by weakening the cellular immune response. This is supported by studies showing that smoking has adverse effects on both systemic and local immunity (48-52). For example, smoking has been correlated with impaired cytotoxic activity of natural killer cells in peripheral blood, and unbalanced systemic production of proinflammatory and anti-inflammatory cytokines (50). In addition, smoking has been associated with a substantial decrease in Langerhans cells (51, 52) and helper/inducer T lymphocytes (52) in cervical epithelium. It is possible that the insufficient availability of these cells may cause a decrease in T-cell response to HPV infections; consequently, the infected cells are less likely to be eliminated, leading to an increase in HPV DNA load.

We found no evidence that baseline viral load was related to long duration, early initiation, and greater intensity of smoking. The credibility of the association between smoking and viral load might be called into question by this absence of a dose-response relationship. A noncausal interpretation is that the results might be confounded by some unmeasured factors, such as passive smoking, tobacco composition, and individual differences in metabolism. It is also possible that smoking might be a marker of recent sexual behavior, which is related to recency of infection and, consequently, the viral load. However, it is more likely that the absence of a dose-response relationship might be due to a low threshold for the effect of smoke on replication of viral genome copies. Support for this comes from recent molecular evidence (34) that links the dosage-related effects of a cigarette carcinogen

Table 3. Correlation between baseline HPV16 and HPV18 DNA load and various measures of cigarette smoking among current smokers

Duration and intensity of smoking	Log_{10} HPV16 E7 copies/1 ng of cellular DNA				Log_{10} HPV18 E7 copies/1 ng of cellular DNA			
	No.	Mean (SD)	*P*	Adjusted *P*-value*	No.	Mean (SD)	*P*	Adjusted *P*-value*
Age of smoking initiation, y								
<16	141	2.87 (1.21)			48	4.34 (1.51)		
≥16	213	2.96 (1.42)	0.57	0.57	71	3.98 (1.78)	0.25	0.31
No. of years smoked								
<6	155	3.07 (1.40)			51	3.86 (1.61)		
≥6	199	2.81 (1.29)	0.07	0.56	68	4.32 (1.72)	0.14	0.09
No. of cigarettes/d								
<20	212	2.88 (1.36)			79	4.15 (1.66)		
≥20	142	2.98 (1.32)	0.50	0.30	40	4.08 (1.74)	0.84	0.66
No. cigarette pack-years								
<4	181	3.01 (1.35)			63	3.95 (1.78)		
≥4	173	2.83 (1.34)	0.21	0.95	56	4.32 (1.55)	0.23	0.13
No. of cigarettes/d, smoked <6 y								
<20	113	3.02 (1.40)			37	3.74 (1.57)		
≥20	42	3.20 (1.42)	0.50	0.79	14	4.18 (1.74)	0.39	0.35
No. of cigarettes/d, smoked ≥6 y								
<20	99	2.73 (1.30)			42	4.51 (1.67)		
≥20	100	2.89 (1.27)	0.36	0.13	26	4.03 (1.77)	0.27	0.19

*Adjusted for age at enrollment, race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types.

Table 4. Associations of baseline HPV16 and HPV18 DNA load with status as a current smoker, stratified by race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types

Characteristic	Log_{10} HPV16 E7 copies/1 ng of cellular DNA					Log_{10} HPV18 E7 copies/1 ng of cellular DNA				
	Never smokers		Current smokers		OR (95% CI)	Never smokers		Current smokers		OR (95% CI)
	No.	Mean (SD)	No.	Mean (SD)		No.	Mean (SD)	No.	Mean (SD)	
Overall	353	2.65 (1.36)	354	2.92 (1.34)	1.15 (1.02-1.30)*	161	3.73 (1.58)	119	4.13 (1.68)	1.23 (1.04-1.45)*
Race†										
White	201	2.77 (1.28)	300	2.92 (1.34)	1.10 (0.96-1.26)	72	3.55 (1.61)	90	4.15 (1.66)	1.26 (1.03-1.53)
Non-White	151	2.48 (1.46)	51	2.96 (1.33)	1.29 (1.02-1.65)	89	3.89 (1.56)	28	4.05 (1.82)	1.07 (0.82-1.40)
Parity										
Nulliparous	179	2.82 (1.27)	157	3.15 (1.23)	1.24 (1.04-1.47)	64	3.76 (1.45)	59	4.37 (1.44)	1.35 (1.04-1.75)
Parous	174	2.47 (1.43)	197	2.75 (1.41)	1.16 (1.00-1.34)	97	3.72 (1.67)	60	3.88 (1.87)	1.06 (0.88-1.27)
Referral Pap										
ASC-US	224	2.53 (1.44)	205	2.82 (1.31)	1.17 (1.02-1.35)	74	3.52 (1.76)	64	3.82 (1.83)	1.10 (0.91-1.33)
LSIL	129	2.85 (1.18)	149	3.07 (1.37)	1.15 (0.95-1.38)	87	3.91 (1.41)	55	4.48 (1.42)	1.35 (1.04-1.74)
Current use of oral contraceptives										
No	171	2.57 (1.44)	191	2.94 (1.41)	1.21 (1.04-1.41)	91	3.88 (1.64)	63	4.11 (1.87)	1.08 (0.89-1.30)
Yes	182	2.71 (1.29)	159	2.91 (1.26)	1.13 (0.95-1.34)	70	3.54 (1.50)	54	4.14 (1.45)	1.34 (1.03-1.73)
Coinfection with other HPV types										
No	92	2.75 (1.28)	106	3.11 (1.33)	1.24 (1.00-1.55)	41	4.11 (1.52)	28	4.98 (1.56)	1.47 (1.03-2.09)
Yes	261	2.61 (1.39)	248	2.85 (1.34)	1.14 (1.00-1.30)	120	3.60 (1.59)	91	3.86 (1.64)	1.11 (0.93-1.32)

