Epidemiology of Human Papillomavirus Infection and Abnormal Cytologic Test Results in an Urban Adolescent Population

Trudee A. Tarkowski,[1,a] Emilia H. Koumans,[2] Mary Sawyer,[3] Antonya Pierce,[2] Carolyn M. Black,[1] John R. Papp,[1] Lauri Markowitz,[2] and Elizabeth R. Unger[1]

[1]National Center for Infectious Diseases and [2]National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention, Public Health Service, US Department of Health and Human Services, and [3]Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia

We determined the prevalence of and the risk factors for human papillomavirus (HPV) infection and abnormal cytologic test results in 312 adolescent girls (mean age, 16.1 years). Subjects had a median of 2 years of sexual activity and 4 lifetime sex partners. Cervical HPV was detected by use of L1-consensus polymerase chain reaction in 64% of subjects; half of those with HPV had >1 type, and 77% had ⩾1 high-risk type. Independent risk factors for HPV were lifetime number of sex partners, age of partner, and douching. Cytologic abnormalities were common (20.9% of subjects had atypical squamous cells of uncertain significance, and 17.0% had high- or low-grade squamous intraepithelial lesions) and were significantly associated with detection of HPV ($P = .0001$); however, most (51.6%) subjects with HPV had normal cytologic test results.

Adolescents' risk-taking behavior places them at high risk for all sexually transmitted infections, including human papillomavirus (HPV). Adolescent girls are a unique and important group for investigating factors that determine the outcome of exposure to HPV. Even though exposure may occur at a young age, cervical cancer and high-grade cervical dyplasias are diseases of older women. The risk of persistence or reappearance of HPV and neoplastic progression may be influenced by behavioral or environmental factors at the time of initial exposure, as well as by the status of mucosal maturity and humoral/mucosal immunity. We studied a high-risk, urban adolescent population near the time of their first sexual intercourse to obtain baseline information about prevalence of HPV infection and cervical disease and to evaluate risk factors for detection of HPV.

Subjects, materials, and methods. Subjects for this study were recruited from patients attending an adolescent clinic at a public pediatric hospital in Atlanta, Georgia. Nonpregnant, human immunodeficiency virus–negative, sexually active females, 12–19 years old, were eligible to participate if a pelvic exam had not been performed within the last 6 months. Those who had received antibiotics in the previous month were also excluded. Enrollment was offered to 80%–90% of eligible subjects, and >85% of them consented and had complete data available. Data for this analysis were from subjects enrolled between January 1999 and January 2001. The present study followed human-experimentation guidelines of the US Department of Health and Human Services. All subjects were volunteers who gave informed consent or assent with parental consent. The study was reviewed and approved by the Institutional Review Boards at the Centers for Disease Control and Prevention and Emory University.

A questionnaire was administered in private by one of several trained interviewers, to obtain data on demographics, sexual and reproductive history, peer norms, attitudes toward and actual condom use, self-esteem, mental health, drug and alcohol use, and characteristics of sex partners. Endo- and ectocervical cells were collected during the pelvic examination, placed in PreservCyt fixative (20 mL; Cytyc), and transported to the hospital cytopathology laboratory for preparation and interpretation of ThinPrep Pap smear (ThinPrep supplies were a gift from Cytyc). The cytologic diagnosis, which was based on the 1991 Bethesda system, was recorded from the resulting clinical report.

Residual PreservCyt cervical material was retrieved from the cytology laboratory. A 3-mL aliquot of resuspended cells was washed with 5 mL of Dulbecco's PBS (Gibco BRL) and was extracted with the Total Nucleic Acid (TNA) Masterpure Extraction kit (Epicentre) by use of minor modifications to the manufacturer's protocol [1]. To monitor cross-sample contamination, 1 blank tube was included for every 10 samples. The TNA was resuspended in 21 μL of dimethyl pyrocarbonate–treated water and was stored at −80°C until amplification.

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[a] Present affiliation: Cytyc Corporation, Marlborough, Massachusetts.

Reprints or correspondence: Dr. Elizabeth R. Unger, Centers for Disease Control and Prevention, 1600 Clifton Rd., MSG41, Atlanta, GA 30333 (eunger@cdc.gov).

