Factors Associated With Human Papillomavirus Infection in Women Encountered in Community-Based Offices

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Objective: To assess risk factors for cervical human papillomavirus (HPV) infection in women presenting to community-based offices because of vaginal symptoms or for preventive screening.

Design: Cross-sectional analysis of history, physical examination, and microbiological infection variables.

Setting: Two community-based family practice offices in southeastern Michigan.

Patients: Two hundred seventy-three women, 18 to 50 years of age, presenting to the study sites because of vaginal symptoms or for a pelvic examination for preventive screening.

Main Outcome Measure: Human papillomavirus infection of the uterine cervix as determined by polymerase chain reaction testing.

Results: Human papillomavirus infection was detected in 21.2% of the women (24.9% and 13.1% of women with and without vaginal symptoms, respectively); 34% of these infections were HPV types 16 or 18. Fifty-four percent of the women with HPV infection who underwent colposcopy had condyloma or cervical intraepithelial neoplasia verified on biopsy. Independent associations were found between HPV infection and the following female risk factors: the presence of vaginal itching, odor, or swelling; knowing the current sexual partner less than 24 months; age less than 40 years; household income of $14 000 or less; and ever having had six or more sexual partners.

Conclusions: In addition to three previously described risk factors for genital HPV infection, two previously unrecognized risk factors were identified in this lower-risk population. These risk factors included the presence of vaginal symptoms of itching, odor, or swelling and having known the current sexual partner less than 24 months. Nevertheless, using risk factors alone, two thirds of the women infected with HPV in this population were not identified as being at high risk of infection. No subset of sexually active women was identified who were at no risk of HPV infection.

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INVASIVE CERVICAL cancer occurs in approximately 13 000 women in the United States each year, leading to 7000 deaths.[1] The risk of premalignant changes in the cervix is many times greater. Although the incidence of invasive cervical cancer has decreased over the past 40 years, evidence suggests that the risk is increasing for women under 50 years of age.[2-6] Risk factors identified with sexual activity have been associated with cervical cancer; however, only recently have data implicated specific types of the human papillomavirus (HPV) as probable causative agents for cervical neoplasia.[7] When polymerase chain reaction (PCR) methods are used for diagnosis, the prevalence of this infection has ranged from 46% to 53% in high-risk populations[8,9] to approximately 22% in lower-risk groups.[10] Because HPV infection of the cervix has not been associated with identifying symptoms and because cervical lesions are often inapparent, most infected women are unaware of their infection status and of their increased risk for cervical abnormalities and carcinoma.

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PATIENTS AND METHODS

PATIENT ENROLLMENT

Our cross-sectional study is part of the University of Michigan (Ann Arbor) Family Practice HPV Study. The study included patients enrolled between March 1, 1990, and June 1, 1992, in an ongoing prospective evaluation of patients, with and without vaginal symptoms, in two primary care offices in the Ann Arbor area. Eligible patients included women, aged 18 to 50 years, who presented with vaginal symptoms (itching, swelling, or odor) or who were asymptomatic and requested a pelvic examination for preventive screening. The concurrent study on vaginitis required that the patients had a current sexual partner and were willing to return for a minimum of four follow-up visits over the next 12 months. Follow-up visits and partner data were not components of our HPV evaluation.

PROTOCOL

The study was approved by the University of Michigan Human Subjects Committee, and all participants gave written informed consent. The HPV testing in the study was not described to patients until after informed consent was obtained for the vaginitis study. A separate informed consent was then obtained for this aspect of the study. Each patient completed a 14-page, self-administered questionnaire that requested information about demographic variables, medical history, exposures to cigarette smoke, medications, and contraceptives, perceived stress, and a detailed sexual history. A pelvic examination was performed, and data were recorded on a standardized form. During this examination, a cervical specimen was collected with a Dacron swab, placed in ViraPap sample collection medium (DiGene, Silver Spring, Md), and stored at −70°C pending analysis for HPV DNA by the PCR method described below. Vaginal specimens were collected for potassium hydroxide and normal saline solution slide preparations, pH testing of vaginal discharge, and whiff test for aromatic amines. These evaluations were performed in the office laboratory. Cervical and vaginal specimens were collected for Papanicolaou's test, and cultures for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, group B β-hemolytic *Streptococcus*, *Staphylococcus aureus*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, and *Candida* species were processed as described below. Results were recorded on coded laboratory data forms. Patients were treated for vaginal or cervical infections by their medical provider based on the office data and the culture results. Patients found to have HPV present were notified of this result and were referred for colposcopic evaluation.

