IMPLEMENTATION OF CYTOLOGIC screening programs has been associated with a 5-fold reduction in the incidence of invasive cervical cancer in North America over the last 4 decades. However, cytologic screening programs have been difficult to implement in many resource-poor regions of the world. Approximately 360,000 cases of cervical cancer were diagnosed in 1990 worldwide, accounting for approximately 190,000 deaths. Even in countries where cytologic screening is routinely performed, cervical cancer continues to be a health problem. This year approximately 12,800 women will be diagnosed as having cervical cancer in the United States and 4800 women will die of this disease. The failure of cytologic screening programs to eliminate cervical cancer in regions where they are widely available is due to many factors including the inherent false-negative results rate of the Pap smear, failure of both clinicians and patients to act on abnormal test results, and, perhaps most importantly, underscreening of the population at risk. More than half of women developing cervical cancer in the United States have not had a Pap smear within the last 3 years, despite contact with the health care system. In many other regions of the world, there is only limited access to cervical cancer screening.

**Objective** To determine whether testing of self-collected vaginal swabs for human papillomavirus (HPV) DNA can be used to screen for cervical disease in women aged 35 years and older.

**Design** Cross-sectional observational study comparing Papanicolaou smears with HPV DNA testing of self-collected vaginal swabs.

**Setting** Outpatient clinics in a periurban settlement outside of Cape Town, South Africa, between January 1998 and April 1999.

**Participants** Screening was performed on 1415 previously unscreened black South African women aged 35 to 65 years.

**Intervention** Women self-collected a vaginal swab for HPV testing in the clinic and were then screened using 4 different tests: Papanicolaou smear, direct visual inspection of the cervix after the application of 5% acetic acid, cervicography, and HPV DNA testing of a clinician-obtained cervical sample. Women with abnormal results on any of the screening tests were referred for colposcopy.

**Main Outcome Measure** Biopsy-confirmed high-grade cervical squamous intraepithelial lesions or invasive cancer.

**Results** High-grade squamous intraepithelial lesions were identified in 47 (3.4%) of 1365 women adequately assessed, and there were 9 cases of invasive cancer. Of women with high-grade disease, 66.1% (95% confidence interval [CI], 52.1%-77.8%) had high-risk HPV detected in self-collected vaginal samples, and 67.9% (95% CI, 53.9%-79.4%) had an abnormal Papanicolaou smear (P = .78). The false-positive rates for HPV DNA testing of self-collected vaginal samples and Papanicolaou smears were 17.1% (95% CI, 15.1%-19.3%) and 12.3% (95% CI, 10.5%-14.2%), respectively (P < .001). A high-risk type of HPV DNA was detected in 83.9% (95% CI, 71.2%-91.9%) of women with high-grade disease and 15.5% (95% CI, 13.6%-17.7%) of women with no evidence of cervical disease using a clinician-obtained cervical sample.

**Conclusions** These results indicate that HPV testing of self-collected vaginal swabs is less specific than but as sensitive as Papanicolaou smears for detecting high-grade cervical disease in women aged 35 years and older, and HPV testing offers an important new way to increase screening in settings where cytology is not readily performed.
Pap smears are often not obtained during routine medical examinations by primary clinicians who provide care. Some possible explanations for this include the inconvenience, time, and discomfort often involved with obtaining Pap smears in older patients. Also, clinicians frequently assume that Pap smears are being obtained elsewhere, and many male physicians feel uncomfortable obtaining Pap smears and taking sexual histories. The availability of a noncytologic screening method not requiring a vaginal speculum examination may reduce underscreening in women who have access to health care. A self-collected screening method may also be expected to increase access to screening in many resource-poor areas where there are limited numbers of clinicians trained in performing speculum examinations.

Human papillomavirus (HPV) DNA testing of clinician-obtained cervical samples has a sensitivity for detection of high-grade cervical squamous intraepithelial lesions (SILs) and invasive cervical cancer that is equivalent or superior to that of a Pap smear. However, clinician-obtained HPV samples share many of the same drawbacks as cytologic screening since they require a gynecological examination. In this study, we evaluated patient-obtained vaginal samples to determine if self-sampling for HPV DNA could provide an alternative for older women.

METHODS

Population Studied

Previously unscreened South African black women between the ages of 35 and 65 years were enrolled from an ongoing cervical cancer screening study between January 1998 and April 1999. All were volunteers and informed of the study through posters placed in health centers and a variety of community-based outreach programs that included presentations at churches, meetings, and radio programs. The study was conducted in a periurban settlement 20 km from Cape Town. Both the Columbia University Institutional Review Board, New York, NY, and the University of Cape Town Ethics and Research Committee, approved this study. All women provided written informed consent and completed a questionnaire at the enrollment.

