RESEARCH ARTICLE

Editorial Process: Submission:03/13/2023 Acceptance:07/02/2023

Comparison of Human Papillomavirus Genotype Detection in Paired Urine and Self-Collected Cervical Swabs: A Pilot Study

Sangram Singh Patel[1]*, Nilanchali Singh[2], Ashima Jamwal[1], Chinmoy Sahu[1], Atul Garg[1], Nisha Rao[2], Nidhi Tejan[1], Rashid Khan[1]

Abstract

Objectives: With the objective of establishing a simple, cost-effective, and effective screening tool for the screening of Human Papilloma Virus infection, the study was undertaken. **Materials and Methods:** This pilot study was conducted on 20 urine samples of women whose cervical swabs were tested positive while screening for Human papilloma virus in asymptomatic women. **Results:** HPV genotypes were detected in 94% (16/17) patients in urine samples by real-time PCR while a 100% detection rate (15/15) was observed in the cervical swab samples. The results of the urine and cervical swab samples, tested by the TRUPCR ®HPV high-risk genotyping kit, are shown in Table 2. HPV genotype 68 was found in 82.3% urinary samples and 100% of self-collected vaginal swab samples. Out of 16 positive urine samples, 2 were positive for HPV genotype 16 and 5 were positive for HPV genotype 18, and in cervical swab testing out of 15 positive samples, 3 were positive for HPV genotype 16, and 5 were positive for HPV genotype 18. Diagnostic accuracy of urine was found to be 98.8% (95% CI 79.43% - 100.00%). **Conclusion:** This pilot study aims to assess the accuracy of urine samples in the screening of HPV infection among asymptomatic women and establish the distribution of prevalent HPV genotypes. This may further contribute to standardizing the urine and cervical swab testing methods for cervical cancer screening strategies.

Keywords: Human Papilloma Virus- cervical swab- Urine samples- HPV genotypes

Asian Pac J Cancer Prev, **24 (7)**, 2427-2430

Introduction

Worldwide, cervical cancer is the fourth most common cancer reported in women and is the second most common cancer in women inhabiting low-income countries (Bhatla and Singhal, 2020). The cancer is associated with the Human papillomavirus (HPV) which is known to affect the sexually active population at some point in their lives. HPV is a small, non-enveloped deoxyribonucleic acid (DNA) virus that has a special predilection for mucosal cells (WHO Cervical cancer, 2022). The viral genome contributes to over 100 HPV genotypes, of which 16 are responsible for 70% of the cervical cancers.

The infection may be self-limiting or may result in pre-cancerous and invasive cancer. In addition, access to treatment for such cancerous lesions is limited in low or middle-income countries which further attributes to a high mortality rate of 13.3/100,000. This, however, can be reduced by effective screening interventions and timely diagnosis.

The PAP (Papanicolaou) test has been a preferred screening tool (MacLaughlin et al., 2019) in the diagnosis of cervical cancer but there are certain limitations like incorrect information regarding the procedure, lack of spousal or family support towards the test and the stigma associated with cervical cancer contribute to the decreased accession of the procedure among the rural population (Taneja et al., 2021).

Screening strategies for cervical cytology or Papanicolaou (PAP) testing require uncomfortable, and pelvic examinations and the cytology results are often susceptible to technical errors, low sensitivity, and false negative results. Therefore, alternative, and supplementary HPV DNA assays are often required in combination with the traditional Pap smear test. Self-sampled vaginal swabs are being used in screening programs at different centres but their limitations like improper technique, non-cooperation further add to the limited implementation in the rural population (Adsul et al., 2019). Testing urine samples may prove to be a successful alternative to screening methods, especially in poor-resource countries. The first void urine sample (Kim et al., 2022) contains a higher percentage of exfoliated cells of the debris compared to the mid-stream collection. Therefore, its self-sampling is comfortable as well as easier compared to the other sampling methods.