LABORATORY TESTING

PCR Testing

Polymerase chain reaction analysis was performed by two investigators (L.G. and W.D.L.) who were "blinded" to the clinical characteristics of the patients. Specimens were transported on dry ice to the laboratory and were stored at −70°C until evaluated for HPV sequences using amplification followed by gel electrophoresis and molecular hybridization. Polymerase chain reaction testing was carried out as described in the accompanying article by Zazove et al.[15]

Several of the reported risk factors for cervical cancer and for HPV infection in high-risk populations are similar. These risk factors include an increased number of sexual partners,[8,11] a younger age at first intercourse,[12] the use of oral contraceptives,[13] and cigarette smoke exposure.[14] However, most women infected with HPV are seen in community-based offices and differ in many characteristics from the high-risk groups typically studied. Some risk factors for infection, such as the duration of a relationship with a sexual partner, age, and marital status, have not been adequately addressed because of insufficient numbers of patients with differing characteristics for these variables in the higher-risk populations studied. Furthermore, most patients in previous studies were either symptomatic (in studies at sexually transmitted disease [STD] clinics) or asymptomatic (in screening studies). Hence, comparison of the risk of HPV infection in symptomatic and asymptomatic groups has not been possible. Information on the factors associated with the presence of HPV in lower-risk populations is needed if detection and appropriate management of this problem are to be achieved in the community where women with HPV infection seek medical care. Our study was designed to evaluate historical and microbiological risk factors associated with HPV infection in sexually active women, with and without vaginal symptoms, who present to community-based primary care practices for gynecological examinations.

See also pages 1227 and 1250

RESULTS

CHARACTERISTICS OF PARTICIPANTS

Between March 1, 1990, and June 1, 1992, all 289 patients in the concurrent vaginitis study were also enrolled

Papanicolaou's Test

After samples were collected for cervical cultures and PCR testing for HPV, specimens were taken for the Papanicolaou test. Single slides were prepared from swabbings of the squamocolumnar junction of the cervix with a wooden spatula and from an endocervical scraping with use of an endocervical brush. The slides were fixed immediately. The adequacy of the sample and the histological features were analyzed at the University of Michigan Clinical Laboratories and were reported by a simple narrative method of interpretation.

Colposcopy

Colposcopy was recommended to all patients who tested positive for HPV DNA by PCR analysis. Prior to performing the procedure, written informed consent was obtained. Colposcopic examination of the perianal, vulvar, vaginal, and cervical areas was performed, using from ×4 to ×25 magnification, green filter review, and 4% acetic acid and Lugol's solution enhancement. Biopsy specimens were obtained from all areas suspected of having HPV-associated disease, and an endocervical curettage was performed. Specimens obtained were transported in formalin to the University of Michigan Pathology Laboratory where the histological fixation and pathological interpretation were performed by certified pathologists.

Microbiological Testing

Microbiological testing for *Candida* species, *G vaginalis*, group B β-hemolytic *Streptococcus*, *S aureus*, *M hominis*, *U urealyticum*, and *N gonorrhoeae* was performed at the University of Michigan Clinical Laboratory using conventional isolation and culture techniques.[16] *Trichomonas vaginalis* was cultured in modified Diamond's media (Remel, Lenexa, Kan) at 35°C for 9 days at the individual clinical offices and was examined microscopically every 1 to 2 days for the presence of motile trichomonads. *Chlamydia trachomatis* was cultured at the clinical laboratories of the McAuley Health Center in Ann Arbor. Cervical specimens were cultured on McCoy cell monolayers for 48 hours, stained with a fluorescein-tagged monoclonal antibody (Kallestad, Chaska, Minn), and examined under ×250 magnification for typical inclusions.

ANALYSIS

All data were identified by a study number and were coded, entered, and quality-assessed on the SPSS Data Entry II program (SPSS, Inc, Chicago, Ill) on IBM-compatible personal computers. Final analysis was conducted using the SPSSX statistical software on the Michigan Terminal System mainframe IBM 3090-600E computer system.

Frequencies of all variables were determined, and univariate associations between potential risk factors and HPV infection were calculated using χ^2 analysis, odds ratios, and 95% test-based confidence intervals. Associations between the potential risk factors were also determined using χ^2 analysis, followed by stratified analysis of the original associations using Mantel-Haenszel χ^2 summary testing to assess potential confounding and interaction variables. Logistic regression analysis was then performed to assess the independent associations between HPV infection and the variables identified by the univariate analyses, including the interaction terms. Also included were factors previously reported by others to be associated with the presence of HPV.[8,11-14]

in the HPV study. Enrollment for both exceeded 90% for patients with vaginal symptoms and approximately 35% for asymptomatic patients. The need to return for four follow-up visits in the concurrent vaginitis study was the reason in more than 80% of the refusals to enroll. Although the patients were unaware of the HPV testing until after first deciding whether to participate in the vaginitis study, potential historical factors that may be associated with a patient's perceived risk for HPV infection were assessed in the two groups. Patients with vaginal symptoms did not differ from asymptomatic patients in having a history of genital warts, abnormal results of Papanicolaou's test, or a partner with genital warts. They also did not differ by age, educational level, use of oral contraceptives, smoking status, and proportion who had known their current partner less than 2 years. The proportion of symptomatic women who had six or more sexual partners in their lifetimes was greater than that of the asymptomatic women (43.1% vs 28.9%, respectively; P=.03).

