Human Papillomavirus Infection in Postmenopausal Women With and Without Hormone Therapy

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Objective: **To determine whether postmenopausal hormone therapy is associated with high-risk human papillomavirus (HPV) infection.**

Methods: **The detection rate of HPV DNA was studied in cellular residue from liquid-based collection tubes taken from 180 postmenopausal hormone users attending a menopausal clinic and 126 postmenopausal nonusers. The samples were analyzed with a hybrid capture technique using a mix of high- to intermediate-risk viral RNA probes. In all patients, information on potential confounding factors for HPV infection, including sociodemographic, reproductive, and gynecologic characteristics, was obtained.**

Results: **The prevalence of HPV DNA in this cohort of postmenopausal women was 1% (three of 306); only two of the 180 current users and one of the 124 nonusers tested positive. Only one of the three women with HPV-positive tests had lesional tissue (ie, vulvar condylomata acuminata). The remaining two HPV-positive women had negative cytology, colposcopy, and biopsy. In all three cases, viral burden was low, about 10 pg per cellular sample. The very low HPV prevalence precluded the analysis of correlation with age, ethnicity, education, sexual history, smoking, history of abnormal Papanicolaou smear, therapy for HPV-related lesions, and contraceptive use.**

Conclusion: **Identification of high-risk HPV types in postmenopausal women is rare, as detected by hybrid capture in cellular residue from the liquid-based cytology-collection system. Postmenopausal hormone therapy does not appear to promote viral replication or the risk of carrying high-risk HPV DNA or related lesional tissue in the lower genital tract. (Obstet Gynecol 1997;90:7–11. © 1997 by The American College of Obstetricians and Gynecologists.)**

In recent years, we have been receiving in consultation an increasing number of cervical smears obtained from postmenopausal women using hormone therapy and found to have atypical glandular cells of undetermined significance, and, less frequently, atypical squamous cells of undetermined significance favoring low-grade squamous intraepithelial lesion (SIL), according to the Bethesda reporting system. It is suspected, although not proven, that these inconclusive smears are not human papillomavirus (HPV)-related, but are due to the stimulatory effect of sex steroids on the endocervical and squamous metaplastic epithelium in hormone users.[1] These issues are important because if none of the above morphologic alterations have clinical significance in these women and are misinterpreted as hormone-promoted HPV effects, then many women may discontinue postmenopausal hormone therapy and augment their risk for developing estrogen-deprivation-related illnesses.[2] As an increasing number of postmenopausal women are encouraged to become long-term hormone users, it is important to gather data on the epidemiology of HPV infection in the postmenopausal years.

The aims of this prospective study were to determine the following: 1) the point-prevalence of infection by high-risk HPV types in postmenopausal women with and without hormone therapy, 2) the viral burden in relation to hormone use, and 3) HPV positivity in relation to the presence of histologically verified lesional tissue of the cervix. The working hypothesis was that in hormone users, the prevalence of both latent and morphologically detectable HPV infections would be higher than in nonusers.

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Materials and Methods

The study was approved by the institutional review board of the Sir Mortimer B. Davis Jewish General Hospital and included 306 postmenopausal women, each of whom had a cervix and provided informed consent. The enrollment period lasted 6 months. Of the 306 women, 180 were current hormone users and 126 were never-users. The latter group approximately frequency age–matched the hormone users. In all the nonusers, serum FSH and estradiol levels were within the menopausal range. All hormone users attended the menopausal clinic of one of the authors (MMG). The nonusers attended the colposcopy clinic of the Jewish General Hospital for either vulvar pathology or borderline Papapanicolaou smears (n = 39), the private practice of NM (n = 73), or the menopausal clinic of MMG (n = 14). All women were interviewed by research nurses, and sociodemographic, reproductive, and gynecologic risk factors related to HPV infections and confounders were recorded. These included age, education, parity, number of lifetime sexual partners, pregnancies, age at first intercourse, smoking, oral contraceptive (OC) use, and history of sexually transmitted disease (STD).

In each patient, an effort was made to obtain a cytologic sample by using an endocervical or exocervical sampler. The sampling head of the device was placed immediately in a liquid-based cytology collection tube. Each specimen was processed for thin-layer cytology within 48 hours of sampling the cervix. After the preparation of the slides in a cell processor as per manufacturer's instructions, the residual cellular material was kept at −20C until HPV DNA testing.