Clinical Examination

At the clinic, women were instructed to collect a vaginal sample for HPV DNA testing. This was done by inserting a sterile Dacron swab into the vagina and rotating it 3 times before placing it into a HPV DNA specimen collection tube (Specimen Transport Media, Digene Corp, Beltsville, Md). The self-collected sample was obtained while the women squatted in the examination room. A gynecological examination was then performed by a certified nurse. The examination followed a specific sequence: (1) a Pap smear was obtained using an Accellon Combi cervical biosampler (MedScand, Hollywood, Fla); (2) an HPV DNA sample was obtained from the cervix using a special conically shaped brush and placed into an HPV DNA collection tube; (3) vaginal swabs were obtained and placed into Diamond’s media for culture of Trichomonas vaginalis and a Dacron swab used to collect material from the external cervical opening that was placed into a specimen collection tube (Digene Corp) for Neisseria gonorrhoeae and Chlamydia trachomatis DNA testing using the Hybrid Capture GC/CT DNA Assay (Digene Corp); (4) direct visual inspection of the cervix was performed with and without magnification using a 2.5 × hand-held magnification device (Selsi, Edmund Scientific, Barrington, NJ) following application of 5% acetic acid solution; and (5) cervicography (ie, 35-mm photograph of the cervix) was taken. All women were asked to return to the clinic within 2 to 6 days after the initial examination.

Laboratory Testing

Human papillomavirus DNA status was initially determined at the University of Cape Town using the clinician-obtained cone brush sample and the second-generation Hybrid Capture II HPV DNA Assay (Digene Corp). The test was run according to the manufacturer’s protocol using the microtiter plate based format and probes for “high carcinogenic risk” HPV types (ie, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Human papillomavirus determinations were quantitative, and women with samples producing readings of 1 or more times the positive control (1 pg/mL HPV DNA or 5000 HPV genome copies per test) were recalled for colposcopy. In addition, all women with positive direct visual inspection examinations (eg, all acetowhite lesions, ulcerations, or exophytic lesions of the cervix) underwent colposcopy when they returned to the clinic 2 to 6 days after the initial examination. Women with negative HPV DNA test results and direct visual inspection examinations were told to return to the clinic again in approximately 8 weeks, for their Pap smear and cervicography results.

Pap smears were evaluated at the University of Cape Town and diagnosed using the Bethesda system. The cytology laboratory at the University of Cape Town uses similar workload limits and quality control rescreening of normal Pap smears as US laboratories. Cervicography results were evaluated at National Testing Laboratories, Inc, and reported using its standard terminology. Women with cytology results of low-grade SIL (LSIL) or high-grade SIL (HISIL), cancer, or who had positive cervicography results were referred for colposcopy, if they had not already been referred on the basis of either their direct visual inspection examination or their HPV DNA test results. Women with cytology results of atypical squamous cells of undetermined significance (ASCUS) were not referred for colposcopy because the criteria for diagnosing ASCUS are highly variable between laboratories and because there is little worldwide consensus on its clinical significance.

Human papillomavirus DNA testing of self-collected vaginal samples was performed at Columbia University using the second-generation Hybrid Capture II HPV DNA Assay. All HPV DNA testing and evaluation of Pap smears, cervical...
biopsies, and cervicography were performed without knowledge of the patient’s other test results.

**Colposcopic Examinations**

Colposcopy was performed on site and lesions were graded using the Reid index.\(^1\) Biopsies were taken for minor-grade lesions (Reid score, \(<3\)), whereas high-grade lesions (Reid score, \(\geq3\)) were immediately treated using loop electrosurgical excision. An endocervical curettage was performed if no lesions were visible, irrespective of the location of the squamocolumnar junction. Although both the vulva and vagina were carefully inspected, a formal colposcopic examination of the vagina using 5% acetic acid and Lugol iodine solution was not performed. All biopsy, loop excision, and endocervical curettage specimens were evaluated blindly at Columbia University (T.C.W).