*[1]Department of Microbiology, SGPGIMS, Lucknow, India. [2]Department of Obstetrics and Gynecology, AIIMS, New Delhi, India. *For Correspondence: sangram87@gmail.com*

Being inexpensive, non-invasive, and simple to collect, urine samples make a promising tool for screening for HPV infection. The majority of the Indian population resides in rural areas with a lack of healthcare facilities and poor accessibility to a well-equipped diagnostic set-ups. Hence, an efficient, effective and affordable tool for screening of cervical cancer is required to significantly reduce the burden of the disease by early diagnosis and management.

This pilot study aims to assess the accuracy of urine samples in the screening of HPV infection among asymptomatic women and establish the distribution of prevalent HPV genotypes. This may further contribute to standardizing the urine and cervical swab testing methods for cervical cancer screening strategies.

Materials and Methods

Study design 1

A cross-sectional study was conducted among asymptomatic women in rural areas surrounding Jagdishpur district, Uttar Pradesh. The asymptomatic women in the age group of 18-65 were enrolled in the study. The study was approved by the Institutional Ethical Committee of Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow with Registration Number: 2021-109-IMP-EXP-38 and Reference Number: PGI/BE/303/2021 before commencing the protocol. Collaboration with the local Primary Health care Centre and subcentre was established to reach the participants.

Recruitment of subjects 1.1

The objectives of the study were explained to the concerned staff at the CHC involved in the recruitment of participants. Households were visited by the ASHA (Accredited Social Health Activists) workers and subjects willing to participate in the study were explained the testing protocol and procedures in their local language. Written informed consent from all the enrolled participants was taken.

Community Health Centre (CHC) Jagdishpur was the primary testing site for the self-collected Cervical swab samples using the Truenat High risk HPV genotyping kit18 (®Molbio Diagnostics, Goa, India) and 20 positive cervical swab samples with their respective urine samples were further tested for PCR genotyping at SGPGIMS to ascertain the role of screening urine samples for HPV detection. This pilot study was conducted on the 20 participants whose cervical swab samples had tested positive by Trunat HPV kit at the Community health center. The respective urine samples of these 20 participants were collected after consent and both cervical swabs and urine samples were sent to the department of microbiology, SGPGIMS for PCR testing. The demographic details for these 20 patients were recorded. The results of the PCR at two centres were not compared as the genotype detection varies in both the kits mentioned;

Specifications of the kits used at the testing sites:

Table 1. Specifications of the Testing Kits Used

Name	Principle	Detection of genotypes
TRUPCR®HPV* High-Risk Genotyping Kit by BlackBio Biotech India Ltd	Real-time amplification kit	14 HPV high/ intermediate risk types 16,18,31,33,35,39,45,5 1,52,56,58 59,66,68
Trunat® HR-HPV* by molbio diagnostics	Real-time amplification kit	4 HPV high risk types 16,18,31 and 45

*Abbreviations: HPV, Human Papilloma Virus; HR-HPV, High Risk Human Papilloma Virus

Testing protocol 1.1.1

An early stream random urine sample and a self-collected cervical swab were collected and transported to the microbiology laboratory at 4-80C in a transport medium.

All (20) urine and vaginal samples were tested by TRUPCR ®HPV High-Risk Genotyping Kit as per the testing protocol (Table 1).

Data analysis 2.0

Out of the 20 samples (urine and cervical swabs), 3 urine samples and 5 cervical swab samples were insufficient in quantity and could not be processed further. Therefore, 17 urine and 15 cervical swabs were tested using the TRUPCR ®HPV High-Risk Genotyping Kit19 (Table 3). Categorical variables were described using percentages and a Chi-square test was applied. All Statistical analyses were performed using SPSS statistical software (IBM SPSS version 26, Armonk, N.Y.).

