Promoter Hypermethylation of Tumor Suppressor Genes in Urine from Patients with Cervical Neoplasia

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Abstract

We examined the feasibility of using detection of high-risk human papillomavirus (HPV) DNA in combination with the presence of aberrantly methylated genes (DAPK1, RARB, TWIST1, and CDH13) for urine-based cervical cancer screening. Urine samples from 129 Senegalese women, aged 35 years or older, 110 with (same day) biopsy-proven cervical neoplasia [cervical intraepithelial neoplasia grade 1 (CIN-1): n = 9; CIN-2–3/carcinoma in situ (CIS): n = 29; invasive cervical cancer (ICC): n = 72], and 19 without cervical neoplasia on biopsy were examined. Hypermethylation of at least one of the four genes identified 62% of ICC and 28% of CIN-2–3/CIS and was present in only 4% of CIN-1 or normal urines. High-risk HPV DNA was detected in urine in 70% of those with biopsy-proven ICC, 59% of those with CIN-2–3/CIS on biopsy, 44% of those with CIN-1 on biopsy, and only 11% of women negative for cervical neoplasia on biopsy. Urine-based detection of either high-risk HPV or hypermethylation of any of the four genes identified 84% of ICC, 64% of CIN-2–3/CIS, 44% of CIN-1, but only 19% of women negative for cervical neoplasia. The sensitivity for detection of CIN-2–3/CIS/ICC by high-risk HPV DNA or aberrant DNA methylation of four genes seems to be comparable to that of an exfoliated cervical cytology. This study shows the potential feasibility of using molecular markers detected in urine for cervical cancer screening. (Cancer Epidemiol Biomarkers Prev 2007;16(6):1178–84)

Introduction

Cervical cancer control has traditionally been based on cytologic screening for, and ablative treatment of, cervical cancer precursor lesions, termed cervical intraepithelial neoplasia grade 3 (CIN-3) and carcinoma in situ (CIS). This approach has been highly effective in preventing progression to invasive cervical cancer (ICC; refs. 1, 2) and has led to a dramatic decrease in the incidence of ICC in areas where routine cytology-based screening has been established. However, identification of cervical cancer precursors relying on microscopic examination of exfoliated cervical cells is associated with many problems, including the low test sensitivity (30-60%) of a single Pap smear to detect high-grade neoplasia, which had led to the practice of frequent repeat screening and referral of women with any abnormality for additional testing. Furthermore, traditionally exfoliated cervical samples have been obtained during a pelvic examination, which requires a visit and/or pelvic examination and are usually performed by trained healthcare providers. Although recently developed prophylactic human papillomavirus (HPV) vaccines for the prevention of infection with high-risk types of HPV found in the majority of cervical cancer will be central to cervical cancer control (3-5), cervical cancer screening will be necessary for the foreseeable future because of the low sensitivity for identification of women with CIN-2–3/CIS on biopsy and only 11% of women negative for cervical neoplasia on biopsy. Furthermore, these vaccines do not offer protection to most of the millions of women already infected with oncogenic HPV. Thus, development of more cost-effective approaches for identification of women at risk of progression to ICC is of considerable importance.

A variety of different approaches have been proposed to simplify cervical cancer screening. HPV DNA–based triage of women with Atypical squamous cells of undetermined significance (ASCUS) has been widely adapted to reduce the number of women undergoing colposcopy and biopsy. A number of studies, including several large ongoing studies, have examined the feasibility of primary HPV detection based on testing of clinician-obtained cervical samples (6-11). Such an approach is of interest because it might eliminate the need for cytology. Furthermore, it would provide high sensitivity for identification of women with CIN-3 (over 85%). However, because up to 30% of young sexually active women will test positive for high-risk types of HPV (6), overall, this approach would require additional testing of a large proportion of those screened.