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Detection of HPV was performed by use of L1-consensus polymerase chain reaction (PCR) with biotinylated PGMY09/11 primers, followed by typing in the Roche line blot assay (gift from Roche Molecular Systems) [2, 3]. The assay followed the manufacturer's guidelines, with the exception that Platinum *Taq* was substituted for *Taq*Gold. Five microliters of TNA was used in the 100-μL PCR. In addition to the negative controls from the DNA isolation step, an additional negative control (water) was added for each PCR set up, to monitor potential PCR contamination. An aliquot (10 μL) of each PCR product was also evaluated by agarose-gel electrophoresis with ethidium-bromide staining, to visualize globin and HPV amplimers. Samples with a gel-detected HPV product that did not hybridize to 1 of the 27 HPV types on the Roche line blot were sequenced to determine HPV type [4].

Positive HPV results were classified into nonoverlapping categories of single (1 type detected) versus multiple (>1 type detected), or high risk (⩾1 high-risk type) versus low risk (only low-risk type[s] detected, no high-risk types). The cytologic test results were classified as normal (diagnoses of within normal limits or benign cellular changes), atypical squamous cells of uncertain significance (ASCUS), or high- or low-grade squamous intraepithelial lesions (SILs). No glandular lesions (atypical glandular cells of uncertain significance) or carcinomas were identified.

The demographic and behavioral characteristics of subjects with HPV were compared with those of subjects without HPV, in univariate analyses, and variables with $P < .1$ were used in backward stepwise regression to arrive at a final multivariate model. We tested for the presence of interactions between variables with $P < .1$, using maximum likelihood estimates [5]. χ^2 tests were used to compare categorical variables, and *t* tests were used to compare continuous variables. Odds ratios (ORs), 95% confidence intervals (CIs), and 2-sided *P* values were calculated by use of SAS software (version 6.12; SAS Institute).

Results. The mean age of the 312 subjects was 16.1 years (range, 12.8–19.9 years); 298 (95.5%) were African American, and 38 (12%) had a child. Subjects had a median of 2 years of sexual activity (range, 0–16 years) and a median of 4 lifetime sex partners (range, 1–50 partners). Previous sexual abuse was reported by 74 (24%), first occurring at a median age of 10 years (range, 2–17 years); 191 (61%) had ever douched, and 80 (26%) used some form of hormonal contraception. Indicators of risky behaviors were frequent: 97 (31%) smoked marijuana during the last 90 days, 49 (16%) smoked tobacco, 42 (13%) had been in juvenile detention, and 25 (8%) had been in jail.

HPV was detected in 200 (64%) of the 312 subjects. Factors significantly associated with the detection of HPV in univariate analysis (table 1) included increases in lifetime number of sex partners, number of sex partners during the last 90 days, difference in age of sex partners (mean difference in age between the subject and all partners during the last 90 days, maximum difference in age between the subject and any partner during the last 90 days, or any partner during the last 90 days who was >20 years old), years of sexual activity, number of sex acts during the last 90 days, as well as a history of sexual abuse, past or current smoking tobacco, smoking marijuana, and douching during the last 90 days (measured as "ever" or frequency).

In multivariate analysis, only lifetime number of sex partners, mean difference in age between the subject and sex partner, and douching were significant risk factors for detection of HPV (table 1). Douching during the last 90 days and a difference in age between the subject and sex partner of >1.5 years were both associated with a 2-fold increase in risk of detection of HPV. Different multivariate models using other measures of age of the sex partner or douching gave similar results (data not shown). There were no significant interactions between any of these variables.

Detection of multiple HPV types, as well as of high-risk HPV, was very common. Of those subjects with HPV, 100 (50%) had ⩾2 different types detected (range, 2–6 types), and 154 (77%) had at least 1 high-risk type detected. Although HPV 16 was the most prevalent (10.2%) type, there was broad representation of most genital HPV types in this group of young women (table 2).

Cytologic test results were available for 305 (97.7%) of the 312 subjects. Some degree of cytologic abnormality was common (20.9% of subjects had ASCUS, and 17.0% had SILs) and was significantly associated with HPV (HPV was detected in 52.3% of subjects with normal Pap smear results, in 71.9% with ASCUS, and in 90.4% with SILs; $P = .001$). However, slightly more than half (51.6%) of HPV-positive subjects had a normal cytologic test, and 5% of HPV-negative subjects had SILs.

Compared with HPV-negative subjects (and excluding those with ASCUS), HPV-positive subjects had an increased risk of SILs: the OR was 8.3 (95% CI, 3.1–21.7). We then calculated the risk of SILs, by HPV risk type and by number of types. Subjects with high-risk type(s) were 1.6 times ($P = .33$) more likely to have SILs than those with low-risk type(s) only (OR, 9.0 [95% CI, 3.4–24.1] vs. 5.5 [95% CI, 1.6–19.0]). Subjects with multiple types were 2.7 times ($P = .007$) more likely to have SILs than those with a single type (OR, 13.5 [95% CI, 4.9–37] vs. 4.7 [95% CI, 1.6–13.6]).