Results

HPV genotypes were detected in 94% (16/17) patients in urine samples by real-time PCR while a 100% detection rate (15/15) was observed in the cervical swab samples (Figure 1). The results of the urine and cervical swab samples, tested by TRUPCR ®HPV high-risk genotyping kit, are shown in Table 2. HPV genotype 68 was found in 82.3% urinary samples and 100% of self-collected vaginal swab samples. Out of 16 positive urine samples, 2 were positive for HPV genotype 16 and 5 were positive for HPV genotype 18 and in cervical swab testing out of 15 positive samples, 3 were positive for HPV genotype 16 and 5 were positive for HPV genotype 18.

Discussion

Cervical screening is crucial, as cervical cancer can be prevented in more than 90% of cases. Cytological screening for the early detection of cervical cancer precursors has been carried out successfully in developed countries. HPV-DNA testing has been shown to be more sensitive than cytological analysis and may thus be an alternative to cervical scrapes (Lorenzi et al., 2018). Vaginal and urine self-sampling methods have been shown to be more acceptable for patients and result in increased participation when available in the screening programs field (Ducancelle et al., 2014). Using urine as a sample for HPV DNA testing is still debated. In this setting, the

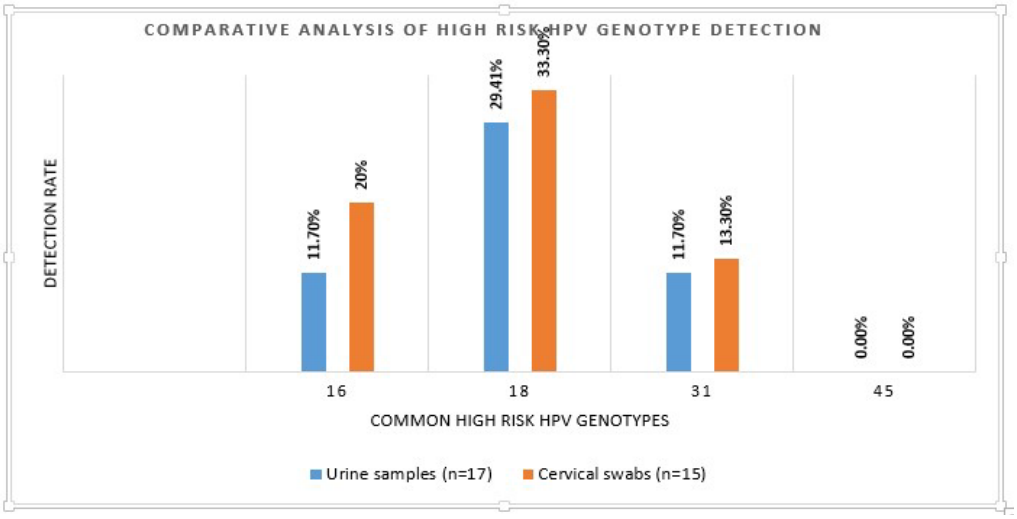

Figure 1. Comparison of Urine and Cervical Swab Samples in the Detection of High Risk HPV Genotypes.

objectives of the present paper were to compare HPV DNA detection in urine and self-collected vaginal swab samples.

Urinary HPV testing is performed on the basis that urine can be used for the detection of HPV as the epithelial wall of the uterine cervix and/or vagina normally sheds exfoliated cells into the urine and if there is HPV or any other microbial infections, the shredded cells should contain viral genomes virions including genomes of other infectious organisms. Therefore first void urine sampling has been utilized for the detection of HPV by several authors (Bober et al., 2021; Leeman et al., 2017; Torres-Rojas et al., 2021; Vorsters, Van Damme, et al., 2014). A study Nilyanimit et al., (2017) suggests that urine represents a viable substitute for cervical swabs in detecting HPV. Urine samples are a good option for self-sampling screening since they are non-invasive and simple to collect.