There has also been considerable interest in approaches that would simplify screening by eliminating the need for a clinic visit and/or pelvic examination by making use of self-collected vaginal (12-21) or urine samples (22-29) as the basis of testing. A few early studies attempted to use microscopic examination of such exfoliated samples for the identification of CIN-2–3/CIS, but found that this approach did not provide adequate sensitivity for identification of women with cancer or CIN-2–3/CIS (30, 31). More recent studies have focused on the detection of HPV DNA in self-collected vaginal or urine samples (13, 20, 24-27, 29, 32, 33). Although the sensitivity for identification of women with CIN-2–3/CIS based on the detection of high-risk types of HPV in self-collected vaginal specimens has ranged from 49% to 100%, given the fact that HPV genital infections are exceedingly common, not surprisingly, as with detection of high-risk types of HPV in clinician-based cervical samples, specificity for the identification of women with CIN-2–3/CIS is low (ranging from 54% to 73%; refs. 13, 20, 25). Furthermore, the relatively few data available...
on detection of high-risk types of HPV DNA in vaginal samples of women with invasive cervical cancer suggest that the sensitivity of HPV detection may be low in this setting (9, 15).

Previous studies examining the detection of HPV in urine for the identification of biopsy-proven CIN-2–3/CIS/ICC (25, 33) reported sensitivities of near 50% for detection of CIN-2–3/CIS. As in cervical or vaginal specimens, detection of HPV DNA in urine seems to lack specificity for detection of CIN-2–3/CIS or invasive cervical cancer. One study reported a specificity of 70% (25). Thus, testing for high-risk types of HPV in urine, by itself, may not be able to provide the necessary sensitivity and specificity for CIN 3/CIS or cancer. To attempt to improve performance of urine-based cervical cancer screening, investigators have attempted, without much success, testing for a variety of different cervical cancer biomarkers such as loss of heterozygosity (LOH) markers (34, 35), telomerase activity (34, 36-38), and several proliferative markers (cyclin E, p16, MIB1, Ki67, MCMs; refs. 34, 38).

In our previous studies, we explored the use of detection of aberrant DNA methylation as a biomarker for cervical neoplasia and identified a panel of four genes that provided sensitivity of 70% specificity of 81% for detection of CIN-2–3/CIS/ICC (39). We hypothesized that cancer-derived methylation markers might be detected in urine of patients with cervical neoplasia and undertook the present ‘proof of principle’ study to determine whether the detection of aberrantly methylated genes in urine, either alone or in combination with the detection of high-risk types of HPV DNA, might be of interest for cervical cancer screening.

**Materials and Methods**

**Study Samples, Cytology, and Histology Methods.** Urine samples with corresponding cervical cytology and same day biopsies were available from 129 Senegalese women (35 years or older) recruited from community-based clinic for a previously reported study examining the prevalence of cervical neoplasia and its relationship to HPV infection. The methods for recruitment of women, collection, and examination of cervical cytology and biopsy specimens have been previously described (39, 40). Briefly, exfoliated cells were collected into PreservCyt (Cytyc Corporation) for cytology examination, and a separate exfoliated sample was placed into STM media for molecular assays. The Thin Prep smears were considered as negative, ASCUS, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), CIS, or ICC. All women who were found to have abnormal cytologic findings (ASCUS or worse), all those who were positive for high-risk types of HPV, and a small subset of those who were negative by both cytology and HPV testing were immediately referred for colposcopy and biopsy (within 1 month). At their return visit, all these women were asked for a urine sample and underwent a colposcopy and biopsy. Biopsy material was placed into formalin for histopathologic examination by the study pathologist (N.K.) as negative, reactive atypical changes, CIN-1, CIN-2, CIN-3/CIS, or ICC. All samples were interpreted by the study pathologist (N.K.) without knowledge of clinical or other laboratory findings. Urine samples were kept at 4°C and processed within 24 h.

Cell pellets were frozen and shipped to Seattle where they remained frozen at −80°C until assayed as described below. All study procedures were approved by the institutional review boards of the University of Washington and University of Dakar. Urine samples were available from the majority of women with biopsy-confirmed CIN-2–3/CIS, CIN-3, or invasive cancer, but from only a small minority of women with negative histology. On average, compared with the original study group, the women in the present study were slightly older (46.8 versus 44.4, \( P = 0.02 \)) and, hence, are less likely to be premenopausal (63% versus 76%, \( P = 0.001 \)) or to practice contraception use (18% versus 29%, \( P = 0.01 \)).