All HPV DNA assays were performed at the Jewish General Hospital. Specimens for HPV DNA testing were recovered from the residual fluid in the Thin-Prep collection tube (PreservCytyc; Cytyc Corporation, Boxborough, MA) by centrifuging the entire cellular residue. Specimen transport medium was added to the pellet, of which 100 μL was used for the hybrid capture HPV DNA assay with only high-risk HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56 (probe B mix), per manufacturer's instructions. For quality control of the assay, three replicates of negative and positive control B (10 pg/mL HPV 16 DNA) were tested first with the probe B mix for each test run. All control results had to have a coefficient of variation below 30% with the use of the DCR-1 luminometer (Digene Corp., Beltsville, MD). To further validate the assay, positive control mean and negative control mean results were used to calculate the positive-negative control mean ratio, which had to be at least 1.5. The cutoff value for determining positive specimens was the positive control, probe B value. Samples were classified as positive for HPV DNA if the relative light unit reading obtained from the luminometer was at least the mean of the positive control values. Samples that produced relative light unit values one to 4.9 times the positive control value were classified as being 1+, those with values five to 20 times the positive control value were classified as being 2+, and those with a value greater than 20 times the positive control value were 3+. In a previous study,[3] we found excellent diagnostic performance indices of both the thin-layer slide technique and HPV DNA testing with liquid-based cytology. In all patients with vulvar pathology, in those in whom the cytologic slide suggested atypical squamous cells of undetermined significance or atypical glandular cells of undetermined significance or worse, and in those whose HPV test was positive, colposcopy and, if appropriate, vulvar biopsy, endocervical curettage, and cervical biopsies were performed by two of the authors (AF and NM). The histologic specimens were read by AF without knowledge of the cytologic diagnosis, colposcopic impression, or HPV DNA results. Similarly, the cytotechnologists reading the cytologic slides and the laboratory technician responsible for HPV DNA testing were blinded as to the purpose of the study as well as colposcopic and histologic findings.

All cytologic diagnoses were recorded according to the Bethesda system and, at the completion of the study, the cytotechnologist revealed the random accession numbers and matched the cytologic slide HPV DNA test pairs and, when appropriate, histologic slides.

Our study was designed to attain sufficient power to detect statistically significant differences between hormone users and nonusers in the rates of cytological abnormalities (assumed at 10 and 3%, respectively) and HPV DNA positivity (assumed at 20 and 10%, respectively). Chi-square or Fisher exact tests were used for the univariate comparison of the two groups of patients with respect to sociodemographic and gynecologic variables and HPV DNA positivity. Exact 95% confidence intervals (CIs) were calculated for HPV prevalence estimates in hormone users and nonusers.

Results

The majority of patients in both groups were between the ages of 50 and 70 years, with mean ages of 56 and 58 years in hormone users and nonusers, respectively. Hormone users tended to have attained higher educational levels than their nonuser counterparts ($P < .001$). Users more frequently than nonusers reported being smokers or having a history of smoking (P = .007) (Table 1). There were no significant differences between

Table 1. Distribution of Users and Nonusers of Hormones According to Selected Variables

Variable	User group		Nonuser group	
	n	%	*n*	%
Age (y)				
<50	7	3.9	16	12.7
50–59	105	58.3	62	49.2
60–69	64	35.6	31	24.6
≥70	4	2.2	17	13.5
Education*				
Elementary	10	5.7	29	23.2
High school	64	36.4	54	43.2
College	40	22.7	15	12.0
University	62	35.2	27	21.6
No. of gestations				
0	21	11.7	10	7.9
1–4	139	77.2	97	77.0
≥5	20	11.1	19	15.1
Cumulative OC use				
Never	101	56.1	68	54.0
1–5 y	43	23.9	40	31.7
6+ y	36	20.0	18	14.3
History of smoking*				
Never	75	42.1	75	60.5
Current	29	16.3	15	12.1
Exsmoker	74	41.6	34	27.4
Lifetime sexual partners				
1	99	55.0	81	64.3
2–3	29	16.1	25	19.8
4–6	23	12.8	11	8.7
≥7	29	16.1	9	7.1
Sexual partners in last 5 y				
0	11	6.1	27	21.4
1	140	77.8	78	61.9
≥2	29	16.1	21	16.7
History of STDs*				
No	160	90.4	114	93.4
Yes	17	9.6	8	6.6
History of abnormal Papanicolaou smear*				
No history	174	97.8	115	92.7
Benign condition	3	1.7	5	4.0
Condyloma	1	0.6	0	
CIN I-II	0		4	3.2

OC = oral contraceptive; STD = sexually transmitted disease; CIN = cervical intraepithelial neoplasia.
Data are presented as *n* (%).
* Data are missing for these variables.

the two groups with respect to marital status, number of gestations or deliveries, and years of OC use.