Polymerase chain reaction analysis for HPV was performed on specimens from each patient; 16 samples were uninterpretable owing to inhibition (n=13) and inadequate cellular material (n=3). Of the remaining 273 patients, 189 (69.2%) had complained of vaginal symptoms (vaginal odor, itching, or swelling) at the time of their initial visit while 84 (30.8%) did not. The demographic characteristics of the participants (expressed as mean±SD) were as follows: age, 31.8±6 years (range, 18 to 50 years); years of education, 14.6±2.6 (range, 9 to 20 years). Sexual history of the participants (expressed as mean±SD) was: age at first intercourse, 18.0±3.1 years (range, 9 to 32 years); number of partners ever, 8.2±12.9 (range, one to 50); and number of months current partner has been known, 104.6±8.6 (range, 1 to 396 months). Other characteristics of the participants are shown in **Table 1**. Human papillomavirus was detected in 21.2% (58/273) of the specimens—24.9% from patients with vaginal symptoms and 13.1% from those without symptoms. The HPV types found using the PCR method were the intermediate-risk (types 31, 33, 35, 52) and the high-risk (types 16 or 18) types

Table 1. Characteristics of Participants (N=273)*

	No. (%) of Patients
Demographics	
Ethnic group	
White	234 (87)
Black	24 (9)
Hispanic	6 (2)
Other	4 (1)
Marital status	
Married	189 (70)
Single	51 (19)
Divorced or separated	29 (11)
Pregnancy history	
Currently pregnant	13 (5)
Ever pregnant	182 (68)
No births	27 (15)
1-2 births	125 (69)
>2 births	28 (17)
Household income	
≤$14 000	31 (12)
>$49 000	78 (29)
Risk factors	
Current smoker	46 (17)
Ever smoked ≥100 cigarettes	98 (37)
Current oral contraceptive user	75 (28)
Ever used oral contraceptives	229 (88)
History of sexually transmitted diseases	
Genital warts	30 (12)
Bacterial vaginosis	124 (47)
Chlamydia trachomatis	26 (10)
Neisseria gonorrhoeae	14 (5)
Genital herpes simplex	25 (10)
Trichomonas vaginalis	47 (18)
Pelvic inflammatory disease	21 (8)
Candidal vulvovaginitis	222 (83)
Partner with history of *Candida, Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*, or herpesvirus in the past 6 mo	40 (17)
Current vaginal symptoms	
Itch	164 (63)
Odor	124 (49)
Swelling	82 (33)
Any combination of the three preceding	189 (69)
Discharge	191 (73)
Organisms present at the initial visit	
Candida species	95 (36)
Gardnerella vaginalis	52 (20)
Chlamydia trachomatis	2 (1)
Neisseria gonorrhoeae	1 (0.4)
Mycoplasma hominis	29 (12)
Ureaplasma urealyticum	110 (45)
Trichomonas vaginalis	2 (1)
HPV by PCR analysis	58 (21)
Evidence of HPV infection on Papanicolaou's test	7 (3)

**N<273 for each variable owing to missing data. Percentages have been rounded. HPV indicates human papillomary virus; PCR, polymerase chain reaction.*

in 32.8% and 34.5% of these patients, respectively, as noted below (percentages have been rounded):

HPV Type	No. (%) of Patients
6 or 11	8 (14)
31	7 (12)
33	2 (3)
35	8 (14)
52	2 (3)
16	15 (26)
16-related	2 (3)
18	5 (9)
18-related	3 (5)
Unknown type	6 (10)
Total	**58 (100)**

The HPV type did not differ significantly between patients with and without vaginal symptoms. Only seven patients (2.6%) had abnormal results of Papanicolaou's test, suggesting condyloma or dysplasia. Of these seven, five specimens were HPV-positive by the PCR method.

Forty-six patients with HPV infection had colposcopy performed. Of the remaining 12, 10 failed to make or keep their appointments for this test, and two were followed up with cytologic smears only by their private physicians. The colposcopies were performed by one of us (B.D.R.) in 42 of the 46 patients and by outside colposcopists for four patients. All patients underwent cervical biopsy and endocervical curettage. Twenty-five patients (54%) were found on biopsy to have HPV-related cellular changes: 22 with low-grade squamous intraepithelial lesions, two with high-grade intraepithelial lesions, and one with squamous intraepithelial lesions of unspecified severity. Ten patients (22%) had cervicitis, and 11 (24%) had no cellular abnormality. Of the 15 patients with HPV types 16 and 18 who had undergone colposcopy, three had normal biopsy findings, six had cervicitis, and six had condyloma or cervical intraepithelial neoplasia 1 (low-grade squamous intraepithelial lesions).