**Genomic DNA Isolation from Cervical Swab and Urine Samples.** For cervical swab samples, genomic DNA was isolated using QIAamp Blood minicolumn as described previously (39). For urine samples, ~5 mL of voided urine sample was collected and stored at 4°C from each patient enrolled into the study. Urine samples were centrifuged within 24 h after collection, and cell pellets were resuspended in 1 mL STM (Digene) and stored at −80°C until being shipped to Seattle. Upon processing in the laboratory, they were digested with proteinase K at 37°C for 1 to 2 h. About 400 μL digested urine sample was used to isolate genomic DNA using QIAamp Blood minicolumn with some modifications as follows: all 400-μL digested urine samples were loaded onto one single column, and the column was washed twice with AW1 and AW2 buffers to remove PCR inhibitors in urine samples and eluted with 100 μL 0.1× AE. The eluted DNA was concentrated down to 25 μL using Speedvac at room temperature.

**HPV Detection and Typing.** Two microliters of DNA were used for PCR amplification of HPV DNA by degenerated primers MY09/MY11/HMB01 and β-globin primers PC04/GH20 (41, 42). PCR products were spotted onto two Nylons membranes and hybridized with a generic HPV probe and GH20 (41, 42). PCR product was hybridized to a Roche line blot containing specific primers to HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, cp6108, and IS39. If a sample was positive by HPV dot blot, negative by Roche line blot, but positive for...
Table 2. Baseline characteristics of study population

<table>
<thead>
<tr>
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<th>N = 129</th>
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<tbody>
<tr>
<td>Age (mean years ± SD)</td>
<td>46.8 ± 10.9</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>78/123 (63%)</td>
</tr>
<tr>
<td>Contraception use</td>
<td>22/125 (18%)</td>
</tr>
<tr>
<td>Prior pregnancy</td>
<td>123/123 (100%)</td>
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<tr>
<td>Current smoking</td>
<td>3/128 (2%)</td>
</tr>
<tr>
<td>Cytology</td>
<td></td>
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<tr>
<td>Unsatisfactory</td>
<td>15/126 (12%)</td>
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<tr>
<td>Negative</td>
<td>15/126 (12%)</td>
</tr>
<tr>
<td>ASCUS</td>
<td>14/126 (11%)</td>
</tr>
<tr>
<td>LSIL</td>
<td>7/126 (6%)</td>
</tr>
<tr>
<td>HSIL</td>
<td>13/126 (10%)</td>
</tr>
<tr>
<td>ICC</td>
<td>57/126 (45%)</td>
</tr>
<tr>
<td>Other cancer pathology</td>
<td>5/126 (4%)</td>
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</table>

β-globin in both assays, the sample was considered positive but “not typed” (NT). In this study, NT was not considered a high-risk type HPV.

Bisulfite Conversion and MethyLight Analysis. Sodium bisulfite conversion of genomic DNA was done as described previously (43). For cervical swab samples, 1 μg DNA was used for bisulfite conversion, and for urine samples, 50 to 100 ng urine DNA was used. Each set of samples was converted in the presence of both human sperm DNA as the unmethylated control (U-DNA) and in vitro SssI-treated human sperm DNA as the fully methylated control (M-DNA). DNA methylation analysis was done by MethyLight (44). For each gene, the primers and probe were designed specifically for bisulfite-converted fully methylated DNA (Table 1). Amplification of bisulfite-converted β-actin (ACTB) was used to normalize for input DNA. Samples that were negative for ACTB were excluded in the methylation analysis. A plasmid-containing bisulfite-converted ACTB gene of known concentration was diluted and used as the standard curve for quantification. The assay for a given set of samples was considered valid only if the converted U-DNA was not amplified, whereas the converted M-DNA was amplified. The percentage of fully methylated reference DNA (PMR) at a specific locus was calculated by dividing the Gene:ACTB ratio of a sample by the Gene:ACTB ratio of fully methylated control DNA and multiplying by 100 (45). In this study, a sample was considered positive for methylation for a specific gene if the PMR was >0. The four gene markers (DAPK1, RARB, TWIST, and CDH13) were each selected for the present analysis based on our previous study of cervical swab and biopsy samples from the same population (39).

