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Urine test for HPV genotypes as a predictor of precancerous cervical lesions and for cervical cancer screening

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Abstract

Objective: To assess the sensitivity of a urine test for high-risk HPV DNA genotypes in the detection of high-grade squamous intra-epithelial lesion (HSIL) and its correlation with pathologic precancerous lesions.

Methods: The present prospective cross-sectional study included women referred to Kasr AlAiny Medical School, Cairo, Egypt, for cervical smear anomalies, a history of cervical smear anomalies, or for suspicious cervix between May 1, 2015, and April 30, 2017. Paired urine tests and cervical smears were performed. HPV DNA was detected in urine using polymerase chain reaction and cervical smears were performed with a cervical spatula and a cytobrush. Agreement between urine test results and pathology was examined.

Results: In total, 1375 women were included. Urine test for high-risk HPV DNA demonstrated 97.8% (95% confidence interval [CI] 92.1%–99.7%) sensitivity and 100% (95% CI 99.7%–100.0%) specificity for HSIL. Overall, 87 women had a positive urine test for high-risk HPV; of these, 82 (94.3%, 95% CI 87.1%–98.1%) had pathologic findings of cervical intra-epithelial neoplasia 2 or 3 (CIN2/3). Similarly, 89 women had HSIL cytology; again, 82 had CIN2/3 (92.1%; 95% CI, 84.3%–96.4%).

Conclusion: There was good agreement between a positive urine test for high-risk HPV DNA genotypes and pathologic findings of CIN2/3.

KEYWORDS

Atypical cells of undetermined significance; Cervical intra-epithelial neoplasia; High-grade squamous intra-epithelial lesion; HPV; Low-grade squamous intra-epithelial lesion; Pap smear

1 | INTRODUCTION

Globally, cancer of the cervix is the fourth most common female malignancy.[1] Its prevalence is high in low-resource countries: 85% of newly reported cases of cervical cancer occur in low-income countries, with higher mortality rates than in other regions of the world.[2] This statistic can be explained by the success of cervical screening programs in high-incomes countries.

Although cervical smear tests are performed extensively to screen for cervical cancer, they do not always detect cervical cancer. Specifically, the cervical smear test only has 53%–80% sensitivity for detecting high-grade lesions on any given single test.[3] Consequently, the preventive power of cervical smear testing lies in regular serial screening. However, the test is not acceptable to many women. In addition, a screening program for cervical cancer can be difficult to implement in low-income countries owing to deficiencies in resources, poor population coverage, and that the fact that some women will not participate in such a screening program.[4]

HPV is the main infection linked to cervical cancer.[5] More than 99% of the disease is concomitant with oncogenic HPV infection,

although other sexually transmitted diseases, such as type II herpes simplex virus, can also be involved.[6] Specifically, high-risk genotypes of HPV (16, 18, 30, and 31) are directly related to cellular changes that lead to precancerous and cancerous lesions.

HPV infection is suspected on the basis of clinical lesions and/or investigations, including cytologic and histologic evaluation, and colposcopic examination. However, these diagnostic tools are subjective (i.e., dependent on observer experience) and often imprecise. Moreover, serology is unreliable and cannot distinguish current infection from historical infections.[7] Culture of HPV is not feasible; thus, diagnosis of infection can be confirmed only by the direct detection of HPV nucleic acids by methods that include in situ hybridization, nucleic acid amplification testing, and polymerase chain reaction (PCR), among others.[8]

Screening for HPV DNA could be more successful in assessing the risk of cervical cancer than cervical smear, and primary screening for HPV could be an easy and less invasive alternative to cervical smear.[9] Screening via a simple and non-invasive method of collection could be one solution to overcome the drawbacks of the cervical smear and screening programs.[10] HPV DNA can be detected in both cervical smear samples and urine samples. Therefore, by using a urine test for HPV DNA, the cervical cancer screening program could become easier, more acceptable to patients, more applicable, and non-invasive.[11]

A recent meta-analysis described the high sensitivity and specificity of the urine test to detect high-risk HPV.[4] However, few studies have correlated HPV DNA in urine with the pathologic effect of HPV on the cervix.[4,11–14] The aim of the present study was to evaluate the sensitivity of the urine test for high-risk HPV DNA types in the detection of high-grade squamous intra-epithelial lesions and to correlate urine test data with pathologic precancerous lesions in Egypt.