UNIVARIATE ASSOCIATIONS

The χ^2 analysis of associations between potential risk factors and HPV infection was performed. Those risk factors found to be statistically associated with HPV infection as well as those reported by others to be associated in previous studies are described in **Table 2**. Other historical factors evaluated that lacked a statistically significant association with HPV infection are listed as follows: ethnic background; educational level; current, past, or passive smoking; number of years smoked; age at first intercourse; parity; douching; barrier contraception use; types of sexual activities; frequency of intercourse; vaginal bleeding; pain with intercourse; picket-fence sign (severe itching); abnormal results of Papanicolaou's test; perceived stress and/or worry; a history of genital warts or pelvic inflammatory disease; tampon use; recent partner with chlamydial infection; and a history of infection with

Table 2. Univariate Associations Between Individual Factors and the Presence of Human Papillomavirus (N=273)*

Factor	N	% With Factor Present	OR	95% CI	Sens	Spec	PPV	NPV
Demographic								
Age, y								
≤29	264	36	1.72	0.94-3.16	46	67	26	83
≤39	264	83	3.03	1.08-8.50	93	20	23	91
Not married	269	30	3.60	1.99-6.50	53	76	38	86
Income ≤$14 000	267	12	3.24	1.52-6.91	23	92	42	82
Exposures								
Current oral contraceptive user	270	28	1.90	1.03-3.50	39	75	29	82
Sexual history								
≥6 sexual partners ever	260	38	2.25	1.24-4.11	54	66	29	85
Known current partner <24 mo	258	18	6.60	3.48-12.51	45	89	53	85
Vaginal symptoms								
Discharge	261	73	2.86	1.26-6.50	87	30	24	90
Any combination of the three symptoms below	261	72	2.99	1.32-6.78	87	31	24	90
Itching	262	63	2.72	1.35-5.49	79	42	26	89
Swelling	245	33	1.45	0.76-2.80	40	68	23	83
Odor	253	49	2.67	1.40-5.07	68	56	27	88
Current cervical infections								
Mycoplasma hominis	248	12	3.06	1.40-6.73	23	91	41	81
Ureaplasma urealyticum	247	45	2.62	1.42-4.83	63	61	31	85
Chlamydia trachomatis	262	<1	18.95	3.79-94.75	4	100	100	79

*N<273 for each variable owing to missing data. OR indicates odds ratio; CI, confidence interval; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; and NPV, negative predictive value.

C trachomatis, Herpes simplex virus, *N gonorrhoeae*, *T vaginalis*, or recurrent *Candida*.

The factors noted on physical examination that were not associated with HPV infection included cervical ectropion; body mass index; cervical friability; and vaginal odor. The factors noted on laboratory evaluation that also were not associated with HPV infection included clue cells (epithelial cells covered with bacteria); vaginal pH; white blood cell count, spores, and hyphae on normal saline solution slide preparation; and the presence of: *G vaginalis*, *Candida* species, group B *Streptococcus*, candidal vulvovaginitis, and *S aureus*.

For four factors—ethnic background, use of tampons, a recent partner with chlamydial infection, and the presence of *Candida* species or candidal vulvovaginitis—a trend toward statistical significance was noted but not achieved ($P>.05$).

STRATIFIED ANALYSIS

Associations between the potential risk factors for HPV infection shown in Table 2 were evaluated using χ^2 analysis. Those found to be statistically associated ($P<.05$) were further evaluated as potential sources of confounding and interaction. Interaction by income level was identified for the association between vaginal itching and HPV infection, as was an interaction by vaginal swelling on the association between any vaginal itching and HPV infection. Therefore, interaction terms of these pairs were included in the logistic regression model to assess the contribution of the interaction to the model. No interaction or confounding was found between the presence of vaginal symptoms and the history of six or more sexual partners and their association with the presence of HPV infection.

Although only 18% of the participants had known their current partner less than 24 months, they constituted 45% of the HPV-infected group. Among those patients who had known their current partner less than 24 months, in no stratum in the stratified analysis was their prevalence of HPV infection less than 46% (**Figure 1**).

The associations between several variables and HPV infection were no longer present when stratification was performed. These variables were marital status (when stratified by whether the patient had known her current partner less than 24 months); use of oral contraceptives (when stratified by the presence of symptoms or the complaint of itching, or by whether the patient had known her partner 24 months or less); the presence of *M hominis* or *U urealyticum* (when stratified by having six or more sexual partners ever, unmarried status, household income of $14 000 or less, whether the woman had known her current partner less than 24 months, or the presence of vaginal odor); and candidal vulvovaginitis (when stratified by the presence of vaginal itching, swelling, or odor or a combination of the three). Although the presence of *C trachomatis* was strongly associated with HPV infection, the small numbers of infected women (n=2) precluded further evaluation.