Statistical Analysis. Pearson’s χ² tests were used in univariate analyses to compare dichotomous variables, and Mantel-Haenszel trend tests were used to compare ordered categorical variables such as histologic diagnosis. Correlations of paired urine and cervical swab specimens were analyzed with McNemar’s test. A two-sided 0.05 level test determined statistical significance for all analyses. In this analysis, a sample was considered positive for methylation for a specific gene if any methylation was detected, that is, if PMR > 0. No post hoc attempt to maximize test sensitivity and/or specificity by optimizing PMR cutoffs which best distinguished cases from controls was made. Cytology sensitivities and specificities were calculated using the histologic diagnosis as the gold standard, with a cytologic diagnosis of HSIL used to determine test positivity. All analyses were conducted using SAS version 9.1 (SAS Institute Inc.).

Results

Demographics and Adequacy of Specimens. The mean age of the 129 women included in this study was 46.8 years (±10.9); the majority (63%) was premenopausal, and 82% did not currently use contraception (Table 2). All women reported at least one prior pregnancy, and 77% had five or more pregnancies. Among 129 women studied, 15 had unsatisfactory Pap smears, and three were missing cytology diagnosis. All but one urine sample were sufficient for HPV, and 113 samples (88%) were sufficient for methylation analysis.

Cytology and Histology Results. Of the 129 women biopsied, 19 were found to have no significant pathology on biopsy, nine were diagnosed with CIN-1, 29 with CIN-2–3/CIS, and 72 with ICC. Of the 111 women who had sufficient material for cytologic diagnosis, 15 had normal cytology, 14 were diagnosed with ASCUS, 7 with LSIL, 13 with HSIL, 57 with ICC, and 5 with other cancer pathology. Thus, the sensitivity and specificity of cytology for CIN-2–3/CIS/ICC were 78% and 84%, respectively, with the sensitivity greater for ICC (88%) than for CIN-2–3/CIS (46%) in this nonrandom sample of specimens, using a cytologic diagnosis of HSIL as the cutoff for test positivity.

Detection of Hypermethylated Genes in Urine Specimens. DNA hypermethylation of any of the four genes of interest (DAPK1, RARB, TWIST, and CDH13) was detected in 47 (42%) of 113 urine samples. Hypermethylation of at least one gene was detected in 62% of samples from women with ICC, 28% of samples from women with CIN-2–3/CIS, and 4% of samples from women with CIN-1 or no evidence of CIN (P < 0.001, test for trend, Table 3). DAPK1, RARB, and TWIST were each significantly more frequently hypermethylated in the urine samples from women with ICC or CIN-2–3/CIS than women with lesser degrees of neoplasia. DAPK1, RARB, and TWIST were methylated in 49%, 40%, and 32% of ICC, 28%, 4%, and 4% of cases of CIN-2–3/CIS, and in only 4% of those with CIN-1 or less. CDH13 was rarely detected in urine samples. However, it was very specific, being detected only in samples from women with ICC (10%) or CIN-2–3/CIS (4%), but never from those with CIN or less (Fig. 1).

Detection of HPV DNA in Urine Samples. HPV DNA was detected in 88 (69%) of 128 urine samples tested, with 73 (57%) being positive for high-risk HPV types. Detection of high-risk HPV DNA increased with level of cervical neoplasia (P < 0.001) and was found in urine samples from 50 (70%) of 71 cases with ICC, 17 (59%) of 29 cases with CIN-2–3/CIS,

Table 3. Detection of hypermethylation of DAPK1, RARB, TWIST, and CDH13 in urine samples from women with and without biopsy-confirmed cervical neoplasia