**Statistical Analyses**

To compare the performance of HPV DNA testing to cytology, we calculated the capacity of each test to detect all cases of biopsy-confirmed HSIL (cervical intraepithelial neoplasia [CIN], 2-3) and invasive cervical cancer detectable during the course of the study. We also calculated the capacity of each test to classify correctly women without disease. Women were considered to be free of disease (eg, no evidence of cervical disease) if any grade SIL and invasive cervical cancer were ruled out on histological sampling following colposcopy among women with 1 or more positive screening test results or if none of the 4 screening test results were available. We compared the sensitivity and specificity of the screening tests by calculating the ratios of sensitivity (true-positive rate) and of false-positivity (1 – specificity) of the HPV DNA test results to cytology and tested whether there were differences using the McNemar test.\(^17,18\)

**RESULTS**

**Prevalence of Disease**

The median age of women recruited into the cervical cancer screening study was 39 years, with 18.4% aged 50 years or older. Sixty-three percent live on sites with basic water and sanitation services (Table 1). Of the 1415 women screened, 550 (38.9%) had positive test results for 1 or more of the screening tests and were referred for colposcopy and biopsy. This included 95 women with a LSIL or higher-grade abnormal Pap smear, 302 women with HPV DNA detected on the clinician-obtained cervical sample assayed in Cape Town, 278 women with a positive direct visual inspection examination, and 132 women with a positive cervicography result. More than 1 positive screening test result was obtained from 181 (12.8%) of the women. Of the 550 women referred for colposcopy, 500 (90.9%) underwent a colposcopic examination and have available biopsy results. Biopsy-confirmed HSIL (CIN, 2-3) was identified in 47 women (3.4%), LSIL (CIN, 1) in 40 (2.9%), and invasive cancer in 9 (0.7%) of the 1356 women screened (excluding the 50 referred for but who did not undergo colposcopy). The demographic profile of the women who underwent screening is displayed in Table 1. *Trichomonas vaginalis* was detected by cultures of clinician-obtained vaginal swabs in 203 (18.6%) of the women and 95 (6.7%) had either *C. trachomatis* or *N. gonorrhoeae* DNA identified in cervical samples.

**Identification of Disease by Different Screening Tests**

Human papillomavirus DNA of a high-risk HPV type was identified among the patient-collected swabs in 37 (66.1%) of the 56 women with high-grade cervical disease (47 cases of HSIL [CIN, 2-3] and 9 cases of invasive cervical cancer) and in 217 (17.1%) of women with no evidence of disease (Table 2). Using the clinician-collected cervical samples, HPV DNA was detected in 47 (83.9%) of the 56 women with biopsy-confirmed high-grade disease and in 197 (15.5%) of the women with no evidence of disease.

Of the 56 women with biopsy-confirmed HSIL (CIN, 2-3) or invasive cancer, 34 (60.7%) had an LSIL or higher-grade abnormal Pap smear and 38 (67.9%) had an ASCUS or a higher-grade-abnormal smear. Only 40 (3.3%) of the women with no evidence of cervical disease had an abnormal smear when only smears diagnosed as LSIL or higher grade were classified as abnormal. This number increased to 154 (12.1%) when Pap smears diagnosed as ASCUS or higher were classified as abnormal. The group of women classified as having no evidence of cervical disease included 865 women with 4 negative screening test results, as well as 404 women with 1 or more abnormal screening test result found to have no cervical disease by colposcopy and cervical biopsy.

**Positive Predictive Value**

Of the 280 women with HPV DNA detected on their self-collected sample who underwent colposcopy, 37 (13.2%) had HSIL (CIN, 2-3) or invasive cervical cancer, 26 (9.3%) LSIL (CIN, 1), and 217 (77.5%) were free of disease (Table 2). Among the 275 women with HPV DNA detected on their clinician-collected sample who underwent colposcopy, 47 (17.1%) had biopsy-confirmed high-grade disease, 31 (11.3%) LSIL (CIN, 1), and...
and 197 (71.6%) were classified as having no evidence of cervical disease. Among 87 women with LSIL or higher-grade Pap smear who underwent colposcopy, 34 (39.1%) had biopsy-confirmed high-grade disease, 13 (14.9%) LSIL (CIN, 1), and 40 (46.0%) had no evidence of cervical disease by colposcopy and biopsy (Table 2).

**Sensitivity and Specificity**

The sensitivity of HPV DNA testing of a self-collected vaginal sample (66.1%; 95% confidence interval [CI], 52.1%-77.8%) for detection of HSIL (CIN, 2-3) or cancer was equivalent to that of conventional Pap smear when LSILs or higher cytologic abnormalities were classified as a positive test result (60.7%; 95% CI, 46.7%-73.2%; P = .58), or when any degree of cytologic abnormality, including ASCUS was classified as positive cytologic test results (67.9%; 95% CI, 53.9%-79.4%; P = .78). In contrast, the sensitivity of HPV DNA testing of a clinician-collected cervical sample (83.9%; 95% CI, 71.2%-96.0%) was significantly greater than the sensitivity of HPV DNA testing of a self-collected vaginal sample (P = .01). Both self-collected and clinician-collected samples tested for HPV DNA had significantly higher false-positive rates than did cytology. The false-positive rate for HPV DNA testing of self-collected cervical samples was 17.1% (95% CI, 15.1%-19.3%) and for clinician-collected samples, it was 15.5% (95% CI, 13.6%-17.7%). In comparison for cytology, it was 12.3% (95% CI, 10.5%-14.2%) when smears diagnosed as ASCUS or higher were classified as positive and 3.2% (95% CI, 2.3%-4.4%) when smears diagnosed as LSIL or higher were classified as positive (P≤ .01, for all comparisons). The false-positive rates for the self-collected samples and the clinician-collected samples did not differ (P = .20).

