

CONCISE COMMUNICATIONS

Use of a Hybrid Capture Assay of Self-Collected Vaginal Swabs in Rural Uganda for Detection of Human Papillomavirus

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A random sample of 960 women aged 15–59 years enrolled in a population-based study in rural Uganda were asked to provide self-collected vaginal swabs for human papillomavirus (HPV) testing by hybrid capture assay. The intensity of HPV infection was assessed by the relative light unit (RLU) ratio in the specimen-to-positive control (PC) ratio. In total, 898 women (93%) provided a swab and 737 provided serum for human immunodeficiency virus type 1 (HIV-1) determination. HPV prevalence was 16.7% and was highest in younger women. HIV-1 prevalence was 17.8%. HPV prevalence was 44.3% in HIV-positive and 10.2% in HIV-negative women (rate ratio, 5.36; 95% confidence interval, 3.81–7.54). The intensity of HPV infection was significantly greater among HIV-positive than HIV-negative women (54.4 vs. 11.1 RLU/PC; $P = .026$); intensity of infection was highest in women aged <30 years. The higher prevalence and intensity of HPV infection in HIV-positive women could facilitate HPV transmission in this population. Self-collected vaginal swabs could be used in population-based screening to identify women at high risk of cervical neoplasia.

Cervical cancer is a major public health problem in the developing world, particularly in sub-Saharan Africa [1, 2], in part because of the inadequacy of screening services for detection of preinvasive and early invasive disease [3]. In addition, human immunodeficiency virus (HIV) infection increases the prevalence and persistence of human papillomavirus (HPV) infection, which is associated with an increased incidence of preinvasive and invasive cervical cancer [4, 5]. Thus, the HIV epidemic has added urgency and complexity to the problems of cervical cancer control, particularly in sub-Saharan Africa,

which has the highest rates of both HIV and cervical cancer in the world [1, 2].

It is well established that cervical cancer is caused by a limited number of cancer-associated HPVs [6, 7]. The detection of infection with cancer-associated HPVs has been proposed as a method of identifying women with cervical neoplasia [8]. Studies suggest that the detection of carcinogenic HPVs in cervical specimens obtained by physicians during pelvic examination are as sensitive and specific as cytology for the detection of high-grade squamous intraepithelial lesions (HSILs) [8]. However, there are important limitations to screening samples obtained during pelvic examination: Such examinations require a trained health professional working in a clinical setting. Health care personnel and facilities are not readily accessible to women in resource-poor settings. In addition, there are often cultural inhibitions that render routine pelvic examination unacceptable to asymptomatic women, limiting the utilization of screening services [9, 10]. Recent pilot studies have shown that HPV detection in self-collected vaginal swabs is comparable to virus detection in swabs obtained directly from the cervical os by a physician during pelvic examination [11–13]. Thus, self-screening for HPV offers a potential opportunity for cancer prevention and population-based research.

We conducted a community-based randomized trial of sexually transmitted disease (STD) control for HIV prevention in a rural area of Uganda [14, 15]. All samples were obtained in

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Written informed consent was obtained from study participants. The study was approved by the Committee for Human Research at Johns Hopkins University, the Institutional Review Board at Columbia University, and the AIDS Research Subcommittee of the Council for Science and Technology, Ministry of Health, Kampala, Uganda.

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Table 1. Prevalence of human papillomavirus (HPV) infection (% HPV) in human immunodeficiency virus (HIV)-positive and HIV-negative women.

Age (years)	Total population		HIV-positive		HIV-negative		Rate ratio (95% confidence interval)
	No.	% HPV	No.	% HPV	No.	% HPV	
15-19	118	25.4	4	75.0	114	23.7	9.67 (0.84-251)
20-29	277	20.6	67	50.8	210	11.0	4.63 (2.95-7.28)
30-39	177	12.4	43	37.2	134	4.5	8.31 (3.47-19.9)
≥40	165	6.7	17	29.4	148	4.1	9.71 (3.30-28.54)
All ages	737	16.3	131	44.3	606	10.2	5.36 (3.81-7.54) ^a

^a Mantel-Haenszel, age-adjusted rate ratio.

the home, and we routinely obtained self-collected vaginal swabs for the detection of *Trichomonas vaginalis* and bacterial vaginosis [15]. Compliance with provision of swabs was excellent, with more than 90% of women consistently providing repeat samples. Therefore, we took advantage of the acceptability of self-administered vaginal swabs in the study communities to evaluate the utility of this approach for HPV detection in a population-based study.

Methods

The community-based randomized trial of STD control for AIDS prevention was conducted in 56 communities in Rakai district, southwestern Uganda. The methods have been described in detail elsewhere [14]. In brief, all consenting resident adults aged 15-59 years were enrolled and followed at intervals of ~10 months. All interviews and sample collection were done in the home, to maximize compliance and coverage. Venous blood was tested for HIV using two EIA assays (Vironostika HIV-1; Organon Teknika, Charlotte, NC, Cambridge Biotech, Worcester, MA), with Western blot confirmation (HIV-1 WB; Bio-Merieux-Vitek, St. Louis).

