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Original Research Article

Concordance of HPV genotype detection in cervical and urine samples among cervical cancer screen positive women

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ABSTRACT

Background: This study investigates the concordance of HPV genotype detection in urine and cervical samples among women undergoing cervical cancer screening.
Methods: Conducted over one year at the Department of Gynaecological Oncology, BSMMU, Dhaka, the study enrolled 74 women aged 30-60 years with positive visual inspection with acetic acid (VIA) results or abnormal Pap test findings. Urine samples (20 ml) and cervical samples were collected from each participant. The samples were analyzed using multiplex real-time PCR to amplify high-risk HPV types (16, 18, and others). DNA was extracted using the Qiagen viral DNA extraction kit. Sensitivity and specificity of HPV detection in urine samples were compared to cervical sampling, the gold standard. Data were analyzed with SPSS 22.0, and agreement was assessed using the Kappa index.
Results: In this study of 74 participants, HPV detection and genotype distributions were compared between cervical and urine samples. The mean age was 40.07 years, with 89.2% of participants being married and 75.7% multipara. There was moderate agreement between HPV detection in cervical and urine samples (Kendall's tau = 0.752, $p<0.001$). Cervical samples identified a greater variety of HPV types, including HPV 16 and high-risk (HR) genotypes, compared to urine samples. The Cohen's Kappa coefficient for agreement was 0.746 ($p<0.001$), indicating moderate concordance. Overall, cervical sampling demonstrated higher sensitivity for HPV detection.
Conclusions: The study demonstrates moderate concordance between cervical and urine samples in HPV detection, with cervical sampling showing higher sensitivity for identifying HPV genotypes.

Keywords: Cervical cancer screening, Genotype distribution, HPV detection, Human papillomavirus, Urine samples

INTRODUCTION

Cervical cancer is primarily caused by persistent infection with human papillomavirus (HPV), a double-stranded DNA virus that infects the deeper layers of skin and the inner mucosal linings of organs. To date, approximately 200 genotypes of HPV have been identified, with more than 40 types specifically infecting the stratified squamous epithelium of the cervix, vagina, vulva, penis, and perianal areas.[1,2] Among women who are HPV-positive, 10% to 20% develop a persistent infection, leading to the shedding of HPV DNA from the genital tract for 24 months or more after the initial infection. However, cervical cancer is considered largely preventable, with more than 80% of cases being avoided through effective screening, early diagnosis, and appropriate treatment. The introduction of screening programs in developed countries has significantly reduced the incidence and mortality of

cervical cancer, underscoring the importance of early detection and prevention.[3]

Historically, the Papanicolaou test (Pap test) has been a cornerstone in the global strategy to prevent cervical cancer. Since its introduction, both the incidence and mortality rates of cervical cancer have markedly decreased.[4] More recently, HPV DNA screening has been shown to be more effective than cytology (Pap test) in detecting premalignant and malignant lesions.[5,6] However, both the Pap test and HPV tests require pelvic examinations, which can cause anxiety, discomfort, pain, distress, and psychological stress for patients.[7,8] The development of non-invasive sample collection methods, such as urine sampling, has the potential to increase acceptance and participation in cervical cancer screening.[9] Recent studies have demonstrated that HPV testing via urine samples induces less physical and psychological stress compared to the Pap test, highlighting its advantages as a screening method.[10,11]

A recent study involving 108 pairs of cervical and urine samples reported HPV prevalence rates of 37.0% (40/108) in cervical samples and 34.3% (37/108) in urine samples. For high-risk HPV (HR-HPV), the prevalence was 22.2% (24/108) in cervical samples and 18.5% (20/108) in urine samples. Although urine samples showed a slightly lower positive rate for HPV compared to cervical samples, the agreement rate for HR-HPV between the two sample types was 94.44%, with a kappa value of 0.823, indicating almost perfect concordance.[12] Similarly, a meta-analysis of HPV infection prevalence in cervical samples showed an overall prevalence of 25.41% (105 studies; 95% CI 22.71–28.32; $I^2 = 98\%$), with HR-HPV genotypes having a prevalence of 17.65% (44 studies; 95% CI 4.80–20.92; $I^2 = 96\%$).[13] Another comparative study found HPV detection rates of 39.6% (65/164) in cervical samples and 32.3% (53/164) in urine samples.[14]

The objective of this study was to evaluate the concordance between high-risk (HR) HPV detection in self-collected urine samples and clinician-collected cervical samples among women who screened positive for cervical cancer.