2 | MATERIALS AND METHODS

The present prospective study was conducted at the Department of Obstetrics and Gynecology, Kasr AlAiny Medical School, Cairo University, Cairo, Egypt, from May 1, 2015, to April 30, 2017. The outpatient colposcopy clinic of the study institution is attended by patients for follow-up for a history of cervical smear anomalies, previous cervical epithelial lesion anomalies, and after being referred to suspicious cervix. To be eligible for inclusion, patients needed to be aged 30–55 year, sexually active, and to have a previous positive cervical smear test result, previous colposcopic lesion, or previous cervical epithelial lesions (diagnosed by colposcopy-guided punch biopsy). The exclusion criteria were no previous sexual activity, a history of cervical cancer, and gross macroscopic lesions of the cervix on examination. Women with a history of treatment for cervical pre-cancerous lesions within the preceding 6 months and those who had undergone total hysterectomy were also excluded. The study was approved by the Kasr Alainy ethics committee and written informed consent was obtained from all participants.

Paired samples (cervical smear and urine test) were collected from all participants. For the urine sample, the first 30–50 mL of voided urine was collected after double swabbing of the external genitalia with betadine. The urine sample was centrifuged at 2000 rpm for 5–10 minutes at 4°C. The supernatant was removed and the remaining pellet was stored at −80°C until DNA isolation.

A pelvic examination, followed by cervical smear, was then performed. The cervix was exposed by introducing a vaginal speculum. If necessary, lubrication with warm saline was applied to avoid patient discomfort. Complete visualization of the external cervical os, squamocolumnar junction, and ectocervical epithelium was necessary for adequate sampling. Transformation zone cells were screened for dysplastic cells because HPV has a preference for this area. A large moistened swab was used to clear the cervix from any discharge without trauma to the cervix. Next, an Ayers spatula was applied to the cervix and rotated unidirectionally through 360°. A cytobrush was then inserted inside the cervix with its bristles still visible at the outer os, and was rotated unidirectionally through 180°.

Each specimen was smeared on a glass slide and fixed with spray fixative or through immersion in 90% alcohol solution. The sample was then sent to the pathology department for cytologic examination. Women with positive findings (atypical cells of undetermined significance [ASCUS], low-grade squamous intra-epithelial lesion [LSIL], or high-grade squamous intra-epithelial lesion [HSIL]) were referred for colposcopic examination and colposcopy-guided punch biopsy for pathologic examination, as part of standard practice at the study institution.

Genetic analysis for HPV detection in the urine sample was performed using the following method. First, DNA was extracted from urine by using an AmpliLute Liquid Media Extraction Kit and linear array (Roche, Basel, Switzerland), in accordance with the manufacturer's instructions. The extracted DNA was stored immediately at −20°C until PCR amplification.

Second, the integrity of the DNA sample was evaluated using PCR of a 268-basepair segment of the β-globin gene with forward (5′-ACACAA CTGTGTTCACTAGC-3′) and reverse (5′-CAACTTCATCCACGTTCACC-3′) primers. β-globin-positive samples were then subjected to PCR amplification of a 450-basepair product for the L1 open reading frame of HPV with forward (5′-TTTGTTA CTGTGGTAGATACTAC-3′) and reverse (5′-AAAAATAAACTGTAAAT CATATTC-3′) primers. HPV-positive and HPV-negative (water) controls were included in each run. The amplified products were visualized by electrophoresis on 2% agarose gels containing ethidium bromide (0.5 mg/mL).[14]

The HPV linear array was used to detect individual HPV genotypes in the presence of multiple infection, a common finding. HPV genotypes were sub-classified as high-risk types linked to cancer and high-grade dysplasia of the cervix (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82) and low-risk types associated with benign low-grade intra-epithelial lesions or condylomas (HPV6, 11, 40, 42, 43, 44, 54, 61, 72, 73, and 81).