Women were asked to provide self-collected vaginal swabs for STD determination [14, 15]. Subjects were instructed to squat, insert a 20-cm Dacron or cotton-tipped swab into the vagina and to rotate the swab high in the vaginal vault. During the third study round (May 1996-February 1997), 7588 women aged 15-59 years consented to participate in the study, representing 92% of all resident women in the communities at the time of the survey. We randomly selected 960 women (12.5% of those enrolled) and asked them to provide additional self-collected vaginal swabs for HPV detection. HPV samples were provided by 898 subjects (93% compliance). After collection, the women handed the swabs to a field worker who placed them in specimen transport medium (Digene Diagnostics, Silver Spring, MD). The specimens were maintained in a cold box ≤8 h until they were frozen at -20°C at the field laboratory. In addition, 82% (n = 737) of the women also provided a venous blood sample for HIV serology.

Vaginal swab specimens were transported to Johns Hopkins University and tested for HPV using the Digene Hybrid Capture II (HCII) probe B microtiter assay according to the manufacturer's instructions [8]. Specimens were denatured, and 75 µL of sample was hybridized with 25 µL of probe B. (Probe B is a pool of full-length RNA probes for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.) During hybridization, the RNA probes bind specifically to the HPV DNA that may be present in the

specimens to form RNA-DNA hybrids. The reactions are then transferred to the capture microtiter plate coated with an antibody specific for the RNA/DNA hybrids. The immobilized hybrids are reacted with a second RNA-DNA antibody conjugated to alkaline phosphatase and detected by the addition of a chemiluminescent substrate. Light emitted when the substrate is cleaved by alkaline phosphatase is measured as relative light units (RLUs). Specimens are classified as positive if the ratio of the RLUs of the specimens to the RLUs of the positive control (PC) of 1 pg/mL (RLU/PC) ratio is ≥1. The intensity of the emitted light is proportional to the amount of target DNA in the specimens, so the RLU/PC ratio also provides a semiquantitative measure of the virus burden and hence an estimate of the amount of HPV in the genital tract, which provides a proxy measure for the intensity of infection.

Data analyses estimated the prevalence and the prevalence rate ratio (RR) of HPV infection in HIV-positive and -negative women. Tests of statistical significance were based on the 95% confidence intervals (CIs) of the RRs or χ² test. In addition, among HPV-infected women, we estimated the median RLU/PC ratios to quantify the total cancer-associated HPV burden. The t test or analysis of variance was used to test statistical significance for differences in RLU/PC ratios among HIV-positive and -negative subjects.

Results

Cancer-associated HPVs were detected by the HCII assay in 150 (16.7%) of 898 subjects; 161 women provided a swab but declined to provide a serum sample. Their sociodemographic and behavioral characteristics were similar to those of 737 women who provided both a swab and blood specimens (results not shown). Therefore, our analyses focus on these latter 737 women, 120 of whom had HPV detected (prevalence, 16.3%); 131 (17.8%) were infected with HIV-1.

Table 1 shows the age-specific prevalence of HPV in HIV-positive and -negative women. The prevalence of HPV was 10.2% in 606 HIV-negative women, compared with 44.3% in the 131 HIV-positive women, and the age-adjusted RR was 5.36 (95% CI, 3.81-7.54). In HIV-negative women, the prevalence of HPV was highest in those aged 15-19 years (23.7%), decreased to 11.0% in those aged 20-29 years, and remained at 4% in women aged ≥30 years. However, in HIV-positive women, the prevalence of HPV was markedly higher in those aged 15-19 years (75.0%) and remained persistently elevated even in women aged ≥30 years. The RRs of HPV infection

among HIV-positive relative to HIV-negative women were statistically significant in women aged >20 years and increased with age thereafter (χ^2 for trend = 101.7, $P < .0001$).

Data on the semiquantitative index of the intensity of HPV infection (the RLU/PC ratio) were available for 120 HPV-infected women: 58 HIV positive, 62 HIV negative. Among the 58 HIV-positive subjects with concurrent HPV infections, the median RLU/PC ratio was 54.37 (SE \pm 13.2), compared with a median ratio of 11.08 (SE \pm 3.6) in the 62 HIV-negative subjects ($P = .026$). The differences in RLU/PC ratios between HIV-positive and -negative women varied markedly with age. In younger women (aged <30 years), the median RLU/PC ratio was 78.32 ± 25.8 for HIV-positive women, compared with 13.30 ± 4.4 in HIV-uninfected women ($P < .01$). In women aged ≥ 30 years, the RLU/PC ratios were 12.36 and 7.81 in HIV-positive and -negative subjects, respectively ($P > .05$).