In the present study, first void urine and cervical swab samples were studied by TRUPCR® HPV High-Risk Genotyping Kit. The cervical samples were already tested positive by the Trunat PCR kit at the testing site in Jagdishpur district. The Trunat PCR HR-HPV genotyping kit is not standardized for the testing of urine samples, hence the urine samples were transported to the microbiology laboratory at SGPGIMS for further testing by real-time PCR. Although the prevalence of HPV infection was slightly higher in the vaginal samples, but it is not significantly different from what has been found in urine samples. The most important finding in our study was that urine samples show a diagnostic accuracy (Table 3) of 98.8% which is significantly higher than the other diagnostic modalities available. While comparing to a meta-analysis done by Peter et al., (2021) and Bober et al., (2021); a heterogeneity was detected between the pooled sensitivities and specificities, i.e., pooled sensitivity for high-risk HPV detection in urine was 78% (70% to 84%) and specificity was 89% (81% to 94%). For any HPV detection in the urine of 87% (74% to 94%) and 91% (83% to 96%), we pooled sensitivity and specificity, respectively. HPV 16 and 18 had a pooled sensitivity of 77% (76% to 77%) and a specificity of 98% (98% to 98%).

The results from our study suggest that using urine sampling to detect genital HPV infection can be a very useful and convenient tool for the non-invasive screening of symptom-free normal healthy women who may have high-risk HPVs. The technique is simple and cost-effective. However, urine samples contain insufficient cervical cells and may vary depending on collection, storage, DNA extraction, and study population (Vorsters et al., 2012; Vorsters et al., 2014) Therefore, it is necessary to develop appropriate collection, storage, and DNA extraction methods to efficiently use urine samples as HPV test samples. The results of testing urine samples

Table 2. Distribution of HPV Genotypes Detected in Urine and Cervical Swab Samples

HPV Genotype detected	Urine samples (n=17) Total tested= 17 Positive= 16	Cervical swabs (n=15) Total tested= 15 Positive= 15
68	14 (82.3%)	15 (100%)
18	05 (29.41%)	05 (33.3%)
59	04 (23.5%)	02 (13.3%)
51	03 (17.6%)	04 (26.6%)
66	02 (11.7%)	05 (33.3%)
16	02 (11.7%)	03 (20%)
31,33	02 (11.7%)	02 (13.3%)
39,52,58	02 (11.7%)	01 (6.6%)
35	01 (5.8%)	01 (6.6%)

*HPV, Human Papilloma Virus

Table 3. Diagnostic Accuracy of Urine Samples in the Estimation of HPV Infection.

Statistical accuracy	Value (%)	95% CI
Sensitivity	94.12%	71.31% - 99.85%
Specificity	100.00%	2.50% - 100.00%
Positive Predictive Value (*)	100.00%	-
Negative Predictive Value (*)	98.55%	91.04% - 99.78%
Accuracy (*)	98.82%	79.43% - 100.00%

(*) based on the prevalence of the disease taken as 20%.

for the determination of HPV genotypes look promising and require thorough research before implementing it as a screening tool.

Strengths and Limitations: This is a pilot study involving only 20 participants. Therefore, these results cannot be directly extrapolated to a screening population due to small sample size. The study highlights the implementation of urine as a useful screening tool which can be further tested on a large population to establish its accuracy in estimation of HPV infection in Indian women.

In conclusion, This pilot study shows that testing of first catch urinary samples is comparable to self-collected vaginal swabs in detecting HPV infection and hence, can make a promising tool in the screening of HPV in comparison to the poorly acceptable cervical sampling procedures. This might show some more hope in bringing out urinary samples as an alternative screening methodology.

Author Contribution Statement

Dr. Sangram, Dr. Nilanchali; Data curation: Dr. Sangram, Dr. Nilanchali; Formal analysis:Dr. Chinmoy, Dr. Atul, ; Investigation: Dr. Nisha, Dr. Nidhi; Methodology: Dr. Sangram, Dr. Nilanchali, Dr. Ashima; Writing - original draft: Dr. Sangram, Dr. Nilanchali, Dr. Ashima; Writing -review and editing: Dr. Chinmoy, Dr. Atul, Dr. Nidhi.