*Adjusted for age at enrollment, race, parity, referral Pap, current use of oral contraceptives, and coinfection with other HPV types.
†Excluded were four HPV16-positive women and one HPV18-positive woman who did not provide race information.

to different stages of the HPV life cycle in a raft culture system; i.e., treatment with low concentrations of benzo (*a*)pyrene (0.001 μmol/L) resulted in the highest magnitude of viral genome copies, whereas viral titers (a measure of infectious viral particles) remained the same as that of the control. Conversely, treatment with high concentrations of benzo(*a*)pyrene (1 μmol/L) significantly promoted virion synthesis but not viral genome amplification. Whereas the increased genome amplification results in more templates from which the viral oncogene transcripts are produced, the increased virion synthesis favors the establishment of a persistent infection by increasing the chances of infecting additional sites. Data on concentrations of benzo(*a*)pyrene in the cervical mucus of smokers have not been available. If the relationship between smoking and viral load mimics the benzo(*a*)pyrene-mediated regulation of the HPV life cycle, the finding of the absence of a dose-response relationship would not be surprising, because increasingly heavy exposures to smoke might favor the production of viral progeny rather than an incremental increase in replication of genome copies.

We noted that baseline HPV16 and HPV18 DNA load did not differ meaningfully between former and never smokers. This agrees with the findings of an association between HPV infection and current, but not former, smoking (16-18). The absence of an association between baseline viral load and former smoker status could be due to a limited number of former smokers. Alternatively, it may suggest that concurrent colocalization of the virus and a smoking-related carcinogen at the cervix is a prerequisite for the effect of cigarette smoking on HPV DNA load. If the latter hypothesis is correct, smoking cessation may help with reduction of viral load among women with HPV infections.

In summary, we observed that in a population of women referred for a minor cytologic abnormality, higher HPV16 and HPV18 DNA load was associated with status as a current but not former smoker. Among current smokers, the viral load did not seem to vary appreciably with the intensity and duration of cigarette smoking. This lack of dose-response relationship may suggest a low threshold for the effect of smoke on HPV DNA load. Data from this study, with viral load as an outcome, and those from others (22, 23, 27, 28), with outcomes of incident and persistent HPV infections, indicate that cigarette smoking affects the early natural history of HPV infections.

Disclosure of Potential Conflicts of Interest

L.A. Koutsky: Commercial Research Grant, Merck Research Laboratories. C. Meyers: supported by the Pennsylvania Department of Health, Health Research Formula Funding program as a part of the Pennsylvania Tobacco Settlement Legislation and by Philip Morris USA Inc., and Philip Morris International.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank the ALTS Group for providing the biological specimens and HPV typing results, and Dr. Kathrin Jansen for providing the HPV16 and HPV18 DNA standard.

References

1. Castle PE, Wacholder S, Lorincz AT, et al. A prospective study of high-grade cervical neoplasia risk among human papillomavirus-infected women. J Natl Cancer Inst 2002;94:1406–14.
2. Tolstrup J, Munk C, Thomsen BL, et al. The role of smoking and alcohol intake in the development of high-grade squamous intraepithelial lesions among high-risk HPV-positive women. Acta Obstet Gynecol Scand 2006;85:1114–9.
3. Deacon JM, Evans CD, Yule R, et al. Sexual behaviour and smoking as determinants of cervical HPV infection and of CIN3 among those infected: a case-control study nested within the Manchester cohort. Br J Cancer 2000;83:1565–72.
4. McIntyre-Seltman K, Castle PE, Guido R, Schiffman M, Wheeler CM. Smoking is a risk factor for cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. Cancer Epidemiol Biomarkers Prev 2005;14:1165–70.
5. Castellsague X, Munoz N. Chapter 3: cofactors in human papillomavirus carcinogenesis-role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 2003:20–8.