The sample size was calculated on the basis of the sensitivity of the urine test to predict HSIL, which has been previously reported as 80%.[15] Consequently, it was presumed that the predicted sensitivity should be at least 80%, and it was postulated that the specificity should be at least 80%. Therefore, at least 90 women with HSIL and 90 patients without

HSIL (control group) would be needed to detect these measures of sensitivity and specificity with a maximal lower 95% confidence interval of 10% less than these values, 80% power, and a type I error probability of 0.05. The prevalence of HSIL in the referred population at the study institution was 6.5% (unpublished data). Therefore, a total sample size of 1375 participants was calculated as being necessary to achieve approximately 90 cases of HSIL, using the equation of Flahault et al.[16]

Statistical analysis was performed with SPSS version 23 (IBM, Armonk, NY, USA). Data were presented as median (range) or absolute number (percentage). as appropriate. Median values were used owing to a non-normal distribution. The Kruskal-Wallis test was used to test for differences in median age and parity. Regarding the accuracy of the urine test, 95% confidence interval were calculated by the binomial exact (Clopper-Pearson) method for all relevant measures. Rates of CIN2/3 diagnoses were calculated among patients with positive urine test results for high-risk HPV and those with positive cytology. $P<0.05$ was considered to be statistically significant.

3 | RESULTS

During the study period, 1375 women underwent paired urine testing and cervical smear testing and were enrolled in the study. All participants were native Egyptians. Participant age, parity, pathologic findings, and results of cervical smear tests and urine tests are presented in Table 1. Overall, 348 women were referred to colposcopy and to colposcopy-guided punch biopsy after a cytologic finding of ASCUS, LSIL, or HSIL. Cervical smear testing identified 89 (6.5%) patients with HSIL, 103 (7.5%) patients with LSIL, 156 (11.3%) patients with ASCUS, and 1027 (74.7%) patients with inflammatory cells.

TABLE 1 Patient characteristics (n=1375).[a]

Characteristic	Value
Age, y	37 (28–43)
Parity	3 (0–6)
Cervical smear results	
HSIL	89 (6.5)
LSIL	103 (7.5)
ASCUS	156 (11.4)
Inflammatory cells	1027 (74.7)
Urine test for HPV	
Positive for HR-HPV	87 (6.3)
Positive for LR-HPV	113 (8.2)
Negative	1027 (74.7)
Pathology results[b]	
CIN3	57 (16.4)
CIN2	34 (9.8)
CIN1	59 (17.0)
Chronic cervicitis	198 (56.9)

Abbreviations: HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; ASCUS, atypical cells of undetermined significance; HR-HPV, high-risk HPV; LR-HPV, low risk HPV; CIN, cervical intra-epithelial neoplasia.
[a]Values are given as median (range) or number (percentage).
[b]In total, 348 women underwent pathologic assessment owing to cervical smear anomalies (HSIL, LSIL, or ASCUS).

TABLE 2 Patient age and parity stratified by urine DNA test findings.[a]

Characteristic	HR-HPV (n=87)	LR-HPV (n=113)	Negative test result (n=1175)	*P* value[b]
Age, y	35 (29–42)	37 (28–43)	36 (28–43)	0.369
Parity	3 (1–5)	3 (0–6)	3 (0–6)	0.895

Abbreviations: HR-HPV, high-risk HPV; LR-HPV, low-risk HPV.
[a]Values are given as median (range) unless indicated otherwise.
[b]Kruskal-Wallis test for difference in medians among the three groups.

Regarding urine HPV DNA testing, 87 (6.3%) patients had high-risk HPV genotypes (two patients with HSIL cytology had negative urine test results) and 113 (8.2%) patients had low-risk HPV genotypes. In total, 1175 (85.5%) patients had a negative urine test result for HPV DNA. No significant difference in age or parity was recorded among patients with different urine HPV test results (Table 2).

Among the 348 patients referred for colposcopy and colposcopy-guided biopsy, 57 (16.4%) were diagnosed with CIN3, 34 (9.8%) diagnosed with CIN2, and 198 (56.9%) diagnosed with CIN1 (Fig. 1).