Table 1 presents selected characteristics of hormone users (n = 180) and nonusers (n = 126) that could influence risk of HPV infection or cytological abnormalities. Despite our attempt to recruit women in the nonuser group to match the age distribution of those taking hormones, there were significant differences in selected sexual characteristics between the two groups. Hormone users tended to have more lifetime sexual partners (P = .012) and more partners in the last 5 years (P = .001) than nonusers. Users were also more likely to have had a history of abnormal Papanicolaou smears than nonusers (P = .044). There were no significant differences between the two groups of women concerning age at first intercourse and history of STD.

Of the hormone users, 32 were taking conjugated equine estrogen alone (0.625 mg/day), 123 were taking either sequential or continuous conjugated equine estrogen (0.625 mg/day) combined with medroxyprogesterone acetate 5 mg, and 25 received intramuscular injections of ethinyl estradiol with testosterone enanthate with or without 5 mg of sequential or continuous medroxyprogesterone acetate.

Table 2 presents the HPV DNA results by selected gynecologic characteristics. The HPV DNA assay was positive in only two of 180 hormone users (1.1%, 95% CI 0.1, 4.0) and one of 124 nonusers (0.8%, 95% CI 0.02, 4.4). In two of the specimens from 126 nonusers, HPV DNA assay could not be performed because of insufficient residual material in the liquid-based cytology collection vial. In all three HPV-positive women, HPV DNA quantitation yielded 1+, or one to 4.9 times, the positive control value of relative light unit per cellular sample.

The small number of HPV-positive women precluded the calculations of odds ratios of HPV positivity associated with ever having used hormones, and we could not gauge the risk of HPV infection by type or duration of hormone therapy. Two of the three HPV-positive

Table 2. Human Papillomavirus Testing Results by Selected Gynecologic Characteristics

Variable	HPV negative		HPV positive	
	n	%	*n*	%
History of any sexually transmitted disease				
No	270	99.3	2	0.7
Yes	24	96.0	1	4.0
History of abnormal Papanicolaou smear result				
No history	284	99.0	3	1.0
Benign condition	6	100.0	0	
Condyloma	1	100.0	0	
HGSIL	4	100.0	0	
History of therapy for cervical dysplasia				
No history	289	99.0	3	1.0
Cryotherapy	5	100.0	0	
LEEP/cone	2	100.0	0	
Cytology				
Negative	297	99.0	3	1.0
AGUS	4	100.0	0	

HGSIL = high-grade squamous intraepithelial lesions (grades 2 and 3 cervical intraepithelial neoplasia); LEEP = loop electrosurgical excision procedure; AGUS = atypical glandular cells of undetermined significance.
Data are presented as *n* (%). Missing values have been excluded.

women were hormone users, one of 6 and the other of 20 years duration (Table 2). Among the three HPV-positive women, only one (54 years old) was found with histologically documented condylomata acuminata of the vulva (her cervix was found to be normal by colposcopy and biopsy). None had a history of abnormal cytology results, and none of the seven patients who were treated for HPV-related cervical lesions in the past tested positive for HPV DNA. Among patients with a previous abnormal Papanicolaou smear (low-grade SIL or worse) or therapy for cervical SIL, one of 178 (0.6%) hormone users and seven of 125 (5.6%) nonusers were treated for low-grade or high-grade SIL with either cryotherapy (five women) or loop electrosurgical excision procedure (two women). The remaining 176 users and 118 nonusers reported negative histories of abnormal Papanicolaou smear and therapy for cervical SIL. The incidence of intercurrent abnormal cytology in both groups of women was 1.7% (three cases) and 0.8% (one case) in the hormone user and nonuser groups, respectively, not a statistically significant difference. In all four cases, thin-layer slides contained atypical cells of the undetermined significance. Histologically, two of three cytologically positive hormone users had endocervical polyps and one low-grade SIL of the cervix. In the nonuser group, the woman with atypical squamous cells of undetermined significance favoring low-grade SIL was found to have a high-grade SIL on histology. In each group of women, two had vulvar condylomata without associated cervical HPV infection.