<table>
<thead>
<tr>
<th>Gene</th>
<th>Negative and atypical</th>
<th>CIN-1 (n = 9)</th>
<th>CIN-2–3 and CIS (n = 25)</th>
<th>ICC (n = 63)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAPK1</td>
<td>1/16 (6%)</td>
<td>0/9 (0%)</td>
<td>7/25 (28%)</td>
<td>31/63 (49%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>RARB</td>
<td>1/16 (6%)</td>
<td>0/9 (0%)</td>
<td>1/25 (4%)</td>
<td>25/63 (40%)</td>
<td>0.0006</td>
</tr>
<tr>
<td>TWIST</td>
<td>1/16 (6%)</td>
<td>0/9 (0%)</td>
<td>1/25 (4%)</td>
<td>20/63 (32%)</td>
<td>0.0046</td>
</tr>
<tr>
<td>CDH13</td>
<td>0/16 (0%)</td>
<td>0/9 (0%)</td>
<td>1/25 (4%)</td>
<td>6/63 (10%)</td>
<td>0.1044</td>
</tr>
<tr>
<td>DAPK1, RARB, or TWIST</td>
<td>1/16 (6%)</td>
<td>0/9 (0%)</td>
<td>7/25 (28%)</td>
<td>39/63 (62%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DAPK1, RARB, TWIST, or CDH13</td>
<td>1/16 (6%)</td>
<td>0/9 (0%)</td>
<td>7/25 (28%)</td>
<td>39/63 (62%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Mantel-Haenszel test for trend.
Detection of HPV DNA and Aberrant Hypermethylation in Urine Samples. Detection of DNA hypermethylation or high-risk HPV detection was noted in urine samples from 52 (84%) of 62 women with biopsy confirmed ICC, 16 (64%) of 25 women with CIN-2–3/CIS, 4 (44%) of 9 cases with CIN-1, and 3 (19%) of 16 women without cervical neoplasia (Table 4). Urine-based detection of both high-risk HPV and aberrant hypermethylation of any of the four genes was noted in 33 cases with ICC, 5 cases with CIN-2–3/CIS, and in none of those without such pathology, whereas 10 cases with ICC, 9 cases with CIN-2–3/CIS, and 18 of those without such pathology were negative for both high-risk HPV and hypermethylation of any of the four genes.

Among CIN-2–3/CIS/ICC samples, 23 samples that were negative for hypermethylation of any of the four genes tested were positive for high-risk type HPV, and 7 cases which were negative for high-risk HPV were positive for DNA methylation. Among these seven cases, two had methylation of DAPK1 only, one had methylation of RARB only, and the remaining four cases had methylation of at least two of the four genes tested. Interestingly, the only normal control that was positive for DNA methylation had concurrent methylation of three genes (DAPK1, RARB, and TWIST1), but was negative for high-risk HPV (Fig. 1).

Correlation between Aberrant Methylation and High-Risk HPV DNA Detected in Exfoliated Cervical Samples and Urine Samples. We correlated aberrant methylation detected in cervical swab samples and urine samples. Overall, agreement between cervical and urine sample–based detection of aberrant methylation of DAPK1, RARB, TWIST1, or CDH13 was seen in 49%, 59%, 80%, and 80% of 97 specimens analyzed, respectively, with 6% to 47% cervical specimens positive for hypermethylation of these respective genes being positive in corresponding urine samples (Table 5). In a minority of cases (n = 3, 6, 2, and 4 for DAPK1, RARB, TWIST1, and CDH13, respectively), DNA hypermethylation was detected in urine but was absent in the corresponding cervical specimens. Similarly, there was a high degree of agreement (73%) for the detection of high-risk HPV DNA between urine and cervical samples. Overall, DNA hypermethylation or high-risk HPV were detected more often in cervical swab samples than in urine samples.

Table 4. Detection of DNA hypermethylation and/or high-risk HPV DNA in urine samples from women with and without biopsy-confirmed cervical neoplasia

<table>
<thead>
<tr>
<th></th>
<th>Negative and atypical (n = 19)</th>
<th>CIN-1 (n = 9)</th>
<th>CIN-2–3 and CIS (n = 29)</th>
<th>ICC (n = 72)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine HPV</td>
<td>5/19 (26%)</td>
<td>5/9 (56%)</td>
<td>22/29 (76%)</td>
<td>56/71 (79%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urine HR-HPV</td>
<td>2/19 (11%)</td>
<td>4/9 (44%)</td>
<td>17/29 (59%)</td>
<td>50/71 (70%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urine—any hypermethylation</td>
<td>1/16 (6%)</td>
<td>0/9 (0%)</td>
<td>7/25 (28%)</td>
<td>39/63 (62%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urine HR-HPV or any hypermethylation</td>
<td>3/16 (19%)</td>
<td>4/9 (44%)</td>
<td>16/25 (64%)</td>
<td>52/62 (84%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urine HR-HPV and any hypermethylation</td>
<td>0/16 (0%)</td>
<td>0/9 (0%)</td>
<td>5/25 (20%)</td>
<td>33/62 (53%)</td>
<td>&lt;0.0001</td>
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*Mantel-Haenszel test for trend.