**Agreement Between the 3 Tests**

There was fair agreement between cytology and either the self-collected vaginal swabs assayed for HPV DNA (κ = 0.28) or the clinician-collected cervical swabs assayed for HPV DNA (κ = 0.35). Of 38 women with biopsy-confirmed HSIL (CIN, 2-3) or invasive cancer who had ASCUS or higher Pap smear, 31 (81.6%) were positive for high-risk HPV DNA using a self-collected vaginal sample. Human papillomavirus DNA testing of self-collected swabs identified 37 women with high-grade disease, 6 of whom would have been missed using cytology alone (Table 3).

There was moderate agreement between the results obtained with HPV DNA testing of self-collected vaginal swabs and clinician-collected vaginal swabs (κ = 0.45) (Table 4). Overall, there was an 82% concordance between the HPV DNA tests performed on the 2 different samples. Of the 47 women with HSIL (CIN, 2-3) or invasive cancer who had HPV DNA identified on their clinician-obtained sample, 36 (77%) also had HPV DNA detected on their self-obtained vaginal swabs. One (11%) of 9 women with high-grade disease who did not have HPV DNA detected on the clinician-obtained sample had HPV DNA detected on the self-collected vaginal swab.

**Age Differences in Test Performance**

No significant differences existed in the prevalence of high-grade disease, prevalence of abnormal cytologic results, or

Table 2. Results of Papanicolaou Smear and Human Papillomavirus (HPV) DNA Testing Stratified by Cervical Disease Status in 1415 Women*

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Evidence of Disease (n = 1269)†</th>
<th>Low-Grade SIL (CIN, 1) (n = 40)</th>
<th>High-Grade SIL (CIN, 2-3) (n = 47)</th>
<th>Invasive Cervical Cancer (n = 9)</th>
<th>Total (N = 1415)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papanicolaou smear results</td>
<td>Unsatisfactory</td>
<td>13 (1.02)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Within normal limits</td>
<td>1102 (86.8)</td>
<td>18 (45.0)</td>
<td>18 (38.3)</td>
<td>0 (0.00)</td>
<td>1174 (83.0)</td>
</tr>
<tr>
<td>ASCUS</td>
<td>114 (8.96)</td>
<td>9 (22.5)</td>
<td>3 (6.8)</td>
<td>1 (11.1)</td>
<td>131 (9.26)</td>
</tr>
<tr>
<td>Low-grade SIL</td>
<td>32 (2.52)</td>
<td>8 (20.0)</td>
<td>4 (8.5)</td>
<td>1 (11.1)</td>
<td>49 (3.46)</td>
</tr>
<tr>
<td>High-grade SIL</td>
<td>8 (0.63)</td>
<td>5 (12.5)</td>
<td>21 (44.7)</td>
<td>4 (44.4)</td>
<td>42 (2.97)</td>
</tr>
<tr>
<td>Cancer</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (2.13)</td>
<td>3 (33.3)</td>
<td>4 (0.28)</td>
</tr>
<tr>
<td>Clinician-collected cervical HPV DNA sample result</td>
<td>Negative</td>
<td>1072 (84.5)</td>
<td>9 (22.5)</td>
<td>8 (17.0)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Positive</td>
<td>197 (15.5)</td>
<td>31 (77.5)</td>
<td>39 (83.0)</td>
<td>8 (88.9)</td>
<td>302 (21.3)</td>
</tr>
<tr>
<td>Self-collected vaginal HPV DNA sample result</td>
<td>Negative</td>
<td>1052 (82.9)</td>
<td>14 (35.0)</td>
<td>17 (36.2)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Positive</td>
<td>217 (17.1)</td>
<td>26 (65.0)</td>
<td>30 (63.8)</td>
<td>7 (77.8)</td>
<td>298 (21.1)</td>
</tr>
</tbody>
</table>

*SIL indicates squamous intraepithelial lesions; CIN, cervical intraepithelial neoplasia; and ASCUS, atypical squamous cells of undetermined significance.
†No evidence of disease includes 865 women with 4 negative screening test results and 404 women with 1 or more abnormal screening test result found to have no disease at colposcopy.
‡Numbers in the 4 columns do not add up to totals because 50 women did not receive follow-up.
testing of HPV DNA using either the clinician-collected or self-collected samples by age. Nor were there significant differences in the performance of the tests by age, except for the prevalence of abnormal cytologic results, which increased with age among those with biopsy-confirmed HSIL (CIN, 2-3) and invasive cancer (P = .05).