6. Plummer M, Herrero R, Franceschi S, et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case-control study. Cancer Causes Control 2003;14:805–14.
7. Rajeevan MS, Swan DC, Nisenbaum R, et al. Epidemiologic and viral factors associated with cervical neoplasia in HPV-16-positive women. Int J Cancer 2005;115:114–20.
8. Ylitalo N, Sorensen P, Josefsson A, et al. Smoking and oral contraceptives as risk factors for cervical carcinoma *in situ*. Int J Cancer 1999;81:357–65.
9. Harris TG, Kulasingam SL, Kiviat NB, et al. Cigarette smoking, oncogenic human papillomavirus, Ki-67 antigen, and cervical intraepithelial neoplasia. Am J Epidemiol 2004;159:834–42.
10. Gunnell AS, Tran TN, Torrang A, et al. Synergy between cigarette smoking and human papillomavirus type 16 in cervical cancer *in situ* development. Cancer Epidemiol Biomarkers Prev 2006;15:2141–7.
11. Phillips DH, She MN. DNA adducts in cervical tissue of smokers and non-smokers. Mutat Res 1994;313:277–84.
12. Holly EA, Petrakis NL, Friend NF, Sarles DL, Lee RE, Flander LB. Mutagenic mucus in the cervix of smokers. J Natl Cancer Inst 1986;76: 983–6.
13. Szarewski A, Maddox P, Royston P, et al. The effect of stopping smoking on cervical Langerhans' cells and lymphocytes. BJOG 2001;108: 295–303.
14. Wiley DJ, Wiesmeier E, Masongsong E, et al. Smokers at higher risk for undetected antibody for oncogenic human papillomavirus type 16 infection. Cancer Epidemiol Biomarkers Prev 2006;15:915–20.
15. Simen-Kapeu A, Kataja V, Yliskoski M, et al. Smoking impairs human papillomavirus (HPV) type 16 and 18 capsids antibody response following natural HPV infection. Scand J Infect Dis 2008;40:745–51.
16. Vaccarella S, Herrero R, Snijders PJ, et al. Smoking and human papillomavirus infection: pooled analysis of the International Agency for Research on Cancer HPV Prevalence Surveys. Int J Epidemiol 2008; 37:536–46.
17. Sellors JW, Mahony JB, Kaczorowski J, et al. Prevalence and predictors of human papillomavirus infection in women in Ontario, Canada. Survey of HPV in Ontario Women (SHOW) Group. CMAJ 2000;163:503–8.
18. Syrjanen K, Shabalova I, Petrovichev N, et al. Smoking is an independent risk factor for oncogenic human papillomavirus (HPV) infections but not for high-grade CIN. Eur J Epidemiol 2007;22:723–35.
19. Bauer HM, Hildesheim A, Schiffman MH, et al. Determinants of genital human papillomavirus infection in low-risk women in Portland, Oregon. Sex Transm Dis 1993;20:274–8.
20. Kjaer SK, van den Brule AJ, Bock JE, et al. Determinants for genital human papillomavirus (HPV) infection in 1000 randomly chosen young Danish women with normal Pap smear: are there different risk profiles for oncogenic and nononcogenic HPV types? Cancer Epidemiol Biomarkers Prev 1997;6:799–805.
21. Herrero R, Castle PE, Schiffman M, et al. Epidemiologic profile of type-specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. J Infect Dis 2005;191:1796–807.
22. Minkoff H, Feldman JG, Strickler HD, et al. Relationship between smoking and human papillomavirus infections in HIV-infected and -uninfected women. J Infect Dis 2004;189:1821–8.
23. Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218–26.
24. Moscicki AB, Hills N, Shiboski S, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA 2001;285:2995–3002.
25. Munoz N, Mendez F, Posso H, et al. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. J Infect Dis 2004;190:2077–87.
26. Sellors JW, Karwalajtys TL, Kaczorowski J, et al. Incidence, clearance and predictors of human papillomavirus infection in women. CMAJ 2003;168:421–5.
27. Koshiol J, Schroeder J, Jamieson DJ, et al. Smoking and time to clearance of human papillomavirus infection in HIV-seropositive and HIV-seronegative women. Am J Epidemiol 2006;164:176–83.
28. Giuliano AR, Sedjo RL, Roe DJ, et al. Clearance of oncogenic human papillomavirus (HPV) infection: effect of smoking (United States). Cancer Causes Control 2002;13:839–46.