Discussion

In this prospective study, we used a hybrid capture system to detect HPV DNA in a cohort of 306 postmenopausal women (304 of whom were tested for HPV DNA) and planned to relate the results to hormone use as well as to a series of variables thought to influence HPV DNA positivity. The very low rate of HPV DNA positivity (1%) in this group of women, irrespective of confounders and hormone use (1.1%) or no use (0.8%), precluded any correlative analysis. The low prevalence of HPV DNA positivity was likely due to the consultation-type practice rather than the unusually low HPV risk of the sample studied. Of the three postmenopausal women who tested HPV positive, none had a history of intercurrent or previous abnormal cytology and only one of three patients had coexistent HPV-related vulvar condylomata.

The prevalence of HPV infection found in the current study is within the range of most previously published studies of elderly women not on hormones. For example, a study from Germany found 3.5% of women over the age of 65 years old had negative cervical cytology but positive HPV DNA in cervical smears[4]; another study,[5] of elderly African-American women with negative cervical cytology, found the prevalence of HPV infection to be 3.1%. In contrast, in those with positive cytology, the prevalence of HPV DNA was 30% in the German study[4] and 17% in the American study.[5] The percentage of postmenopausal women aged 40 years and older with latent HPV infection was approximately 3% in a Dutch study.[6] The somewhat lower rates of HPV DNA positivity in our subjects compared with those reported by others may be due to differences in study populations and the technique of cell sampling rather than the HPV testing techniques used. Indeed, the relatively insensitive filter hybridization, the more-sensitive dot-blot hybridization, and the ultrasensitive polymerase chain reaction technology used in the German,[4] the American,[5] and Dutch[6] studies, respectively, using adequate numbers of HPV probes, all found similar rates of latent HPV infection.

It is noteworthy that all three HPV-positive women had low quantities of viral DNA, near the cutoff point, which corresponds to 10 pg/mL HPV DNA per cellular sample. Low viral burden has been associated with greater likelihood of latent HPV infection than high levels in the range of 20 pg/mL HPV DNA per cellular sample.[3,7] High levels denote the presence of histologically verifiable, either low- or high-grade cervical cancer precursors.

Because our HPV DNA study examined prevalence rates, it prevented us from knowing whether the two of three postmenopausal women with latent HPV DNA represent recently acquired or persistent infection. Nevertheless, the comparatively higher rates of HPV infection among women under 30 years old concur with the current concept that, in the majority of positive cases, HPV infection is a transient phenomenon.[8–10] The data also suggest that HPV is unlikely to remain clinically important "forever," as it is either destroyed by the host's cell-mediated immune system or viral shedding reaches low levels that prevent detectability by the currently used molecular probing techniques.

There are a number of limitations of this study, particularly with respect to power and the use of a cross-sectional design. The study had a power of 80% based on our assumption of 10% abnormal cervical cytology rates in the hormone users and 5% in nonusers; we assumed 20 and 10% HPV DNA positivity in users and nonusers, respectively. However, the number of women with disease and HPV DNA was very low, so the power was considerably less for detection of increases in rates of HPV-related lesions as well as latent HPV infection. It may be worthwhile to repeat the study in a sample with a larger number of postmenopausal

women with and without hormone therapy. Similarly, because of the very small number of HPV-positive women, we could not determine any association between education, income, sexual behavior, pregnancy history, OC use, and history of STD.

The cross-sectional nature of this study precludes assessment of temporal relationships between HPV exposure and duration of hormone use. One could argue that patients' recall of their history of abnormal Papanicolaou smears or therapy for HPV-related lesions may be inaccurate in the postmenopausal period. However, none of the patients who gave a positive history of therapy for cervical SIL had residual disease by thin-layer cytology, colposcopy, biopsy, and HPV DNA testing, suggesting that these patients were disease- and HPV-DNA free. Although we have not used colposcopy on women whose thin-layer cytology and HPV DNA tests were negative, the very high negative predictive value of double-negative tests provides assurance of an HPV-negative population.[3] Also, the sensitivity of the HPV DNA assay used in cellular residue from liquid-based cytology specimens appears similar to that obtained by the HPV DNA assay manufacturer's collection kit. Indeed, in our previous study,[3] which examined hybrid capture in liquid-based cytologic samples, HPV DNA positivity was 60% in low-grade SILs and 77% in high-grade SILs, whereas 23% of histologically negative patients tested HPV DNA positive.

Despite the aforementioned limitations, it is comforting to know that unlike OC use in premenopausal women, in whom an increased risk of developing cervical neoplasia was found by some,[11] the use of hormones in their postmenopausal counterparts does not appear to be associated with higher rates of either latent or subclinical high-risk HPV infection of the cervix than in their nonuser counterparts. Our study results support the safety of postmenopausal hormone therapy with respect to a lack of an increased susceptibility of HPV infection.

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