METHODS

This comparative cross-sectional study was conducted in the Department of Gynaecological Oncology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka, over one year from June 2022 to May 2023. The study population included women aged 30-60 years who tested positive for visual inspection with acetic acid (VIA) or had abnormal cytology (Pap test) results. Based on sample size calculation using a 98% confidence interval, a prevalence of 42.1% for HPV positivity in urine samples, and an allowable error of 30%, the required sample size was 83. However, 74 participants were ultimately selected using purposive sampling method.

Inclusion criteria

Inclusion criteria were women aged 30-60 years with VIA-positive results or abnormal cytology tests (Pap test) who consented to participate in the study.

Exclusion criteria

Exclusion criteria included women previously vaccinated against HPV, those who had received prior treatment for cervical disease, pregnant women or those who had given birth within the last three months, and those who did not consent to participate.

Participants were instructed not to urinate or wash their genitalia one hour before sample collection. Urine samples (20 ml) were collected before pelvic examination, and cervical samples were obtained using sterile polypropylene swabs under aseptic conditions. Samples were promptly transported in temperature-controlled conditions to the Department of Virology for analysis. Urine samples underwent modified aliquoting prior to DNA extraction using the Qiagen viral DNA extraction kit. Multiplex real-time PCR was performed to amplify the LCR/E6/E7 regions of high-risk HPV types (16, 18, and others). For viral nucleic acid purification, the QIAamp MinElute Virus Spin Kit was used, ensuring minimal elution volumes for higher sensitivity. All procedures were conducted at room temperature with stringent safety protocols.

Statistical analysis

Data were analyzed using SPSS 22.0. Demographic data and baseline characteristics were summarized using frequency and percentages. Continuous variables were represented by mean ± standard deviation or median with interquartile range, depending on normality. Sensitivity and specificity of HPV DNA detection in urine samples were calculated, using cervical sampling as the gold standard. The Kappa index was employed to determine the agreement between paired samples.

Ethical approval was obtained from the BSMMU IRB, and informed consent was secured from all participants. Data confidentiality was maintained, with anonymized records stored securely. Each patient was assigned a unique ID number for all study procedures, ensuring privacy and traceability throughout the study.

RESULTS

Table 1 shows sample size (n) was 74. And 20.3% (15 individuals) fell within the age range of 25-34. 54.1% (40 individuals) fell within the age range of 35-44. 25.7% (19 individuals) were aged 45 or older. The mean age was 40.07 years with a standard deviation (SD) of 7.58. The median age was 39 years, ranging from 25 to 62. 89.2% (66 individuals) of the participants were married. 4.1% (3 individuals) were divorced, 6.8% (5 individuals) were

widow/widower. 24.3% (18 individuals) were primipara and 75.7% (56 individuals) were multipara. Information about the use of oral contraceptive pills (OCPs) indicates that a vast majority used OCPs for less than five years (97.3%), while only a very small percentage reported using them for five years or more (2.7%).

Table 1: Distribution of the participants according to socio demographic characteristics (n=74).

Variables	Frequency (N)	Percentage (%)
Age		
30-39	43	58.1
40-49	17	23
≥50	14	18.9
Mean±SD	40.26±7.14	
Median (min-max)	39 (30-60)	
Marital status		
Married	66	89.2
Divorced	3	4.1
Widow/widower	5	6.8
Parity		
Primipara	18	24.3
Multipara	56	75.7
Occupation		
Housewife	50	67.6
Student	1	1.4
Service holder	22	29.7
Others	1	1.4
Use of OCP (years)		
<5	72	97.3
≥5	2	2.7

Figure 1 overview the distribution of the participants according to educational qualification. About 36% participants were secondary level followed by 27% primary pass, 26% higher secondary pass, 10% graduate and only 1% illiterate.

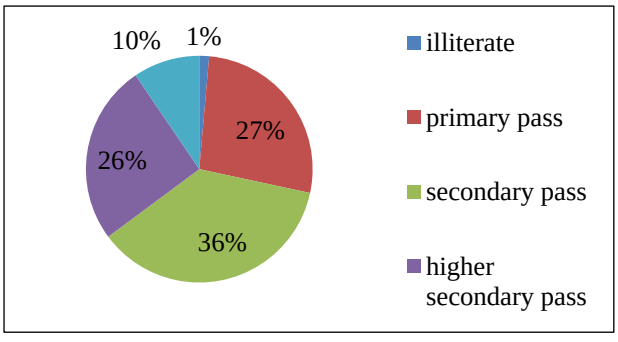

Figure 1: Distribution of the participants according to educational qualification.

Table 2 describes the correlation of agreement between the urine and cervical sample HPV report. The Kendall's tau was 0.752 which indicated towards moderate agreement. The significance was 0.001 which was highly significant.