Acknowledgements

The authors would like to thank the staff at CHC Jagdishpur for their constant support.

Ethical Approval

The study was approved by the Institutional Ethical Committee of Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow with Registration Number: 2021-109-IMP-EXP-38 and Reference Number: PGI/BE/303/2021 before commencing the protocol.

Conflict of interest

None declared.

References

Adsul P, Srinivas V, Gowda S, et al (2019). A community-based, cross-sectional study of hr HPV DNA self-sampling-based cervical cancer screening in rural Karnataka, India. *Int J Gynaecol Obstet*, **146**, 170–6.

Bhatla N, Singhal S (2020). Primary HPV screening for cervical cancer. Best Practice & Research. *Clin Obstet Gynecol*, **65**, 98–108.

Bober P, Firment P, Sabo J (2021). Diagnostic Test Accuracy of First-Void Urine Human Papillomaviruses for Presence Cervical HPV in Women: Systematic Review and Meta-Analysis. *Int J Environ Res Public Health*, **18**, 13314.

Cervical cancer (n.d.) (2022). WHO. Human Papillomavirus (HPV) and Cervical Cancer; World Health Organization: Geneva, Switzerland. Retrieved January 3, 2023, from https://www.who.int/news-room/fact-sheets/detail/cervical-cancer.

Ducancelle A, Legrand MC, Pivert A, et al (2014). Interest of human papillomavirus DNA quantification and genotyping in paired cervical and urine samples to detect cervical lesions. *Arch Gynecol Obstet*, **290**, 299–308.

HPV Information Centre (n.d.) (2023). ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Retrieved January 3, from https://hpvcentre.net.

Kim DH, Jin H, Lee KE (2022). Analysis of HR-HPV Infection Concordance Rates in Cervical and Urine Specimens; Proposal of Additional Cervical Screening Process for Women Who Refuse Invasive Cervical Sampling. *J Pers Med*, **12**, 1949.

Leeman A, Del Pino M, Molijn A, et al (2017). HPV testing in first-void urine provides sensitivity for CIN2+ detection comparable with a smear taken by a clinician or a brush-based self-sample: Cross-sectional data from a triage population. *BJOG*, **124**, 1356–63.

Lorenzi AT, Fregnani JHT, Dockter J, et al (2018). High-Risk Human Papillomavirus Detection in Urine Samples From a Referral Population With Cervical Biopsy-Proven High-Grade Lesions. *J Low Genit Tract Dis*, **22**, 17–20.

MacLaughlin KL, Jacobson RM, Radecki Breitkopf C, et al (2019). Trends Over Time in Pap and Pap-HPV Cotesting for Cervical Cancer Screening. *J Womens Health*, **28**, 244–49.

Nilyanimit P, Chansaenroj J, Karalak A, et al (2017). Comparison of human papillomavirus (HPV) detection in urine and cervical swab samples using the HPV GenoArray Diagnostic assay. *Peer J*, **5**, e3910.

Taneja N, Chawla B, Awasthi AA, et al (2021). Knowledge, Attitude, and Practice on Cervical Cancer and Screening Among Women in India: A Review. *Cancer Control*, **28**, 107327482110107.

Torres-Rojas FI, Mendoza-Catalán MA, Alarcón-Romero LDC, et al (2021). HPV molecular detection from urine versus cervical samples: An alternative for HPV screening in indigenous populations. *Peer J*, **9**, e11564.

Vorsters A, Micalessi I, Bilcke J, et al (2012). Detection of human papillomavirus DNA in urine. A review of the literature. *Eur J Clin Microbiol Infect Dis*, **31**, 627–40.

Vorsters A, Van Damme P, Clifford G (2014). Urine testing for HPV: Rationale for using first void. *BMJ*, **349**, g6252–

Vorsters A, Van Den Bergh J, Micalessi I, et al (2014). Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. *Eur J Clin Microbiol Infect Dis*, **33**, 2005–14.