The sensitivity, specificity, positive predictive value, and negative predictive value of the urine HPV DNA test for the detection of cytologic anomalies (HSIL, LSIL+, ASCUS+) are presented in Table 3. The urine test demonstrated sensitivity and specificity of 97.8% (95% confidence interval [CI] 92.1%–99.7%) and 100% (95% CI 99.7%–100.0%), respectively, for the detection of HSIL.

There was good agreement between the high-risk HPV urine test and cytologic HSIL diagnosis in terms of identifying patients with pathologic findings of CIN2/3 (Table 4).

4 | DISCUSSION

The present study aimed to establish a novel procedure that is faster, easier to perform, and more acceptable as a screening method for CIN and its precancerous lesions. This procedure needed to overcome drawbacks of the cervical smear, including its occasional difficulties, relatively invasive nature, and non-acceptability to some patients. Good agreement was found between a positive urine test for high-risk HPV and a pathologic finding of CIN2/3 by colposcopy-guided punch biopsy, confirming that the urine test was a reliable method for predicting precancerous cervical lesions without performing a cervical smear. It should be noted that the high prevalence of CIN2/3 among women with a positive urine HPV test or HSIL cytology could be due to the characteristics of the current study population, many of whom were referred for a suspicious cervix.

Majid et al.[17] and Jacobson et al.[18] also reported agreement between a positive urine test for high-risk HPV and HSIL cytology. In a study of women with infiltrating squamous cell carcinoma, HPV

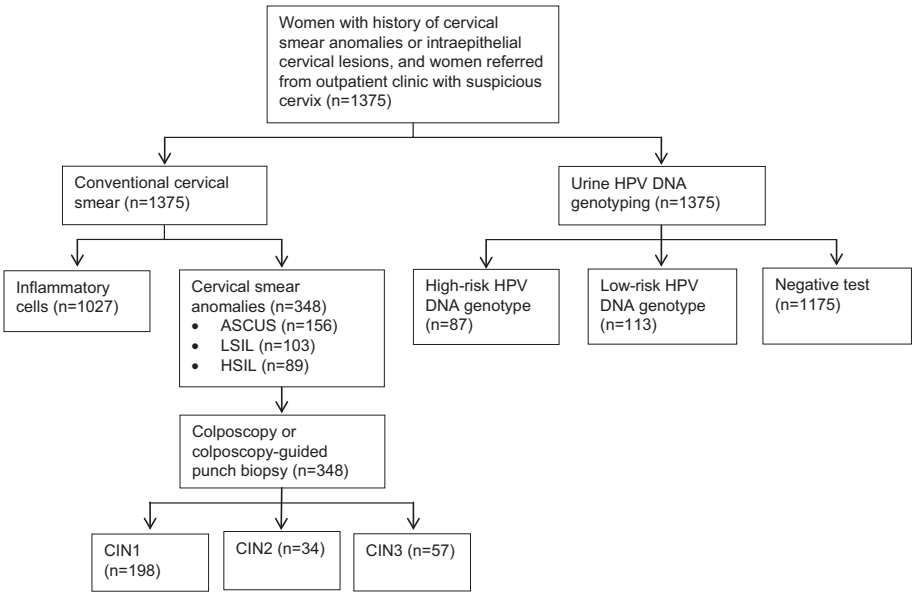

FIGURE 1 Flow of the study population. Abbreviations: ASCUS, atypical cells of undetermined significance; LSIL, low-grade squamous intra-epithelial lesion; HSIL, high-grade squamous intra-epithelial lesion; CIN, cervical intra-epithelial neoplasia.

TABLE 3 Accuracy measures of urine testing for high-risk HPV in the prediction of HSIL, LSIL+, and ASCUS+ cytology.[a]

Cytology finding	Sensitivity (95% CI), %	Specificity (95% CI), %	PPV (95% CI), %	NPV (95% CI), %
ASCUS+	25.0 (20.5–29.9)	100 (99.6–100)	100 (95.9–100)	79.7 (77.4–81.9)
LSIL+	45.3 (38.1–52.6)	100 (99.7–100)	100 (95.9–100)	91.9 (90.2–93.2)
HSIL	97.8 (92.1–99.7)	100 (99.7–100)	100 (95.9–100)	99.8 (99.4–100)

Abbreviations: HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; ASCUS, atypical cells of undetermined significance; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[a]Cervical smear testing was used as the reference standard.