29. Richardson H, Abrahamowicz M, Tellier PP, et al. Modifiable risk factors associated with clearance of type-specific cervical human papillomavirus infections in a cohort of university students. Cancer Epidemiol Biomarkers Prev 2005;14:1149–56.
30. Hildesheim A, Schiffman MH, Gravitt PE, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994;169:235–40.
31. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423–8.
32. Molano M, Van den Brule A, Plummer M, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. Am J Epidemiol 2003;158:486–94.
33. Kjaer SK, Munk C, Winther JF, Jorgensen HO, Meijer CJ, van den Brule AJ. Acquisition and persistence of human papillomavirus infection in younger men: a prospective follow-up study among Danish soldiers. Cancer Epidemiol Biomarkers Prev 2005;14:1528–33.
34. Alam S, Conway MJ, Chen HS, Meyers C. The cigarette smoke carcinogen benzo[a]pyrene enhances human papillomavirus synthesis. J Virol 2008;82:1053–8.
35. Melikian AA, Sun P, Prokopczyk B, et al. Identification of benzo[a]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. Cancer Lett 1999;146:127–34.
36. Gravitt PE, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000;38:357–61.
37. Peyton CL, Gravitt PE, Hunt WC, et al. Determinants of genital human papillomavirus detection in a US population. J Infect Dis 2001; 183:1554–64.
38. Schiffman M, Adrianza ME. ASCUS-LSIL Triage Study. Design, methods and characteristics of trial participants. Acta Cytol 2000;44:726–42.
39. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 2003;188:1383–92.
40. Xi LF, Kiviat NB, Hildesheim A, et al. Human papillomavirus type 16 and 18 variants: race-related distribution and persistence. J Natl Cancer Inst 2006;98:1045–52.
41. Xi LF, Kiviat NB, Galloway DA, Zhou XH, Ho J, Koutsky LA. Effect of cervical cytologic status on the association between human papillomavirus type 16 DNA load and the risk of cervical intraepithelial neoplasia grade 3. J Infect Dis 2008;198:324–31.
42. Xi LF, Koutsky LA, Castle PE, et al. Human papillomavirus type 18 DNA load and 2-year cumulative diagnoses of cervical intraepithelial neoplasia grades 2-3. J Natl Cancer Inst 2009;101:153–61.
43. Slinker BK, Glantz SA. Multiple linear regression is a useful alternative to traditional analyses of variance. Am J Physiol 1988;255: R353–67.
44. Ananth CV, Kleinbaum DG. Regression models for ordinal responses: a review of methods and applications. Int J Epidemiol 1997;26:1323–33.
45. Xi LF, Edelstein ZR, Meyers C, Ho J, Cherne SL, Schiffman M. Human papillomavirus types 16 and 18 DNA load in relation to coexistence of other types, particularly those in the same species. Cancer Epidemiol Biomarkers Prev 2009;18:2507–12.
46. Patrick DL, Cheadle A, Thompson DC, Diehr P, Koepsell T, Kinne S. The validity of self-reported smoking: a review and meta-analysis. Am J Public Health 1994;84:1086–93.
47. Prokopczyk B, Cox JE, Hoffmann D, Waggoner SE. Identification of tobacco-specific carcinogen in the cervical mucus of smokers and nonsmokers. J Natl Cancer Inst 1997;89:868–73.
48. Kalra R, Singh SP, Savage SM, Finch GL, Sopori ML. Effects of cigarette smoke on immune response: chronic exposure to cigarette smoke impairs antigen-mediated signaling in T cells and depletes IP3-sensitive Ca(2+) stores. J Pharmacol Exp Ther 2000;293: 166–71.
49. Sopori M. Effects of cigarette smoke on the immune system. Nat Rev Immunol 2002;2:372–7.
50. Zeidel A, Beilin B, Yardeni I, Mayburd E, Smirnov G, Bessler H. Immune response in asymptomatic smokers. Acta Anaesthesiol Scand 2002;46:959–64.
51. Barton SE, Maddox PH, Jenkins D, Edwards R, Cuzick J, Singer A. Effect of cigarette smoking on cervical epithelial immunity: a mechanism for neoplastic change? Lancet 1988;2:652–4.
52. Poppe WA, Ide PS, Drijkoningen MP, Lauweryns JM, Van Assche FA. Tobacco smoking impairs the local immunosurveillance in the uterine cervix. An immunohistochemical study. Gynecol Obstet Invest 1995; 39:34–8.