Table 2: Kendall's correlation of agreement between cervical and urine sample HPV report.

Urine sample HPV report	Cervical HPV report (%)		Kendall's tau correlation	P value
	Positive	Negative		
Positive	9 (90)	1 (10)	c0.752	0.001s*
Negative	4 (6.2)	60 (93.8)		

Data expressed as frequency (percentage); c= Kendall's tau correlation; s*= statistically significant at 0.01 levels

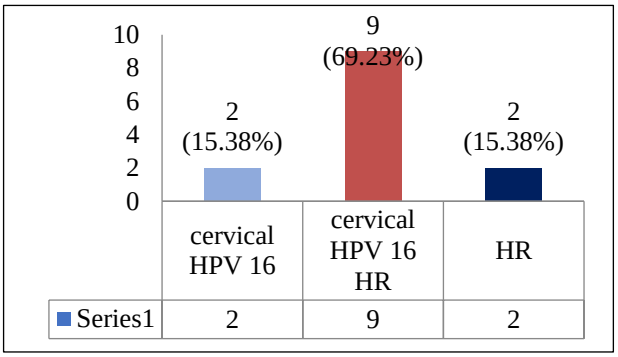

Figure 2: Distribution of the participants according to genotype detection by cervical HPV sample.

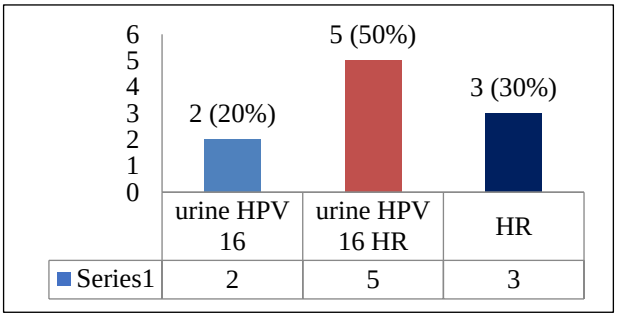

Figure 3: Distribution of the participants according to genotype detection by urine HPV sample.

Figure 2 and 3 shows distribution of the participants according to genotype detection by cervical and urine HPV sample. Cervical sample detected 2 HPV16, 4HPV 16 HR and 2 HR. Urine sample detected 1 HPV HR and 1 HR patient.

Table 3 describes the agreement between the urine and cervical sample HPV genotype report. The most frequently detected HR genotype in cervical sample was HPV 16 and HR (co-injection) 69.23% (9/13), HPV 16 (15.38%, 2/13) and HR only (15.38%, 2/13). The most frequently detected HR genotype in urine sample was HPV 16 and HR (co-injection) 50% (5/10), HPV 16 (20%, 2/10) and HR only (20%, 2/10). The Kappa agreement was moderate (0.746, 0.001).

Table 3: Genotype detection by urine sample and cervical sample.

Variables	Cervical HPV genotype (%)				Cohen's Kappa of agreement	P value
	No genotype detected	HPV 16	HPV 16 HR	HR		
Urine HPV genotype						
No genotype detected	61 (95.3)	1 (1.6)	3 (3.1)	0	0.746	[k]0.001[s]
HPV 16	1 (50)	1 (50)	0	0.		
HPV-16 HR	0	0	5 (100)	0		
HR	0	0	1 (33.3)	2 (66.7)		

Data expressed as frequency (percentage); k= Cohen's kappa for agreement test; s= statistically significant

DISCUSSION

The present study aimed to evaluate the concordance between HPV DNA detection in self-collected urine samples and clinician-collected cervical samples among women aged 30-60 years who were positive for VIA or had abnormal Pap test results. Our findings showed that the overall agreement between HPV detection in urine and cervical samples was substantial, with a Kappa value of 0.746. This result aligns with and, in some cases, surpasses those reported in similar studies, indicating the potential utility of urine samples as an alternative or complementary method for cervical cancer screening.

Several studies have explored the reliability of urine samples for HPV detection, particularly in resource-limited settings where traditional cervical sampling may be less feasible. Pathak et al. reported a concordance rate of 77% ($\kappa = 0.62$) between urine and cervical samples in detecting high-risk HPV (HR HPV) genotypes in a similar population of women aged 30-60 years.[15] The slightly lower Kappa value in their study compared to ours may be attributed to differences in sample processing, laboratory techniques, or population characteristics.