DNA positivity was higher in the cervical sample (98%) than in the urine sample (71%).[19] Majid et al.[17] also reported a lower detection rate of HPV DNA genotype in urine than in cervical samples; 28% of their study population had HSIL and 40% had ASCUS, and none of the women with ASCUS tested positive for HPV in a cervical or urine sample. In the present study, no women with a normal cervical smear test result had a positive urine test, whereas a previous study detected HPV in 15% of urine samples from women with a normal cervical smear result; this could be related to infection in anogenital sites other than the cervix.[19]

The high sensitivity and specificity of the urine test for HPV DNA make it an excellent procedure for cervical cancer screening owing to the high detection rate of high-risk HPV genotypes.[4] In addition, a previous study showed that the detection of high-risk HPV DNA genotypes in urine has criteria similar to the detection of cytology anomalies in cervical smear examinations.[20]

The present study confirmed that urine HPV DNA genotype analysis can predict the presence of pathologic lesions of the cervix, thereby bypassing the step of cervical smear. Most previous studies investigated the ability of the urine test based on an HPV Cobas test or linear array to predict the presence of high-risk HPV DNA genotypes, rather than to predict the pathologic lesions. For example, one study compared the detection of high-risk HPV DNA between urine and cervical smear samples and reported a positive correlation ranging from 79% to 80%.[21] Similarly, Khunamornpong et al.[15] reported a substantial agreement in the detection of high-risk HPV DNA using the Cobas test between urine and cervical samples, with discordance of 2 in 24 (8.3%).[15] Bernal et al.[21] also compared the performance of Cobas test in paired urine and cervical smear samples, reporting

TABLE 4 CIN2/3 diagnoses among women with urine tests positive for high-risk HPV and positive cytology.[a]

Urine test/cytology result	No. of women	Patients diagnosed with CIN2/3
Urine test positive for HR-HPV	87	82 (94.3; 87.1–98.1)
HSIL	89	82 (92.1; 84.3–96.4)
LSIL	103	6 (5.8; 2.2–12.2)
ASCUS	156	3 (1.9; 0.3–5.5)

Abbreviations: CIN, cervical intra-epithelial neoplasia; HR-HPV, high-risk HPV; HSIL, high-grade squamous intra-epithelial lesion; LSIL, low-grade squamous intra-epithelial lesion; ASCUS, atypical cells of undetermined significance; CI, confidence interval.
[a]Values are given as number or number (percentage; 95% CI).

a high rate of agreement in high-risk HPV detection (88%). Another study of the detection of HPV genotypes in urine and cervical smear samples reported that discordance could result from the exfoliation of HPV-infected cells from the cervix, anogenital region, or urethra.[22] Use of urine samples for detecting high-risk HPV DNA genotypes has been examined as a follow-up test for women with abnormal cervical smear test cytology.[13,21] Lastly, a population-based study of 1305 women in a rural area of India detected only 5 (0.4%) patients had urine test results positive for HPV DNA using PCR detection when cervical HPV detection was not included to serve as a reference for HPV prevalence.[23]

The present study had some limitations. It was not possible to correlate the specific genotypes of HPV to the pathologic findings or to follow up the women with lesions long term to revise the sequelae. In addition, the study population of women with cytology anomalies included those with inflammation, who might not be the best sample to form a control group. Furthermore, no histologic analysis was done for this negative control group. Therefore, only agreement values (rather than sensitivity and specificity) between cytology and urine HPV testing could be measured for the accompanying histologic diagnosis.

In summary, the present study found good agreement between a positive urine test for high-risk HPV DNA genotypes and pathologic findings of CIN2/3. To the best of our knowledge, the present study was the first to link the detection of HPV genotypes in urine to the findings of both cervical smear and pathologic examination. In the future, researchers should use the urine test to identify individual types of high-risk HPV and correlations between each type and pathological cervical lesions.

AUTHOR CONTRIBUTIONS

AMM contributed to designing the study, and manuscript writing and revision. HS contributed to designing the study and manuscript revision. ES, HM, MA, and AE contributed to data collection and manuscript writing. EO contributed to data collection, data analysis, and manuscript writing.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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