Discussion

This study examined the feasibility of cervical cancer screening based on detection of high-risk HPV or aberrant methylation of four cervical cancer–associated genes (DAPK1, RARB, TWIST1, and CDH13) in urine. Using this approach, we were able to detect 84% of the biopsies confirmed ICC and 64% of CIN-2–3/CIS lesions, with a specificity of 72% with respect to a biopsy showing CIN-1 or less, and a specificity of 81% with respect to negative biopsies. Furthermore, we found 49% to 80% concordance between the detection of DNA hypermethylation or high-risk HPV in exfoliated cervical samples and urine samples.

Previous studies examining approaches that would eliminate the need for a pelvic examination have generally focused on the detection of high-risk types of HPV in self-collected vaginal samples. Studies examining the potential utility of HPV DNA testing of self-collected vaginal samples have shown that similar to the performance of HPV testing of exfoliated clinician cervical samples, detection of high-risk types of HPV DNA in vaginal collected provides high sensitivity, with sensitivities ranging from 49% to 100% (13, 20, 25) with most studies reporting sensitivities of above 80%. However, the specificity of this approach for CIN 3/CIS is low, ranging from 54% to 73% (13, 20, 25), making such an approach impractical because of the high percentage of women that would need to either have additional testing or treatment. Furthermore, studies examining detection of high-risk HPV DNA in self-collected vaginal and clinician-collected samples have reported that whereas overall agreement is high (78-96%), agreement among HPV-positive specimens is low (37-39%; refs. 12, 17, 46). Lastly, because few cases of invasive cervical cancer were included in most such studies, the sensitivity of such an approach for the detection of ICC is unknown. Among 17 cases of ICC included in a study by Lorenzato et al., only 41% (7/17) were detected by HPV DNA testing in a self-collected vaginal sample (15).

Only a few, relatively small studies have examined the use of a urine sample as the basis of cervical cancer screening (22, 23, 25, 28, 33), although few have used biopsy as their gold standard. The present study is the first to examine the use of a combination of biomarkers in self-collected urine samples for the detection of various degrees of histologically confirmed cervical neoplasia. Several early studies reported high numbers of inadequate specimens due to the presence of PCR.
inhibitors. In the present study, through the Qiagen columns to efficiently eliminate PCR inhibitors and by concentrating the eluted DNA, only 1 out of 129 urine samples was insufficient for HPV detection. Nevertheless, in our study, as in previous studies, HPV was detected less often in urine samples than in cervical samples. Of 125 women in this study who had both HPV testing of cervical and urine, high-risk types of HPV were present in 74% of cervical sample, but in only 68% of corresponding urine sample (for a relative sensitivity of 68% and overall concordance for detection of high-risk HPV DNA of 73%). Similarly, a study among HIV-infected women, who might be expected to have more frequent detection of HPV (47), reported 58% of cervical swabs specimens, but only 48% of urine specimens were HPV DNA positive (22), whereas any HPV DNA was detected in 90.0% of cervical and 75% of urine samples from adolescent girls (23). Other studies have reported levels of overall concordance of detection of HPV DNA in cervical swabs as compared with urine similar to that seen in the present study (ranging from 70% to 82%; refs. 22-24, 33). In one of the most recent studies, Dapante examined 77 women with abnormal biopsy (n = 9 with ICC; n = 29 with high-grade; n = 39 with low-grade lesions). In this group of 77 women, 37 (48%) had cervical samples that were positive for HPV DNA, whereas only 26 (33%) of the corresponding urine samples were HPV DNA positive, for a relative sensitivity of detection of HPV of 70% (similar to the present study). HPV type–specific concordance has generally somewhat lower (40-79%; refs. 23, 26), although Daponte et al. reported 100% type concordance in 26 HPV positive urine/cervical swab pairs (33). In the present study, type-specific concordance for HPV 16 was 86%.