Figure 1 shows the median and interquartile range of RLU/PC ratios by age and HIV status. There was considerable variability in the upper quartile range of the RLU/PC ratios. In the younger HIV-positive women, it is likely that the upper RLU/PC range was compromised by the inability of the HCII test to accurately measure RLU/PCs $>10^7$. However, among HIV-infected women aged <30 years, the median and lower quartile range was substantially higher than in HIV-negative women or in older women (aged ≥ 30 years), irrespective of HIV status. Thus, despite the variability in this measure, there is evidence of a heavier genital tract HPV burden in younger HIV-positive women.

Discussion

This cross-sectional study suggests that self-collected vaginal swabs can provide useful information on the prevalence of HPV infections in general populations and on cofactors such as HIV infection. This approach to specimen collection was well accepted by the women, with compliance rates $>90\%$. Small pilot studies in North American and European populations also suggest that self-collected swabs or tampons are acceptable to women and that results are comparable to those of physician-collected swabs for HPV detection [11–13].

We could not conduct pelvic examinations to obtain cytology smears or perform colposcopy in the home, so we do not know whether the women with HPV-positive swabs had evidence of cervical neoplasia. However, other investigators have shown that HPV detection is comparable to or more sensitive than Pap smears for detection of cervical neoplasia [8]. Thus, in this resource-poor setting, detection of cancer-associated HPVs from self-collected vaginal swabs could minimize the needs for health personnel and facilities and may provide an alternative means of sample collection for cervical cancer screening.

Self-collected vaginal swab specimens could help overcome barriers to cervical screening. In many settings, particularly in developing countries, access to clinical facilities is limited, and

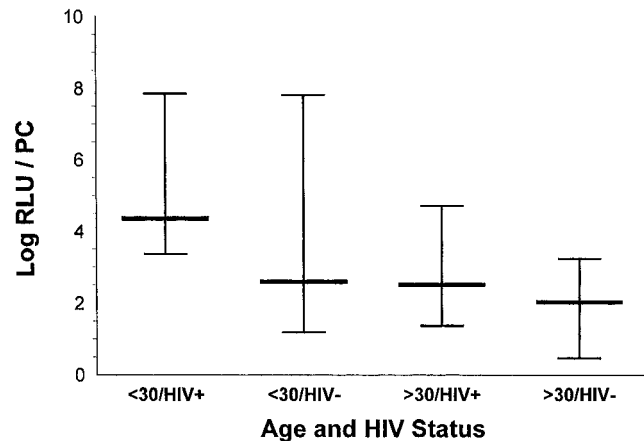


Figure 1. Intensity of human papillomavirus infection in genital tract, shown as median and interquartile range of log light units in specimen relative to positive controls (log RLU/PC) by age (<30 or >30 years) and human immunodeficiency virus (HIV)–type 1 status.

women are reticent to undergo pelvic examinations unless they have symptoms of genital pathology. This results in late presentation of advanced and often incurable malignancies [9]. Access to self-collected swabs might provide a more acceptable method of screening and improve service utilization by asymptomatic women. Better screening of such asymptomatic subjects would facilitate early detection of preinvasive cancers and could, potentially, be cost-effective.

To the best of our knowledge, these data on HPV infection are the first available from a general population in rural Africa. The results suggest that HPV infection is highly prevalent in younger women and declines markedly with age in HIV-negative women. However, infection in women aged ≥ 30 years was relatively common in Rakai, especially among HIV-positive subjects. The latter observation is cause for concern, as other studies indicate that persistent HPV infection in older women is associated with HSILs or invasive carcinoma [8]. Also, cancer registry data show that east and central Africa have the highest rates of invasive cervical cancer in the world, and Ugandan surveillance studies suggest that the rates of invasive cervical cancer have been increasing markedly in recent years [2].

Rakai has an advanced HIV epidemic, and in the present study sample, 17.8% of women were infected with HIV-1. We observed high rates of HPV prevalence and intensity of HPV infection in women with concurrent HIV infection (table 1; figure 1), which is consistent with data on high incidence and persistence of HPV infection in HIV-positive women observed in clinic-based studies [4, 5]. Thus, it is possible that the HIV epidemic, which is particularly severe in southwestern Uganda, may be promoting a secondary epidemic of HPV by increasing the proportion of women who shed HPV and the intensity of infection in these women. This, in turn, may result in invasive cervical cancer both in the HIV-positive and possibly in HIV-

negative women who experience higher HPV exposure through their partners than would occur in a population with little or no HIV.

In conclusion, self-collection of vaginal samples for HPV detection is feasible and acceptable to women in a rural African setting. This approach offers potential for future cervical cancer screening programs in resource-poor settings with limited access to cytology services. We plan to further evaluate self-collected vaginal swabs for HPV detection and cytology as a method of detecting colposcopically diagnosed HSILs or invasive cancer.

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