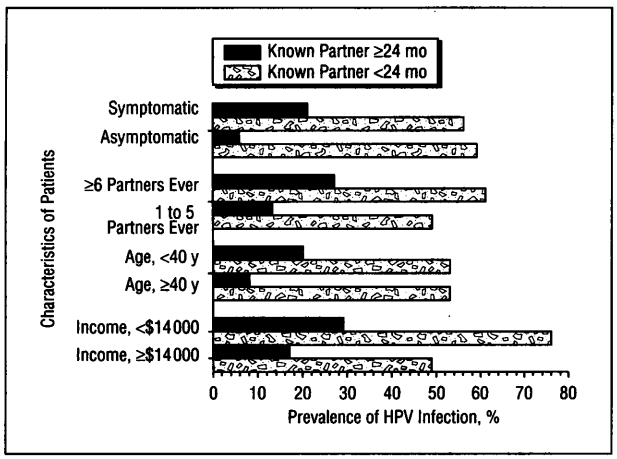

Figure 1. *Prevalence of human papillomavirus (HPV) cervical infection in women with various risk factors, stratified by the length of time they had known their partners.*

Table 3. Factors Present in Final Logistic Regression Model*

Variable	β	SE	OR	95% CI	P
Known current partner <24 mo	1.71	.41	5.53	2.49-12.3	.000
≥6 sexual partners ever	1.07	.37	2.91	1.42-5.96	.004
Presence of vaginal itching, odor, or swelling	1.09	.51	2.99	1.10-8.13	.032
Age <40 y	1.38	.68	4.01	1.05-15.30	.042
Household income <$14 000	1.02	.50	2.78	1.04-7.46	.042
Constant	−4.47	.86	...	...	.000

**Accuracy of the model, 82.6%; sensitivity, 30.6%; specificity, 96.3%; positive predictive value, 68.2%; and negative predictive value, 84.1%. OR indicates odds ratio; CI, confidence intervals.*

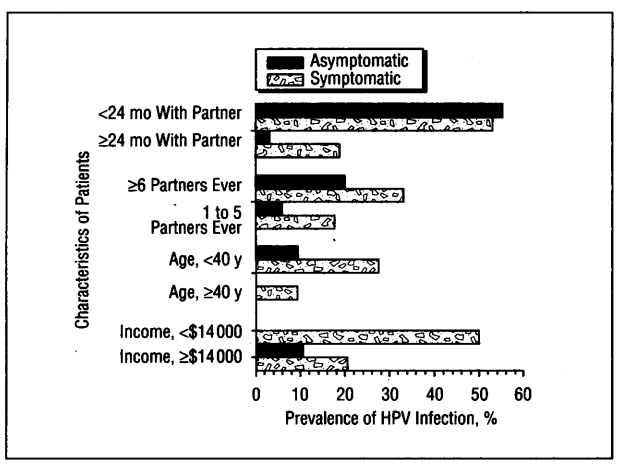

Figure 2. *Prevalence of human papillomavirus (HPV) cervical infection in women with various risk factors, stratified by the presence of vaginal itching, odor, or swelling.*

The variables no longer associated with HPV infection on stratified analysis were not included in the logistic regression model. The history of either genital warts or abnormal Papanicolaou's test results were not associated with HPV infection and were not included in the multivariate analysis. The age at first intercourse was not used in the logistic regression analysis because of the lack of association noted and the lack of data in the literature supporting its inclusion.[8]

LOGISTIC REGRESSION ANALYSIS

The variables entered in the logistic regression model included (1) age less than 40 years, (2) household income level of $14 000 or less, (3) six or more sexual partners ever, (4) whether the woman had known her partner less than 24 months, (5) the presence of symptoms of vaginal itching, swelling, or odor, and (6) the presence of a vaginal discharge. The interaction terms described above were also included but were deleted when found to be noncontributory. A model in which all factors were entered was used. This model was followed by forward inclusion and backward elimination models to assess differences. The final backward elimination model that maximized sensitivity and classification accuracy without elimination of variables that were independently associated with the outcome is shown in **Table 3**. Each of these five factors were associated with HPV infection in these women, independent of the values of the other four factors.

Two of these associations are shown graphically in Figure 1 and **Figure 2** using the stratified data. The associations between the remaining risk factors and HPV infection, stratified by the presence of vaginal itching, odor, or swelling, are shown graphically in Figure 2. The prevalence of infection was increased if these symptoms were present, and the effect of the other risk factors within the symptomatic and asymptomatic groups are also evident.