Discussion. Multiple studies have found that HPV is the most prevalent sexually transmitted infection in adolescents. The observed prevalence of HPV varies by the population studied, the type of sample collected, and the assay used. The 64% prevalence in this study is quite high but is within the range reported in other studies of sexually active, immunocompetent, urban adolescents that used PCR detection in cervicovaginal samples (54% [6] and 90% [7]). The risk factors for HPV

Table 1. Univariate and multivariate analysis of risk factors for detection of human papillomavirus (HPV) in an urban adolescent population ($n = 312$).

Characteristic	No.	HPV positive, no. (%)	Univariate analysis		Multivariate analysis
			OR (95% CI)	*P*	OR (95% CI)
Age group, years					
<15	110	60 (55)	Reference	…	
15	53	36 (68)	1.7 (0.9–3.5)	.11	
16	100	70 (70)	1.9 (1.1–3.4)	.02	
⩾17	49	34 (69)	1.9 (0.9–3.9)	.08	
Lifetime sex partners					
1	47	16 (34)	Reference	…	
2–3	87	51 (59)	3.0 (1.5–6.1)	.0027	2.8 (1.3–5.8)
4–7	111	111 (73)	5.7 (2.8–11.7)	.0001	3.9 (1.8–8.2)
⩾8	61	61 (84)	10.8 (4.4–26.3)	.0001	7.4 (2.9–18.5)
Sex partners during last 90 days					
0	30	11 (37)	Reference	…	
1	163	102 (63)	2.9 (1.2–6.5)	.01	
⩾2	119	87 (73)	4.7 (2.0–10.9)	.0003	
Age difference, mean, years[a]					
⩽1.5	126	63 (50)	Reference	…	
>1.5	186	137 (74)	2.8 (1.7–4.5)	.0001	2.1 (1.2–3.6)
Age difference, maximum, year(s)[a]					
⩽1	138	74 (54)	Reference	…	
1–3	77	53 (69)	1.9 (1.1–3.4)	.01	
>3	97	73 (75)	2.6 (1.5–4.7)	.0009	
Sex partner >20 years old					
No	188	115 (61)	Reference	…	
Yes	93	73 (78)	2.3 (1.3–4.1)	.004	
Age at first sexual intercourse, years					
<14	130	84 (65)	1.5 (0.8–2.9)	.21	
14	84	56 (67)	1.7 (0.8–3.4)	.16	
15	45	31 (69)	1.8 (0.8–4.2)	.15	
⩾16	53	29 (55)	Reference	…	
Sexual activity, years					
⩽1.5	123	66 (54)	Reference	…	
1.6–2.5	62	42 (68)	1.8 (1.0–3.4)	.07	
>2.5	127	92 (72)	2.3 (1.3–3.8)	.002	
First sexual intercourse					
Before menarche	85	59 (69)	1.8 (1.0–3.4)	.05	
⩽2 years after menarche	131	88 (67)	1.7 (1.0–2.9)	.07	
>2 years after menarche	96	53 (55)	Reference	…	
Sexual intercourse during last 90 days, times					
<7	151	88 (58)	Reference	…	
⩾7	161	112 (70)	1.6 (1.0–2.6)	.04	
Sexual abuse					
No	238	142 (60)	Reference	…	
Yes	74	58 (78)	2.5 (1.3–4.5)	.004	

(continued)

Table 1. ***(Continued.)***

Characteristic	No.	HPV positive, no. (%)	Univariate analysis		Multivariate analysis
			OR (95% CI)	*P*	OR (95% CI)
Marijuana use (ever)					
No	214	127 (59)	Reference	…	
Yes	97	72 (74)	1.9 (1.2–3.4)	.01	
Cigarette use (ever)					
No	174	101 (58)	Reference	…	
Yes	138	99 (72)	1.8 (1.1–3.0)	.01	
Douching during last 90 days					
No	182	99 (54)	Reference	…	
Yes	129	100 (78)	2.6 (1.6–4.2)	.0001	2.1 (1.2–3.6)
Douching frequency during last 90 days, times					
Never	118	59 (50)	Reference	…	
0	66	36 (62)	1.6 (0.9–3.1)	.13	
1 or 2	65	47 (72)	2.6 (1.4–5.0)	.004	
⩾3	62	52 (84)	5.2 (2.4–11.2)	.0001	

NOTE. CI, confidence interval; OR, odds ratio.