**COMMENT**

In the setting of primary screening for cervical disease, testing of self-collected vaginal samples for high-risk HPV types detected as many cases of high-grade SIL (CIN, 2-3) and invasive cervical cancer as did a conventional Pap smear. Of the cases of HSIL (CIN, 2-3) and cervical cancer identified in this study, 66.1% would have been detected through HPV DNA testing of a self-collected vaginal sample, whereas 67.9% would have been detected by a Pap smear alone. These findings suggest that in clinical settings where cytologic screening is not routinely available, self-collected HPV DNA tests could be used to identify older women at high risk for having cervical disease. For example, in resource-poor areas such as most of sub-Saharan Africa, it has proved difficult to train sufficient numbers of clinicians to implement a comprehensive cytologic screening program. In these areas, HPV DNA testing of self-collected vaginal samples could be used to rapidly screen women attending community-based clinics for other reasons. Women found to be positive for “high carcinogenic risk” HPV-types could be either rescreened using a Pap smear or another screening test such as direct visual inspection of the cervix or immediately treated using cryosurgery, once the safety and effectiveness of this approach has been documented. Under screening accounts for a significant number of cases of invasive cervical cancer in the United States. Among the approaches that could be taken to reduce underscreening, one is to encourage primary care providers to obtain annual or biannual Pap smears. However, as long as screening requires a speculum examination, possible barriers to screening such as lack of proper examination tables, time constraints on practitioners, the misconception that screening will be performed elsewhere, and a patient’s aversion to undergoing

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pelvic examinations may be difficult to overcome. Testing for HPV DNA by self-collected vaginal swabs at the time a woman provides a routine urine sample would eliminate the need for a speculum examination and would convert cervical cancer screening to a simple laboratory test. In addition to being used for HPV DNA testing, self-collected vaginal swabs have also been shown to be acceptable as a method to detect C. trachomatis, bacterial vaginosis, and T. vaginalis.23-26

Although HPV DNA testing of self-collected vaginal samples has a number of advantages for selected populations, this approach is not without limitations. One limitation is that simply identifying women as being high-risk for HPV does not guarantee that they will return for either colposcopic evaluation or treatment. Another potential limitation of screening using self-collected vaginal samples is that HPV DNA testing has a lower specificity than cytologic screening and that the sensitivity of HPV DNA testing of self-collected specimens is significantly less than that of HPV DNA testing of clinician-obtained cervical specimens.

There are many differences between the African women included in this study and older women in the United States and Europe. The prevalence of high carcinogenic risk HPV DNA positivity was 17% in the Cape Town women with no evidence of cervical disease and other sexually transmitted diseases. There was a very high rate of biopsy-confirmed SIL and sexually transmitted diseases including trichomoniasis, chlamydia, and gonorrhea. In addition, 12% of the conventional Pap smears collected from women with no evidence of cervical disease were diagnosed as ASCUS or higher. A much lower prevalence of HPV DNA positive results has been observed among older women with no evidence of cervical disease in studies of lower risk women from the United Kingdom and Europe.8,27,28

For example, Meijer et al29 detected HPV DNA of any type using a polymerase chain reaction method in only 4.8% of Dutch women older than 35 years reporting for routine cytologic screening. Although we have not yet evaluated the clinical utility of HPV DNA testing of self-collected vaginal swabs in such lower risk populations, we would expect that fewer women would be HPV DNA positive, although the sensitivity is unlikely to change. In populations with a high prevalence of HPV infections, such as in Cape Town, HPV DNA testing of self-collected vaginal samples might be useful for identifying women at greatest risk for having cervical disease. Human papillomavirus DNA testing of self-collected vaginal samples provides an opportunity to extend cervical cancer screening coverage to large numbers of unscreened women in the United States and in resource poor areas.

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Financial Disclosure: Dr Lorincz is scientific director of and holds stock options in Digene Corp, which produces and distributes the Hybrid Capture II assay used in this study. All HPV DNA assays were performed at either the University of Cape Town or at Columbia University. Dr Lorincz had no direct oversight over any of the assays.

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