The strength of the association between the time women had known their partners and prevalence of HPV infection is shown in Figure 1. Although only 18% of the participants had known their current partner less than 24 months, they made up 45% of the HPV-infected group. Among those participants who had known their current partners less than 24 months, in no stratum in the stratified analysis was their prevalence of HPV infection less than 46% (Figure 1).

COMMENT

Sexually transmitted diseases are more common and more readily suspected in office practices serving young, single, sexually active adolescents or adults, such as STD clinics and university health centers. Previous studies of risk factors for HPV infection have been described in such settings. If approximately 10% to 20% of the 54 million women in the United States between 18 and 44 years of age are infected with HPV, then over 5 to 10 million women are infected, the majority of whom are not seen in high-risk centers. Because STDs are less prevalent in women seen in community-based offices and because of the unrecognized nature of many predisposing factors, risk for HPV infection may be less obvious to these women and their physicians. Thus, informa-

tion about risk factors for this infection in women seen in centers serving low-risk populations is imperative. Although some of these factors would be expected to be similar to those in high-risk populations, women in populations with a lower prevalence of STDs differ in characteristics regarding their health, sexual activities, and development and duration of relationships—factors that may also play a role in their acquiring HPV infection.

The participants in our study differed greatly from those typically evaluated in higher-risk groups. Compared with previous studies, most participants in our study were older, white, more likely to be married and to have been pregnant previously, more likely to be in long-term relationships, more educated, and had higher household incomes (Table 1). These characteristics are usually not associated with increased risk for STDs, and these patients would usually not be evaluated for unrecognized disease. Many did complain of vaginal symptoms (69%), but very few had classic STDs such as infection with *C trachomatis*, *N gonorrhoeae*, *T vaginalis*, or genital herpes simplex virus. Furthermore, risk factors for HPV infection, such as a history of genital warts or abnormal findings on cytologic smears, did not differ between women without vaginal symptoms and women with symptoms. These potential risk factors were not associated with current HPV infection or the presence of vaginal symptoms in these patients, possibly owing to resolution of infection over time, and hence were not controlled for in the analysis.

The univariate analysis revealed associations between HPV infection and individual risk factors that are similar to those identified in high-risk populations and in patients with cervical cancer. These factors included the number of sexual partners ever,[8,11] income status, use of oral contraceptives,[8,17,18] and age.[2,8,17] Other risk factors were also found that have not been previously evaluated (such as the presence of vaginal symptoms) or that have been unevaluable because too few patients exhibited the characteristic in question (such as, prolonged relationship with current partner and married status).[8] Thus, the importance of these factors as predictors of infection in lower-risk populations and the implications for current understanding of the pathogenesis of HPV infection have been unclear.

We found five factors that were significantly associated with HPV infection in the logistic regression analysis, two of which have not been previously observed.

PATIENT HAS KNOWN CURRENT PARTNER LESS THAN 24 MONTHS

Patients who had known their sexual partners less than 24 months were more likely to have HPV infection, independent of age, income, vaginal itching, odor, or swelling, and number of previous partners. Although few data have been reported regarding the impact of partnership duration on the prevalence of HPV infection, decreased time of contact between male partners and female partners who have abnormal Papanicolaou's test results has been shown to be independently associated with higher rates of HPV-related genital lesions in the male partners.[19]

In our study, although women who had known their current sexual partner 24 months or less accounted for only 18% of the patients enrolled, they made up 45% of those with HPV infection of the cervix. The prevalence of HPV infection of the cervix in this subgroup was 46% or greater regardless of the other risk factors (Figure 1). Several possible theories could explain these findings. Genital HPV infections may be transient with increased risk for infection with recent exposure to an infected partner and subsequent clearing of infection over time.[7] There may also be variations in effectiveness of transmission related to the stage of infection. Evidence from studies on the transmission of the human immunodeficiency virus (HIV) suggests that the risk for seroconversion is related to the timing and type of the contact relative to the point in time when the HIV-infected partner becomes symptomatic.[20] This increased risk may be related to different levels of viral replication or to the level of immunocompetence in either partner. Symptomatic HIV-infected patients are more likely than asymptomatic patients to have positive HIV cultures from peripheral lymphocytes[21,22] and an increase in free virus.[23] Similar timing for increased HPV infectivity related to HPV replication and transmissibility may occur. If a period exists after infection with an STD in which a person is most infectious and if that infectivity decreases over time or if new infections resolve over time, a decreased prevalence of infection would be expected as the duration of a relationship increases. Therefore, either decreased transmission over time or resolution of infection may result in the association demonstrated herein.

Characteristics of partnership formation may also influence transmission probabilities. If choice of partner and onset of sexual activity result in longer periods of dating prior to sexual activity, less frequent exposure to new partners, and selection of partners with a lower risk for STD infection, decreased risk for infection may also occur. In our study, characteristics of the patients, such as age, income level, and number of past sexual partners, did not explain the association between HPV infection and length of time the women had known their partners. Evaluations of partner selection and onset of sexual activity are indicated to more clearly define these relationships.