In the current study, detection of high-risk HPV DNA in urine had a sensitivity of 79%, 76%, and 56% for biopsy-confirmed ICC, CIN-2–3/CIS, and CIN-1, respectively. Brinkman (22) reported detection of HPV in urine (of HIV-infected women) to have a sensitivity of 67% for an abnormal Pap smear, and a specificity of 68% with respect to normal Pap smear, and 53% with respect to ASCUS. Jacobson et al. (23) similarly showed that HPV detected in urine had a sensitivity of 73% to detect an abnormal Pap, with a specificity of 57% with respect to a normal Pap smear, and 40% with respect to ASCUS. Two other studies have compared the detection of HPV in urine to biopsy diagnosis. Sellors et al. (25) showed that HPV detection in urine had a sensitivity of 45% to detect CIN-2–3/CIS/ICC, with a specificity of 70% with respect to negative or histologic CIN-1. More recently, Daponte et al. (33) reported that detection of HPV 16 or 18 in urine had a sensitivity of 45% to detect CIN-2–3/CIS/ICC while detecting only 13% of low-grade cases. In summary, our results and those of others suggest that the detection of HPV DNA in urine lacks adequate sensitivity and specificity for the identification of women with CIN-2–3/CIS or ICC.

To increase the sensitivity and specificity of urine-based screening, we examined the utility of combined detection both high-risk HPV DNA and hypermethylation of several genes we previously reported to be predictive of cervical neoplasia. Detection of DNA hypermethylation in urine samples has previously been proposed for detection of bladder, kidney, and prostate cancers (48-60), with recent studies suggesting that such an approach could provide a sensitivity and specificity of 69% to 90% and 60% to 100%, respectively, for bladder cancer (48-51). Hypermethylation of GSTP1 in urine samples has also been used to detect prostate cancer (52-55) and a combination of four genes (p16, ARF, MGMT, and GSTP1) detected 87% of prostate cancers with 100% specificity (56). DNA hypermethylation markers were also detected in urine samples from renal cancer patients (57-60).

In the present proof of principle study, the combined detection of DNA hypermethylation and high-risk HPV DNA had a sensitivity for detection of ICC similar to that of cytology. The majority of DNA hypermethylation detected in urine was also present in corresponding cervical swabs, demonstrating that these changes were specific to cervical tissue. The few discrepancies observed between the cervical and urine samples may reflect tumor heterogeneity with differential likelihood of tumor clone shedding.

The sensitivity of a urine-based biomarker assay to detect ICC might be improved in several ways, for example, by increasing the amount of urine assayed. In the present study, the equivalent of only a 2-mL urine was processed to isolate genomic DNA and used for DNA methylation analysis. Because cells in the urine sample were concentrated first by centrifugation, it may be possible to increase the sensitivity by using a larger quantity (10 mL) of urine. Repeat testing of multiple urine samples taken at different times might also increase the chance of detecting tumor-associated DNA hypermethylation changes because cervical tumor cells might not constantly shed into urine. Furthermore, a urine sample taken without prior washing of the genital area might contain more exfoliated cervical cells, and thus, more tumor-specific DNA.

In conclusion, this study and previous studies discussed above show the feasibility of urine-based screening for cervical neoplasia. The combination of high-risk HPV and cervical cancer–specific DNA hypermethylation markers may have considerable potential for urine-based cervical cancer screening. Larger studies are needed to confirm our observations and assess the utility of DNA hypermethylation detection of these and other genes in urine for cervical cancer diagnosis and management. Although the current gold standard for DNA methylation analysis is based on sodium bisulfite conversion, several novel approaches have been developed independent on bisulfite conversion (61-63), and it is likely that rapid, low-cost clinical assay formats for detection of DNA methylation markers will become available within the near future. If the utility of such an approach is confirmed, it may be of particular interest in resource-poor settings where screening tests that eliminate the need to maintain an extensive clinical and laboratory infrastructure including large numbers of examination rooms, many highly trained clinicians and pathologists would be of considerable importance.
References
Promoter Hypermethylation of Tumor Suppressor Genes in Urine from Patients with Cervical Neoplasia

Qinghua Feng, Stephen E. Hawes, Joshua E. Stern, et al.


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