[a] Between subject and sex partner(s).

infection in this urban adolescent population were lifetime number of sex partners, increasing number of sex partners during the last 90 days, increasing average difference in age between the subject and partner(s), increasing years of sexual activity, higher number of sex acts during the last 90 days, history of sexual abuse, past or current smoking tobacco, smoking marijuana, and douching during the last 90 days. However, in the multivariate analysis, only lifetime number of sex partners, mean difference in age between the subject and sex partner(s), and douching were significant risk factors for detection of HPV. The association between HPV and lifetime number of sex partners has been a consistent observation in previous studies. The difference in age between the subject and sex partner is most likely an indirect measure of the sexual experience of the partner and the increased likelihood that older partners are infected with HPV from previous exposures.

Douching has not been previously reported as a risk factor for detection of HPV, although it has been found to be associated with cervical cancer [8] and other sexually transmitted diseases [9–13]. The high frequency of douching in this population (54% of subjects had douched during the last 90 days), combined with the use of trained interviewers with pretested questionnaires for accurate ascertainment of douching behaviors, may have allowed us to detect this association. The reasons for the association between douching and detection of HPV are not clear. Increased detection of HPV may reflect increased susceptibility to infection because of alterations in the vaginal pH, microflora, or cervical mucous. However, even in the absence of infection, douche-induced cervical friability could increase the cellular yield of scrapes and indirectly increase detection of HPV. Further study is needed to determine whether douching is associated with detection of HPV in other populations, the relation of douching to persistence of HPV, and whether douching products differ in their effects on detection and persistence of HPV. More than 95% of subjects in the present study who douched used an over-the-counter commercial product (data not shown).

There was a broad range of HPV types detected in this population. Overall, high-risk types were more prevalent than low-risk types. HPV 16 was the most prevalent type, detected in 10.6% of samples. Relatively few subjects had types other than the 27 included in the line blot assay, a finding that is in agreement with those of a previous study concluding that the line blot assay covers HPV types most often detected in US populations [14]. Half of all subjects with HPV detected in the genital tract had >1 type present, with a range of 2–6 types.

Although cytologic abnormalities were common in this population, nearly all were low-grade changes that are more likely to be a reflection of cytopathic effect of HPV than of neoplastic progression. As expected, cytologic abnormalities were associated with detection of HPV. Of note, however, most subjects (51.6%) with detectable HPV did not have an abnormal cytologic test result. This has been observed before and indicates that HPV testing would not be effective as a primary screen for cervical neoplasia in this population. The risk of SILs was similar for high-risk and low-risk HPV types. However, this

Table 2. Prevalence of individual human papillomavirus (HPV) types in an urban adolescent population ($n = 312$).

HPV type	No. (%) of subjects[a]
High-risk types	
HPV 16	32 (10.2)
HPV 59	28 (8.9)
HPV 52	24 (7.7)
HPV 35	22 (7.0)
HPV 58	22 (7.0)
HPV 39	18 (5.8)
HPV 18	16 (5.1)
HPV 68	15 (4.8)
HPV 31	14 (4.5)
HPV 83	14 (4.5)
HPV 82	13 (4.2)
HPV 45	10 (3.2)
HPV 51	7 (2.2)
HPV 55	7 (2.2)
HPV 73	7 (2.2)
HPV 33	5 (1.6)
HPV 56	3 (1.0)
HPV 26	2 (0.6)
Low-risk types	
HPV 66	25 (8.0)
HPV 6	24 (7.7)
HPV 84	19 (6.1)
HPV 53	14 (4.5)
HPV 42	12 (3.8)
HPV 54	12 (3.8)
HPV 40	10 (3.2)
HPV 11	2 (0.6)
HPV 57	1 (0.3)
Sequenced types	
HPV 70	3 (1.0)
DL 416	2 (0.6)
HPV 61	1 (0.3)
HPV 69	1 (0.3)

[a] The column totals and percentages do not add up to 312 (100%), because subjects could have 0–7 types detected.

risk was significantly higher for subjects with multiple HPV types, compared with those with a single HPV type. At least 1 previous study found multiple HPV infections to be a risk factor for dysplasia [15].

The cross-sectional study design does not allow differentiation between detection of transient and persistent HPV. Almost all HPV infections resolve and result in no clinical disease, particularly in young populations. Understanding factors that differentiate infection from neoplastic progression will require longitudinal studies of persistence of HPV and of cytologic abnormalities in different populations.

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