COMPLAINT OF VAGINAL ITCHING, ODOR, OR SWELLING

The presence of vaginal complaints, such as itching, odor, or swelling, was associated with an increased risk for HPV infection in this population. Most studies of HPV infection in women have enrolled asymptomatic women at the time of a routine gynecological examination[8,11]; none of these studies assessed the presence of vaginal symptoms as a risk factor.

Patients with vaginal symptoms were more likely to agree to participate in our study than were asymptomatic women. If this altered participation were related to the likelihood of

having HPV present, bias would be introduced into the analysis. However, patients were not aware that HPV testing would be part of the study until after consent was obtained for participation in the vaginitis study, at which time an additional informed consent was signed for the HPV testing. In addition, the symptomatic and asymptomatic groups did not differ in the historical factors that would indicate the patient knew of an altered risk for HPV infection—these factors being abnormal results of cytologic smears, genital warts, or a partner with warts. Therefore, no plausible association between enrollment in the study by symptomatic women and the presence of HPV is postulated.

The presence of symptoms may indicate that another genital infection is present that might increase the risk for HPV exposure or HPV infection when exposed. The risk for HIV infection is increased in the presence of other genital infections, such as syphilis, chancroid, and herpes.[24] However, these particular infections are uncommon in a low-risk, community-based population. Most of the symptomatic women we studied were found to have common vaginal syndromes such as bacterial vaginosis (BV) and candidal vulvovaginitis. Bacterial vaginosis has not been described as a risk factor for HPV infection, although altered vaginal flora (usually found in cases of BV) has been associated with cervical intraepithelial neoplasia.[25] Our data indicate an association between the complaint of vaginal odor (also associated with BV) and HPV infection. An increased prevalence of HPV infection in women with vaginal anti-*Candida* antibodies has been described,[26] as well as an increased prevalence of *Candida* species in women with HPV infection of the cervix.[27] In our study, the presence of candidal vulvovaginitis was weakly associated with HPV infection, but the occurrence of vaginal itching, odor, or swelling was associated with HPV infection, even in the absence of *Candida* infection.

The symptoms associated with HPV infection may be caused by immunological mediators such as IgE or histamines, which may be liberated in response to hypersensitivity reactions as well as to the infectious agent itself. These mediators may be associated with local immunosuppression and may result in an increased susceptibility to viral replication.[26,28]

The increased detection rate of HPV in women with vaginal symptoms could alternatively be explained by altered viral load or cellular shedding. Further study is needed to assess these possibilities.

PATIENT AGE LESS THAN 40 YEARS

Although the incidence of cervical cancer has decreased over the past 40 years, the rate in women less than 50 years of age has not decreased and is predicted to increase.[5,6] Furthermore, the mortality rate from cervical cancer appears to be increased in women less than 40 years of age.[4,29]

Less is known about younger women's risk for precancerous lesions or HPV infection as determined by DNA detection methods.[2,30] Ley et al[8] found younger age to be an independent risk factor for HPV infection (determined by the PCR method) in a university health center population. Several mechanisms could explain this association between age and risk for HPV infection, such as cervical ectropion, presence of other STDs, the resolution of infection over time, or a cohort effect.

Everted endocervical tissue, commonly present in younger women, has been associated with other STDs, including HIV[31] and *C trachomatis*[32] infections, and may contribute to the increased risk for HPV infection in younger women.

The incidence of HPV infection is increased in patients with other sexually transmitted infections, such as herpes simplex,[33,34] *C trachomatis*[35] *N gonorrhoeae*,[36] and HIV,[37-39] and in patients with altered vaginal flora.[25] Younger women are more likely to have been infected with these viruses in the recent past than older women.[40] Although the information obtained in the patient questionnaire about previous infections was not statistically associated with the risk for HPV infection in our study, inaccurate past clinical diagnoses and past asymptomatic infections may create classification errors that could obscure an association.[41-43]

Evidence suggests that genital HPV infections may be transient owing to elimination or latency of the virus.[7] If most incident HPV infections occur in younger women and if elimination or reduction in quantity of the virus occurs over time, an age-related prevalence risk for HPV infection would be expected.