A study by Franceschi et al conducted in rural India found a concordance rate of 85% ($\kappa = 0.69$) between urine and cervical samples, which is comparable to our findings.[16] This study also highlighted the feasibility of using urine samples for HPV detection in low-resource settings, where cervical sampling might not be readily available or acceptable to women. The higher agreement rate in our study could be due to the more controlled environment in which our samples were collected and processed.

In contrast, Leeman et al found a lower concordance rate of 65% ($\kappa = 0.54$) in a high-income setting, suggesting that population differences, including sexual behavior, HPV prevalence, and screening practices, might influence the sensitivity of urine samples.[17] The authors of this study posited that the lower concordance rate might also reflect the lower viral load typically found in urine compared to cervical samples, which could affect the detection of certain HPV genotypes.

The HPV positivity rates observed in our study were slightly different between urine and cervical samples. Specifically, 10% of HPV-positive urine samples were negative in cervical samples, while 6.2% of cervical-positive samples were negative in urine samples. These findings are consistent with those of Stanczuk et al, who observed that 12% of HPV-positive cases in urine samples were missed by cervical sampling.[18] This phenomenon underscores the potential of urine sampling to detect HPV infections that might be overlooked by traditional cervical methods, possibly due to differences in the site of infection or sampling technique.

A systematic review by Mbulawa et al, assessed the sensitivity and specificity of urine samples for HPV detection across multiple studies, finding that sensitivity ranged from 70% to 90%, with specificity generally higher than 90%.[19] Our study's findings fall within this range, suggesting that urine sampling can be a reliable method for detecting HPV, especially when combined with other screening methods. The consistency of our findings with global trends further supports the integration of urine sampling into cervical cancer screening programs, particularly in settings where cervical sampling is underutilized.

When considering specific HPV genotypes, our study found that HPV 16 and HR genotypes were detected in 50% of cases in urine samples and 69.23% in cervical samples. This detection rate is comparable to the results of Tisci et al, who reported that HPV 16 was detected in 45% of urine samples and 70% of cervical samples in women with abnormal cytology.[20] This close alignment between studies reinforces the relative reliability of urine samples in detecting high-risk HPV genotypes, particularly HPV 16, which is most commonly associated with cervical cancer.

Vorsters et al, also reported similar findings, with HPV 16 being the most commonly detected genotype in both urine and cervical samples.[21] They noted that the slightly lower detection rates in urine could be due to a lower viral load or dilution effects, similar to the potential explanations in our study. The ability of urine samples to detect co-infections of HPV 16 with other HR genotypes in 50% of cases, compared to 69.23% in cervical samples, highlights their potential utility, though with slightly reduced sensitivity compared to cervical samples.

The implications of these findings are significant for public health, particularly in the context of cervical cancer

screening. Non-invasive sampling methods, such as urine collection, could greatly increase screening coverage, especially in populations where traditional cervical sampling is less acceptable due to cultural or logistical barriers. A study by Louvanto et al found that over 80% of women preferred urine sampling over cervical sampling, suggesting that urine-based screening could improve compliance and early detection rates.[22] The potential for urine samples to serve as a complementary tool in cervical cancer screening programs is further supported by the growing body of evidence from studies like ours and others.

However, methodological differences across studies should be considered when interpreting these findings. For example, Palefsky et al., emphasized the importance of sample collection timing, noting that first-morning urine samples yielded higher HPV detection rates compared to random samples.[23] This could explain variations in concordance rates between studies and suggests that standardizing collection protocols could enhance the reliability of urine-based screening.

Differences in laboratory techniques, such as the DNA extraction methods and PCR protocols used, might also contribute to variations in detection rates. Some studies, like those by Schiffman et al used different viral DNA extraction kits or targeted different HPV regions, which could explain discrepancies in sensitivity and specificity.[24] Additionally, population characteristics, such as age, sexual behavior, and HPV vaccination status, could influence HPV prevalence and detection rates, as noted in studies like Arbyn et al.[25]

This study has few limitations. The study had a relatively small sample size of 74 participants, which may limit the generalizability of the findings. A larger sample size could provide more robust results and improve the statistical power of the study. The use of purposive sampling may introduce selection bias, as the participants were not randomly selected. The study focused on high-risk HPV types (16, 18, and others), which may overlook the detection of other potentially significant HPV genotypes. A broader range of HPV types should be considered in future research.

CONCLUSION

This study demonstrated a significant level of concordance between HPV genotypes detected in cervical and urine samples among women who tested positive for cervical cancer screening. While cervical samples remain the gold standard for HPV detection, urine-based testing showed promising results, offering a non-invasive and potentially more accessible alternative. The findings suggest that urine samples could be effectively integrated into existing screening programs, particularly in settings where traditional cervical sampling is less feasible or accepted.

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