Finally, a cohort effect may explain the association of HPV infection in younger women if the prevalence of the virus in the general population has increased over time. If so, the current risk for infection would be expected to be maximal in those women who have had a more recent exposure to new sexual partners. In our study, women over 40 years of age were almost as likely to be infected as those under 40 years of age if they had known their current partners less than 24 months, suggesting recent exposure affects risk considerably. Whether the prevalence of HPV infection is actually increasing or whether the perceived increase is due to more sensitive detection methods remains controversial.[44,45]

HOUSEHOLD INCOME OF $14 000 OR LESS

Few data are available regarding the relationship between HPV infection and income status, although the incidence of cervical cancer is greater in women with a lower socioeconomic status.[46] Household income status is probably a marker for risk factors that have not yet been identified or measured accurately. Past infections with other sexually transmitted organisms are difficult to measure by historical information alone, but evidence suggests that increased rates of infection are associated with low-income status.[41,47] Although in our study low income was associated with other factors (such as the history of their partner having had *C trachomatis* or *T vaginalis* infection in the previous 6 months, having known their partner less than 24 months, and the presence of *M hominis*

and *U urealyticum* infection), controlling for these factors did not explain the relationship between income and HPV infection. More precise identification of past STDs, such as identification using serologic data, needs to be included in future assessments. Other important factors that may clarify the association between income and HPV infection include the characteristics of previous sexual partners and the length of time each was known prior to sexual intercourse, the use of intravenous drugs, stress levels, and personal hygiene practices.

NUMBER OF SEXUAL PARTNERS EVER OF SIX OR MORE

An increased number of lifetime sexual partners is a known risk factor for cervical cancer[48] and has been associated with HPV infection in other studies in which DNA probes[11,17] or PCR analyses[8] were used for HPV testing. This association was seen in our study despite the older average age of the participants, the length of time they had been with their current partners, and the paucity of other recent partners (only 4% had had more than one partner in the previous 2 months).

Other characteristics of the relationships with previous sexual partners may also be important in determining risk. These may include the length of time each relationship continued prior to sexual activity, as well as the sexual history, toxic exposures to cigarette smoke and medications, and infectivity of each partner. Detailed information about these factors needs to be addressed further.

Three risk factors were noted on univariate analysis that were no longer associated with HPV infection when evaluated by stratified analysis or when included in the logistic regression model. These factors included unmarried status, oral contraceptive use, and the presence of other genital infections. Previous studies have suggested associations between these factors and HPV infection[8,13,18,25,33-36,49-51] but have been limited by small numbers of patients with the desired characteristic (married status) and incomplete controlling for other risk factors.[50,51] In our study, stratification by whether the woman had known her sexual partner less than 24 months resulted in no independent association between these factors and HPV infection, suggesting that the duration and the stability of the relationship as well as the length of time since exposure to a potentially infected individual may explain the findings.

No association was found between HPV infection and current smoking, past smoking, number of years smoked, or number of cigarettes smoked per week. Although smoking has been reported as a risk factor for cervical cancer,[14,52] other studies have similarly failed to find significant associations on multivariate evaluation.[8,17] Therefore, although smoking may act as a cofactor for disease progression, no evidence suggests this exposure is associated with HPV infection in this population. Furthermore, no association between the history of genital warts or abnormal results of cytologic smears and HPV infection was found. This finding may reflect resolution of viral infection over time in this cohort of women.

Limitations to our study exist. The prevalence of HPV infection was determined by PCR analysis on a single sample obtained from the cervical os. Repeated PCR analysis of specimens collected over time and testing of multiple sites might result in higher detection rates. Although the patients made up a lower-risk group compared with those in most published studies, they are also not representative of the general population seen in primary care settings. The predominance of long-term, stable partnerships, with an anticipated lower rate of HPV infection, was expected. Conversely, the higher participation rate of symptomatic women, compared with that of asymptomatic women, resulted in a reversed prevalence of the two groups in the sample, compared with a typical office practice and, hence, may have increased the HPV infection rate found. Therefore, the prevalence of HPV infection in specific community-based populations may differ somewhat from our values, although other studies on low-risk women suggest this variability is not large.[10] Furthermore, no data suggest that the participation ratios of patients with HPV infection to those without would differ in the symptomatic and asymptomatic groups, and hence no bias in results is expected despite the differing participation rates.

CONCLUSIONS

Human papillomavirus infection, as determined by the PCR method, was present in 21.2% (58/273) of the participants who were evaluated in two community-based offices (24.9% in women with vaginal symptoms and 13.1% in those without; $P<.01$). The presence of HPV infection of the cervix was associated with histological abnormalities in over half of those tested as confirmed by colposcopically directed biopsies. Multivariate analysis indicates that independent risk factors for HPV infection in this group include having known the current sexual partner less than 2 years; the symptoms of vaginal swelling, odor, or itching; a history of 6 or more sexual partners ever; age of less than 40 years; and a household income of $14 000 or less. Nevertheless, no individual or grouped risk factors were highly predictive of HPV infection in this population, and less than one third of HPV-infected women were identified by these risk factors. Further research is needed to assess the reproducibility of these findings in other low- and high-risk populations, to clarify the partnering characteristics associated with risk, and to further identify the underlying pathophysiological implications of these findings.

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